Strategy towards producing relevant and reliable data for the hazard assessment of micro- and nanoplastics in agricultural soils

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Strategy towards producing relevant and reliable data for the hazard assessment of micro- and nanoplastics in agricultural soils

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ABSTRACT

Micro- and nanoplastics (MNPs) are widespread emerging contaminants with many potential direct and indirect effects on soil ecosystems. Ecological soil MNP hazard assessment is thus crucial for a proper risk assessment and the development of environmental protection regulations. However, current hazard assessment testing approaches are hampered by the absence of guidelines, harmonization, and standard reference materials. This article discusses the need for improving testing approaches and provides specific recommendations to increase the relevance and reliability of ecotoxicity data. Our recommendations focus on environmentally relevant experimental designs, guidelines for MNP test materials selection and characterization, analysis of MNPs and additives in soil and biota, and a proposal for relevant soil physicochemical properties to be assessed during ecotoxicity testing. This article brings novelty to the field of ecological hazard assessment of MNPs in soil by providing specific recommendations much needed in this field.

1. Introduction

Recent research is unveiling microplastics as prominent contaminants of soils. Soils receive significant quantities of microplastics and nanoplastics (herein jointly referred to as micro- and nanoplastics, MNPs) from a range of sources, pathways and processes, such as use of plastics and sewage sludge-based fertilizers in agriculture, compost, runoff, atmospheric deposition and littering [1–6]. This has already resulted in widespread contamination, with evidence showing that concentrations increase over time [4,7–15]. In contrast, knowledge on the effects of MNPs on soil ecosystems is fragmentary, mainly due to the lack of guidelines, harmonization, and reference materials to conduct an ecological risk assessment.

Existing studies show that MNPs can induce a range of direct and indirect effects on both abiotic and biotic constituents of soils. For instance, different types of MNPs were shown to affect soil aggregate stability, bulk density, soil water holding capacity and soil microbial communities [12,16–23]. In this way, MNPs can act as habitat modifiers, for instance when their interactions with abiotic constituents produce physical or chemical alterations of the soil environment that pose a

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stress to inhabiting soil organisms [23–26]. A number of different types of effects on terrestrial invertebrates and plants at environmentally plausible levels of MNPs have already been observed but much is still unknown [23,27].

Micro and nanoplastics are present in soil in complex mixtures of debris, and vary greatly in size, shape and chemical composition. Characterizing hazard and risk for such complex anthropogenic stressors is challenging [7]. Nevertheless, ecological risk assessment of MNPs in soil is likely to be a component of future environmental protection regulations. The scientific community, therefore, is urged to refine approaches to enable hazard and risk assessments to be performed in a rigorous and reproducible way. This will require identifying 1) strategies to reduce complexity of testing, 2) representative exposure scenarios to address (case by case) the range of questions inherent to the risk of different types or mixtures of materials, and 3) sensitive and representative endpoints and risk thresholds for both direct and indirect effects.

Standardized single-species toxicity tests will likely be a valuable component of future ecological risk assessment for MNPs in soils even though they cannot exhaustively address the full spectrum of potential direct and indirect effects posed by MNPs. These tests are based on the framework of representative sensitive species [28] and are currently used tools to evaluate the risk of chemical contaminants. However, data reliability, relevance and reporting in ecotoxicological studies have been identified as a critical issue preventing data (re-)use in hazard assessment [29]. Therefore, it has been proposed that data used in hazard assessment require a measure of quality [30]. In general terms, this implies how well a study was conducted (i.e., reliability) and how relevant the observations are to the question (i.e., relevance). Several recommendations have been suggested to advance the quality of MNP research in freshwater ecosystems [31,32], but for the soil environment, this has not yet been holistically approached. At this stage, it is therefore pivotal for scientists to understand how studies based on single species can be meaningfully designed to deliver responses at the population level that could be useful to extrapolate risk at higher levels of ecological complexity.

The aim of this paper is to address the needs to arrive at more complete and environmentally relevant test strategies for assessing the environmental hazards of MNPs in soils, especially using single-species or simplified community toxicity tests, and simultaneously provide recommendations for the practical implementation of measures to fulfill quality criteria in environmentally relevant ecotoxicology tests on MNP effects. In the following sections, we present: 1) relevant MNP test materials, 2) approaches regarding the MNP hazard testing, 3) analysis of biota and soil, and 4) quality assurance/quality control for soil MNP hazard assessment.

2. Micro- and nanoplastic test materials

2.1. Environmental relevance of test materials

Effective testing for MNP ecotoxicity in soil requires environmentally relevant test materials (test MNPs). Because of the virtually infinite possibilities, it is very difficult to theoretically define which test materials should be used in ecotoxicological tests where MNPs are added to the system in a controlled manner. Pristine spherical MNPs composed of a narrow range of polymer types have dominated thus far in ecotoxicological studies [33], which is linked to the commercial availability of such materials. Yet, spherical MNPs rarely represent an important component of MNP contamination in soil environments and they are likely to impart a rather different ecotoxicological profile compared to more environmentally relevant MNPs [34]. Test materials should instead represent relevant particle typologies linked to dominant sources to soils; for example, fibers which are prevalent in sewage sludge [35,36] or fragments from the degradation of agricultural plastic products [37] or introduced via organic fertilizer.

The constellation of different MNP particle properties present in soil environments is likely to exceed the capacity for ecotoxicological testing; hence, a prioritization is necessary to identify the most important – from both an exposure and hazard perspective – particle types for testing. For example, for agricultural plastics, products/applications that have a higher likelihood of forming MNP residues, where those residues also have a high likelihood of entering soils, should be prioritized. In some cases, the use of a mixture of different types of MNPs in ecotoxicological testing can be justified to mimic the relevant MNPs pollution in the environment [5,6]. This type of testing has not been regularly undertaken in soil.

Future ecotoxicity studies should critically evaluate the environmental relevance and significance of the test materials used, which should encourage a shift away from the use of pristine spherical particles towards, for example, more complex morphologies and more relevant size ranges and material origins. This should draw from the characterization of particles observed in soil monitoring activities, such as those that help to define exposure concentrations (Section 3.1), to identify environmental relevant particle typologies (relevant size distributions, relevant morphologies, relevant polymer types or potential sources).

2.2. Production of microplastic test materials

Currently, MNPs used for ecotoxicity testing are produced by various methods (Fig. 1). There are often several trade-offs associated with producing MNPs; for example, producing sufficient quantities versus producing sufficiently small particles or conserving important physical or chemical properties. It is possible that different methods are needed for different starting materials or desired final particle properties; for example, to produce fibers from textiles versus fragments from agricultural plastics. However, it is suggested that environmentally relevant MNPs should be produced by the fragmentation of plastics into MNPs (i.e. top-down production), as their formation in the environment is the consequence of a variety of natural biotic and abiotic processes that lead to random fragmentation and generation of polydisperse MNPs with different sizes and shapes.

From an ecotoxicological perspective, many of the ways in which environmentally relevant test materials are expected to differ from commercially available MNPs relate to the material properties or MNP characteristics: for example, particle size or morphology, degree of aging or additive composition [34]. A major challenge in producing or obtaining environmentally relevant test materials is the lack of a thorough characterization of real soil MNP contamination. There is therefore a paucity of information on target particle attributes to refine production methods. An assessment of the particle characteristics – such as the size distribution or extent of ageing – from monitoring studies would facilitate a prioritization of environmentally relevant reference materials for ecotoxicological testing. In addition, there is a need for international collaboration and harmonization on the production of test materials. This should also include a description of production methods that can facilitate reproducibility, as well as a critical evaluation of the particles produced by each method to identify both opportunities and shortcomings.

2.3. Characterization of microplastic test materials

Regardless of the objective of the study – whether ecotoxicological effects on terrestrial or aquatic organisms are investigated – the basic physicochemical characterization of MNPs should be performed in a similar manner [34]. It includes material characterization, investigation of surface morphology, determination of size and shape, additive analysis and, in some studies, also additional parameters, such as for example surface charge or ζ-potential (Fig. 1). This is recommended because even supposedly identical particles differ in their properties and thus also may differ in their interaction with organisms [38].

Some of the methods to characterize microplastics are widely used

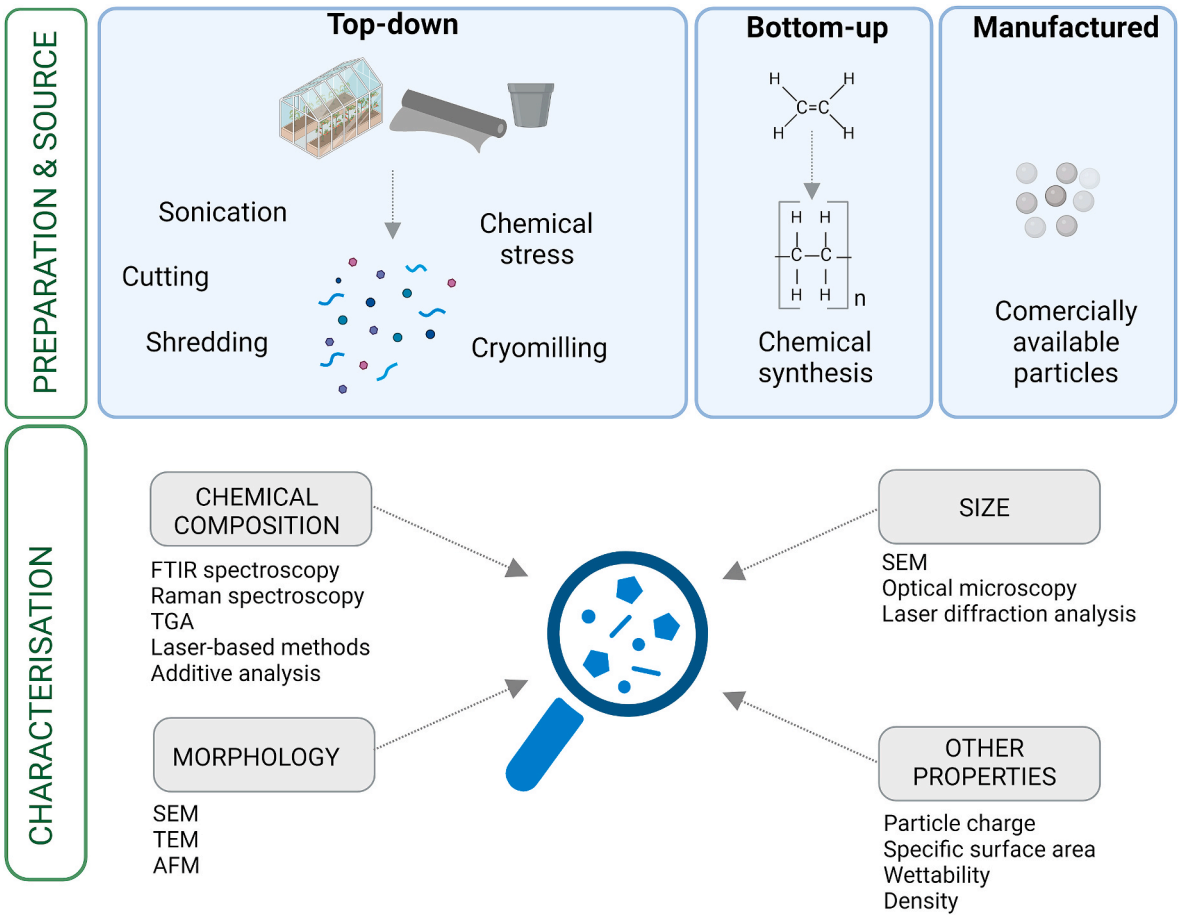

Fig. 1. List of methods used for preparation and characterization of micro- and nanoplastic (MNP) test materials. The figure was prepared based on [52–57]. FTIR - Fourier-transform Infrared Spectroscopy, TGA - Thermogravimetric analysis, SEM - Scanning Electron Microscopy, TEM - Transmission Electron Microscopy, AFM - Atomic Force Microscopy. Additive analysis is presented in detail in Fig. 3.

and are robust analytical tools in MNP research (e.g., Fourier-transform Infrared Spectroscopy (FTIR) and Raman spectroscopy, Scanning Electron Microscopy (SEM), particle size/ζ-potential analyzers), but some can have important limitations. For example, optical microscopy is commonly used for determining particle size distributions, but it is not suitable for the determination of smaller particles in sub-micron sizes. In such a case, the presence of nanoplastics in the prepared test materials can be overlooked. Therefore, it is important to understand the limitations of the methods used for characterization of MNPs and to combine them with other analytical tools as needed to reliably assess their properties.

Micro- and nanoplastics can also contain various additives used during polymer synthesis and production, while residues of monomers and other contaminants may also still be present. The use of plastic additives (PAs) is necessary to improve the flexibility, durability and endurance towards the ambient conditions that plastics may be exposed to, such as sunlight and humidity. These additives form a component of the chemical composition of MNPs and are important to consider in ecotoxicological testing. Plastic additives encompass a large (>10 000 compounds [39]) and physiochemically diverse group of molecules. Some of them (e.g., phthalates, flame retardants or bisphenols) have been widely studied both in terms of toxicity and their analytical determination in soil [40,41]. However, this is not the case, for example, for plastic additives derived from agricultural plastics, highlighting a need to map these molecules and better understand their presence in different agricultural plastic products and MNPs that may subsequently be formed.

In detail, based on their function, PAs commonly used in agricultural plastics can be divided into antioxidants, slip agents, light stabilizers and plasticizers. Antioxidants increase the lifetime of plastic materials by delaying their oxidative degradation when exposed to UV light [42]. Various molecules have been used including different Irganox® and Irgafos® analogues (there is a whole spectrum of chemicals belonging to these two commercial names). Slip agents are used to reduce the surface coefficient of friction lubricating the film surface, which prevents mechanical problems such as films – an important material type in agriculture – from sticking together. Examples of common slip agents are fatty acid amides and metallic stearates [43]. Light stabilizers are responsible for plastic protection from sun and weather exposure. They protect agricultural plastics from UV light, protecting against color changes and embrittlement. Among them, the hindered amine light stabilizers (HALS) are considered the best performing ultraviolet stabilizers and long-term heat stabilizers in polyolefins [44]. Of note is the lack of new commercially available stabilizers as HALS feature robust performance and cost efficiency, setting a great challenge in the synthesis of new molecules [45]. Plasticizers are added to polymers to reduce brittleness, improve flexibility and toughness, and enhance plastic processability [46]. For this reason, plasticizers are used not only in agricultural plastics but in many other applications and according to a recent United Nations (UN) report on plastic pollution is the most common type of additives [47]. All in all, the discussed additives differ from those typically considered within ecotoxicological testing of common, non-agricultural, plastic types. This represents a knowledge gap which can hinder the hazard assessment of agricultural plastics, where further testing of compounds relevant to agricultural plastics is needed.

Overall, the characterization of MNPs used in ecotoxicological

studies is important because they can aid in interpreting their potential hazard. In particular, the additive composition and content is interesting to discern a challenging task whether the effects of MNPs are due to particles or additives leaching from particles. However, recent reports have shown that the characterization of MNPs in existing ecotoxicity studies is often insufficient [34,48,49]. On the other hand, it clear that extensive characterization of each type of MNPs used in ecotoxicological studies is not feasible because many methods and devices are not available in every laboratory or require very specific skills. In particular, the detection and identification of additives in MNPs is a major challenge for analytical chemistry due to their low concentrations and unknown composition. This problem could easily be solved if plastic manufacturers were required to list the additives used in the production of plastics in the safety data sheets, as is common practice for other chemicals.

We recommend reporting at least a minimum of information about MNPs used in ecotoxicological research, including the size, shape, chemical composition, origin of the plastic material and the preparation procedure. It is also recommended to keep the test material in stock and available, to allow for future testing and verification of reproducibility of results. Where possible, as much additional information as feasible should be reported to provide further context for understanding potential ecotoxicological effects [50]. For example, the characterization of the eco-corona on MNPs, which has been shown to alter their interaction with organisms and cells, might aid in interpreting modes of MNP interactions with organisms [51]. Finally, further work is needed to establish and optimize protocols for the production and characterization of MNP test materials. The production of reference/certified materials (reference MNPs) with known composition, size and shape is crucial for reliable quality assurance of MNP research.

3. Testing the hazards of micro- and nanoplastics

3.1. Exposure concentrations

To obtain ecologically relevant results from ecotoxicity tests on soil invertebrates based on realistic exposure scenarios, it is essential to address effects not only in environmentally plausible shapes, size distributions and chemical compositions, but also in a range of environmentally relevant concentrations of MNPs.

The definition of the appropriate test concentrations is complicated by the fact that MNP concentrations in environmental samples are typically reported in two different units: mass and particle number concentrations. Based on current knowledge, MNP mass concentrations in soils depend on location (e.g., levels decrease from urban to rural regions), land use, anthropogenic influence and the analytical technique used, and show large variations also due to the inherent heterogeneity of soils [58–60]. Independent of the comparability between the examples given here, MNP mass concentrations range from 0.01 mg/kg [61] to 67 500 mg/kg [60] corresponding with 1×10^{-6} to 6.75 % (w/w) of soil, respectively. MNP particle concentrations in soils range from non-detectable levels [60] to 3.7×10^{6} [62] particles per kg soil. In agricultural soils in particular, MNP concentrations range from 0.008 mg/kg [61] to 540 mg/kg [63] corresponding with 0.8×10^{-6} to 0.054 % (w/w) and particle concentrations from non-detectable [64] to 3.7×10^{6} [62] particles per kg soil. This gives the framework for the ecotoxicity testing with the highest concentrations in units of % (w/w) in soil and millions of particles per kg.

The reported data on environmental MNP concentrations vary considerably as different methodologies are used for MNP detection, including sample processing and MNP extraction methods [65]. This creates major difficulties for data comparability and a great need for cross-validation and harmonization of the analytical methods used. Moreover, only a few methods are available to analyze particles in smaller size ranges (e.g., submicron and nanoscale), and are only rarely used. Thus, current data on MNP concentrations include particles mostly down to a certain minimum size threshold [66]. This may lead to an underestimation of MNP concentrations (note: mass concentration is less influenced by smaller particles) by several orders of magnitude as particle numbers tend to increase exponentially with decreasing particle size [67]. Hence, adequate risk assessment continues to be hampered by the lack of methods to reliably determine real environmental concentrations. Especially in terms of ecotoxicity, the particle size and shape are likely to be among the properties of MNPs that are crucial for interaction with organisms [38]. Smaller particles might be more readily ingested, and nanoplastics, to some extent, may cross biological barriers [68]. Thus, data from environmental samples generated by particle-specific analytical techniques deliver essential information for the design of realistic exposure scenarios in ecotoxicological experiments, whilst mass-based environmental data is needed for decisions regarding the exposure concentrations spiked in soils. In conclusion, both methodological techniques are complementary options for the characterization of the MNP contamination of environmental samples.

Very different ranges of MNP exposure concentrations have been used in ecotoxicity studies so far. In some studies, only two or three concentrations are used, which is not sufficient for hazard assessment. Some studies used very high exposure concentrations, for example, up to 12 % w/w [69], 50 % w/w [70] and even 80 % w/w [71]. Such high exposure concentrations are only to be expected in certain environments, e.g. municipal landfills or in the immediate vicinity of plastic industries and artificial turf fields and thus, do not represent common exposure scenarios. However, in most studies on MNP ecotoxicological effects in soils, test concentrations between 0.001 % w/w and 1.5 % w/w were chosen, corresponding to the concentrations reported in the environmental context and expected in future worst-case scenarios [68]. These differences show that the currently available soil ecotoxicity studies on MNPs are not based on a harmonized testing approach. This, in turn, is an essential prerequisite for comparability between studies and an important challenge for future research.

Following the hazard assessment approach, toxicity tests should cover a gradient of concentrations to define dose-response relationships and determine effect concentrations (e.g., LCx/ECx values). As with the standard hazard assessment of chemicals, it is recommended to choose a range of concentrations that cover a full dose-response relationship, including at least one low and one high concentration and spanning several orders of magnitude (as suggested by OECD and ISO guidelines for soil invertebrates). Considering literature data on MNP levels in soil, we propose testing ecologically relevant (from 0.001 % to 0.05 %) and worst-case scenario concentrations (up to 5 %) of MNPs in agricultural soils. To limit efforts, a preliminary test with concentrations spaced by a factor of 5–10 can be used to identify the concentration window where the effects occur before conducting a final test with finer concentration spacing (e.g., factor of 2). The gradient of concentrations chosen also depends on the particularities of the toxicity tests. For example, some organisms are difficult to collect or culture and the test requires a large working effort. In this case, fewer concentrations are tested. Thus, a fully harmonized test design for all test organisms is probably not possible.

3.2. Spiking the test media

Harmonization of spiking procedure to mix the test substance in with soil is important to ensure the reproducibility of soil ecotoxicity testing. For traditional chemicals, guidelines for toxicity testing describe standardized procedures, e.g., when and how solvents are used and how to achieve a homogenous distribution of the chemical in the soil [72]. Such procedures, however, are less well developed for MNPs, with authors spiking MNPs as dry powders or as suspensions into dry or premoistened soil.

First of all, care should be taken to prevent MNP background contamination from entering the test soils. Standard lab practices, such as wearing cotton clothing, using equipment, jars and containers made of steel or glass and weighing the MNPs on paper or aluminum foil,

should be followed as routine. The use of equipment to neutralize static electricity on MNP samples before weighing is recommended to ensure greater accuracy in spiking.

Another major challenge is to properly homogenize the MNPs through the test soil. Regardless of the choice of test soil, the procedure for soil MNP spiking should be the same. The common procedure to apply chemicals into the soil for testing involves adding the chemical in pre-moistened soil or bringing the soil to the desired moisture content for testing by adding the chemical in an appropriate amount of water [72]. However, in our experience, mixing dry MNPs in with dried soil (<1 % water content) works better, as this prevents the conglomeration of soil particles and or the formation of MNP aggregates during the mixing. When mixing MNPs into dry soil, however, care needs to be taken to prevent statically charged particles from being lost, due to attachment to mixing equipment. Another risk is that de-homogenization occurs with smaller or larger size fractions separating from the rest, necessitating moistening of the soil as soon as the MNPs are mixed in. We observed the need to prevent adding too much water at once, as this would cause MNPs composed of low-density polymer types to float and conglomerate at the soil surface, preventing proper homogenization.

We recommend mixing the dry MNPs in dry soil also when testing the combinations of MNPs and chemicals by adding the chemicals in aqueous solutions only after soil has been spiked with the MNPs. However, if chemicals need to be spiked in the soil using a solvent, the soil should be spiked with chemicals first and MNPs only added after the solvent has evaporated. In the latter case, the chemical is first dosed to a small portion of the total amount of soil needed, and the rest of the soil is added after evaporation of the solvent. This approach has been used previously [73].

There are different ways of preparing the soil with MNPs, either in one large batch and then distributing to test jars or weighing and mixing MNPs with the soil separately for each test jar. We are in favor of preparing larger batches because this can prevent the need of weighting small amounts of MNPs, which can result in errors. Also, a larger batch of initial test soil enables to measure physico-chemical properties, like pH and WHC (see section 4.3). If the test MNPs have a broad size range and the amount of soil to be spiked is small (e.g. for small organisms), it is also possible to fractionate the MNPs and dose the different size fractions separately into the soil. This normalizes as much as possible the mass and particle number concentrations used in each exposure concentration, especially when preparing small concentrations. If the test organisms are exposed to MNPs via food, similar procedures as described for soil could be applied to ensure homogenous distribution of the MNPs in the diet.

3.3. Mode of exposure

Terrestrial invertebrates may be exposed to pollutants along various routes: via pore water, ingestion of food and soil particles and inhalation of air present in the soil pores. The relative importance of these uptake routes is determined by morphological (e.g., structure of the epidermis), physiological (e.g., mode of uptake of water; drinking vs. uptake via the skin, mode of uptake of oxygen, feeding habits) and behavioral traits of the organisms and the properties of the pollutant [74]. When testing the hazards associated with chemicals using soil invertebrates, the soil is typically spiked with the chemical and dermal exposure is considered one of the potential routes of exposure [74]. Exposure to MNPs, however, differs from chemical exposure, since insoluble particles cannot easily penetrate the organism's surface in the same way as water-soluble or fat-soluble chemicals because organisms have developed various anatomical features, like extra-cuticular matrices, to prevent natural particles entering their body [75]. For example, for some organisms like arthropods with chitin-rich cuticles, surface mechanical damage due to contact with MNPs and their uptake are less likely than for soft-bodied annelids with permeable cuticles [75,76].

It is thus expected that the uptake of MNPs in invertebrates occurs mainly through the oral route. The impact of MNPs on soil invertebrates may arise directly from the physical or chemical harm after ingestion [77] or indirectly via MNP-deviated changes in soil properties or via physical harm by particles outside the organism. Namely, it has been previously demonstrated that the effects of MNPs on organisms can be induced also if they are not ingested [24,78]. The size of the studied particles compared to the size of the oral opening and structure of the mouth parts of the organism should be considered, as it determines whether oral exposure is even possible. For example, the size of the mouth opening of a commonly used small test organism, the springtail *Folsomia candida* (adult size ~1–2 mm), was estimated to be in the range of 200–300 μm [79] which is a common size of test MNPs. Some organisms, like earthworms and enchytraeids, are more prone to ingest plastic particles in soil than some other invertebrates such as springtails or nematodes [74].

So far, the most common mode of exposing soil invertebrates to MNPs is spiking the soil [80], but in some studies invertebrates have been exposed via spiked food (sometimes in combination with spiked soil [24]). The mode of exposure in ecotoxicological testing should be selected according to the research question and the biology of the organisms. If the objective is to mimic environmentally realistic exposure situations, the distribution of MNPs in the environment should determine whether spiking the soil, or exposure via food, is the most relevant choice. To unravel whether the impacts arise from the changes in the soil physicochemical properties or ingestion of the particles, the organism can be exposed separately via food and soil. Also some organisms do not readily ingest soil [77].

In the case of plants, MNPs are taken up via roots [81,82] and leaves [83]. The most common exposure methods are via soil (pot-plant) or in hydroponic solutions, with a recently stronger focus on pot-plant experiments [27]. Roots are the primary uptake route of MNPs by plants during hydroponic and pot-plant experiments. In addition, foliar uptake via the leaves can occur through the stomata. This exposure method is also important because of the possible importance of atmospheric MNP deposition on plants.

3.4. Organisms and endpoints

The most common approach in ecotoxicology is single-species testing, and this also is the case for MNP studies both in the aquatic environment [31] and in soil [27,84]. For an ecologically relevant risk assessment via single-species soil toxicity testing, the selection of different species from different taxonomic positions and with different anatomical and physiological features is important. Namely, as has been discussed in the previous section (3.3.) the extent of MNP interactions with organisms through body surface, ingestion, and water uptake differs between organisms [75,76]. We therefore recommend that, when possible, an array of test organisms is used in testing the hazards of MNPs, covering different taxonomic groups, life histories, body size, feeding strategies or functional feeding groups (primary producers, detritivores, grazers, predators, etc.) and vertical habitat stratification in the soil [31,85]. For plants, differences between the effects of MNPs on monocots and dicots have been observed, also highlighting the need for an array of plant species to be tested [27]. This increases the ecological relevance of the data.

Another important aspect of designing an ecotoxicological experiment is the choice of endpoints. The most common endpoints used in ecological hazard assessment frameworks are typically mortality, growth and reproduction [32]. However, as whole-organism level effects are the result of effects at lower levels, measures of effects at the below-individual level (molecular, biochemical, physiological, etc.) should be implemented. This information is important to derive hypotheses about the MNP mode of toxic action and to develop different risk assessment frameworks, such as Adverse Outcome Pathways (AOP) and Integrated Approaches to Testing and Assessment (IATA) [86].

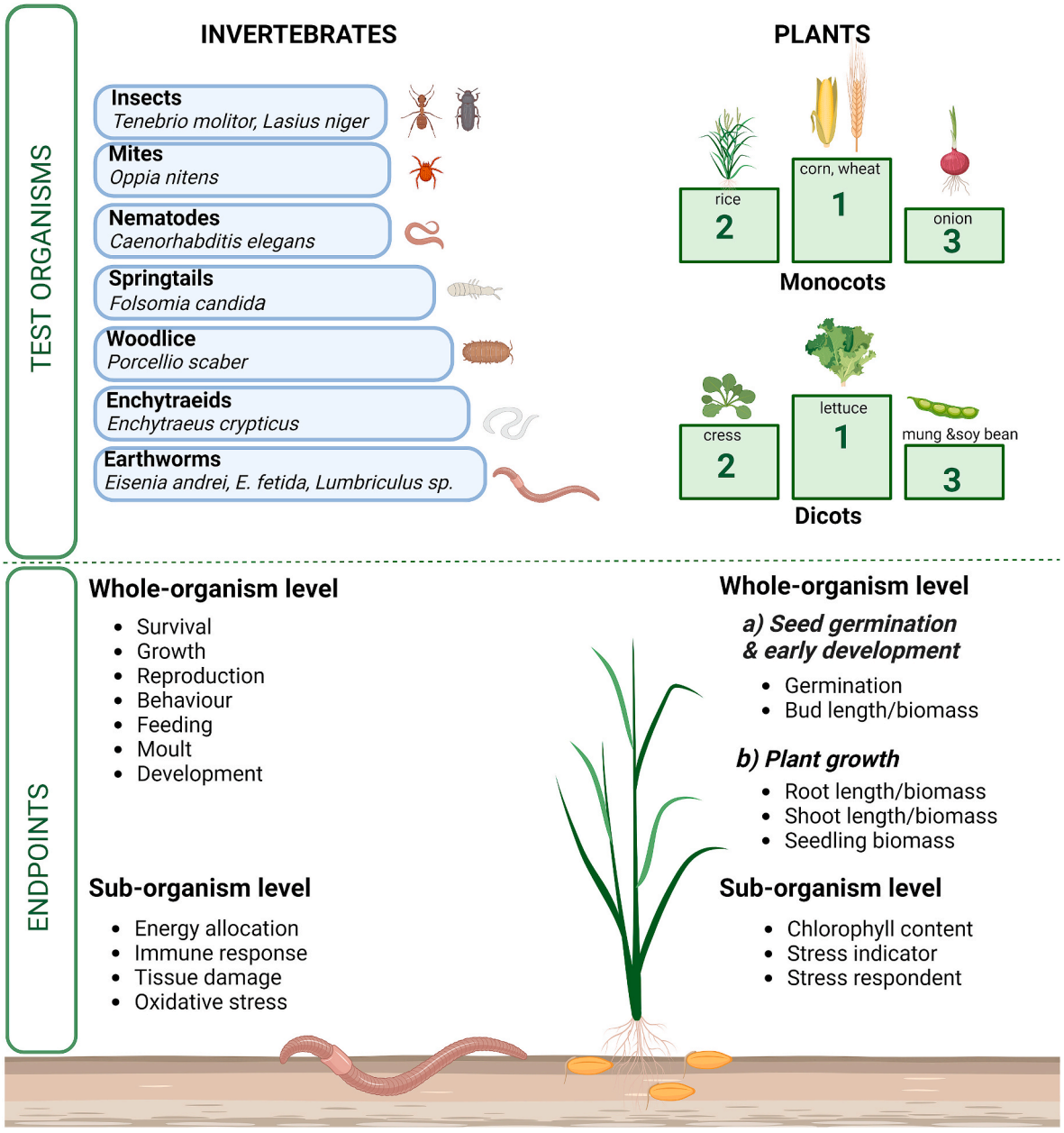

Fig. 2. The most common invertebrate and plant test organisms and endpoints applied in soil micro- and nanoplastic (MNP) ecotoxicity research. The ranking of species was done based on two published reviews [27,84]. For the plants, the numbers in the boxes refer to the 1st, 2nd and 3rd place according to how commonly they have been used in MNP ecotoxicity studies. For the soil invertebrates, the size of the blue box denotes the ranking in the number of studies available, with earthworms being the most used, and insects/mites/nematodes the least studied. Figures of organisms are schematic and do not represent exactly the species or life stage under investigation.

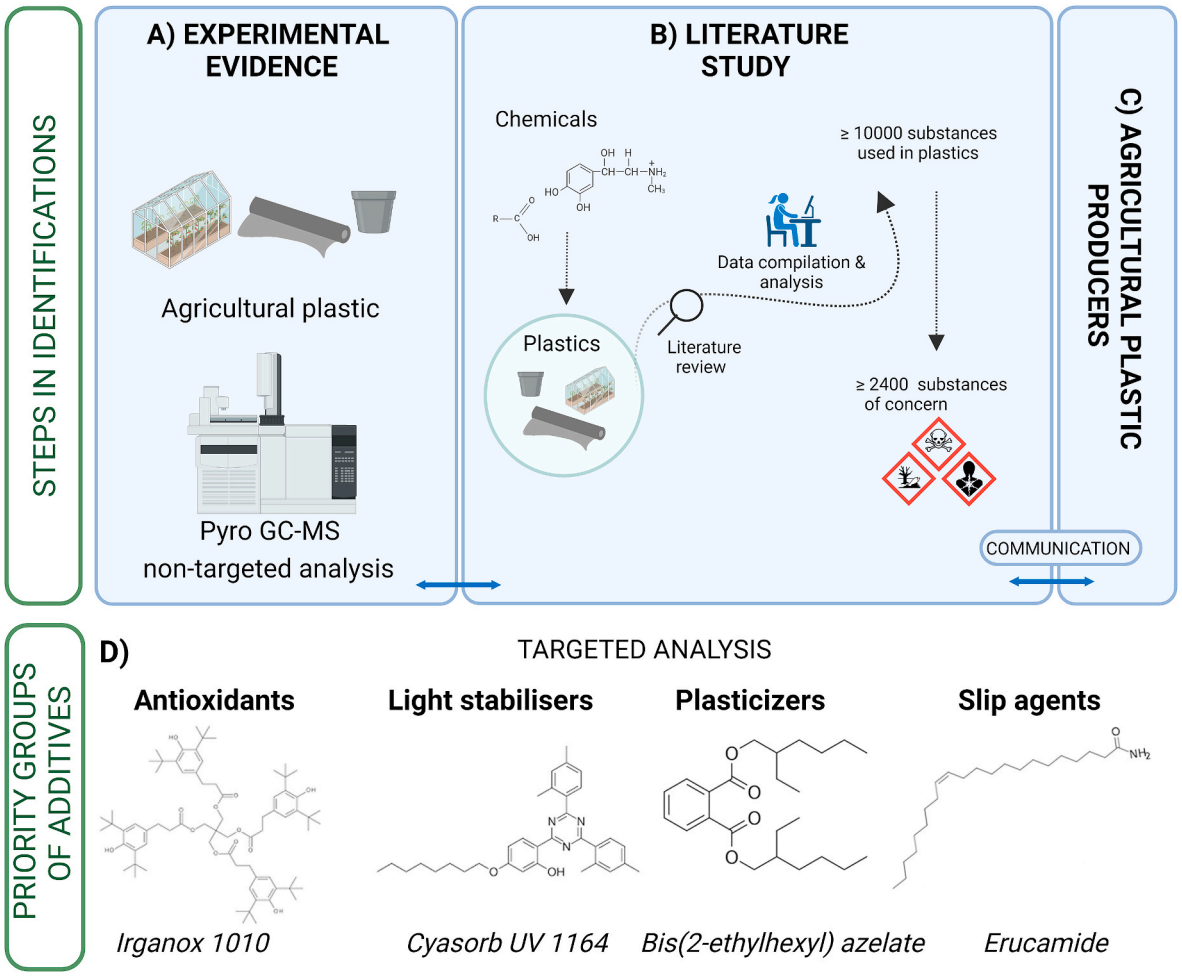

Fig. 3. The recommended strategy to identify and monitor plastic additives derived from agricultural soil. Combining A) experimental data obtained through non-targeted analysis to B) in-depth investigation of the available literature is necessary as more than 10 000 substances have been reported as plastic additives. The picture in panel B) is redrawn according to Ref. [39]. C) Communication with agricultural plastic manufacturers is also necessary within the effort to focus only on compounds used in agriculture. D) Classes of widely used plastic additives in agricultural plastics classified based on their function, alongside a characteristic molecule in each case.

Importantly, it is key to select below-individual endpoints which can be directly linked to individual or population-level responses to avoid losing the ecological relevance of the measured endpoints.

The choice of a certain endpoint also depends on the species or type of organism used. For example, some species reproduce very slowly and are therefore less appropriate for assessing reproductive effects, while fast reproducing species (e.g., springtails, nematodes, enchytraeids, earthworms) are more practical for use in multigeneration testing approaches [87]. Some organisms (e.g., woodlice) are relatively easy to dissect allowing histological analysis or analysis of immune response to be more easily undertaken [88]. Some endpoints are unique to certain groups of organisms, for example the colony founding and queen survival in ants [89] and metamorphosis in insects [90]. Most studied organisms and endpoints in MNP invertebrate and plant ecotoxicity studies are listed in Fig. 2.

Finally, an important consideration in the design of tests is also their duration. Most standard single-species invertebrate ecotoxicity tests last for 3–4 weeks, but for nematodes the duration is as short as 24 h. For plants, the durations vary considerably from several days to 4–5 months, but most commonly from 7 to 28 days [27]. Since MNPs are generally persistent in soil, the common test duration and focus on assessing effects on sub-lethal endpoints may not be sufficient to determine their hazard. Instead or in addition, longer durations, chronic, full-life cycle, multigenerational or even transgenerational (effects passed on from an exposed parental generation to the next, non-exposed, generation without direct genetic inheritance) tests may be needed [91].

3.5. Increasing environmental relevance

Most studies on the impact of MNPs have been performed under highly controlled conditions with only a limited number of species [27, 84]. However, when assessing the hazards of MNPs, we should verify whether the results of these laboratory tests can be translated to more environmentally realistic conditions. The focus on single-species testing limits the ability to predict complex interactions between species in the natural environment. To study species interactions, mesocosm experiments can be used [31]. Such systems are either artificially composed by adding selected species or use indigenous communities by taking intact field-collected soil cores [92]. In both cases, the indigenous microbial community is an essential part of the setup. Field exposures using higher-tier experimental approaches are the next level to increase the environmental relevance of MNP hazard assessment [93].

Current ecotoxicity testing mostly investigates only the potential effects of MNPs without considering other stressors in soil. However, this is far from realistic. Ideally, testing approaches would also consider co-exposure to other types of stress. For example, agricultural soils are sinks for many organic contaminants, especially pesticides [94]. During the interactions of MNPs with co-contaminants, the properties of plastics

can be changed which can change the fate and toxicity of plastics and/or contaminants in exposed soil organisms [24,95,96]. Another aspect is also the fact that organisms are never exposed to pristine particles but MNPs in the environment are instantly covered by an eco-corona, which is the initial layer of biomolecular compounds adsorbed onto the MNP surface [97]. Thus, the environmental relevance of testing could be increased by pre-exposing MNPs in soil to form an eco-corona and expose organisms to these aged MNPs.

Recently, there has been an increasing focus on showing the relationship between MNP pollution and climate change [98,99]. It has been suggested that MNPs increase greenhouse gas emissions either directly by degradation or indirectly by affecting microbial soil respiration. On the other hand, climate change contributes to increasing drought and floods which may change the fate and effects of MNPs in soil. Therefore, further studies on the stress-on-stress effects resulting from the interactions between climate change conditions, pesticides and other chemical pollutants on one hand and soil plastic pollution on the other hand are needed.

4. Analysis of biota and soil

4.1. Analysis of MNPs

Various analytical approaches have previously been used for MNP analysis of test soils (MNP characterization methods were already summarized in section 2.3) [65,100]. We present several important aspects to consider when applying MNP analysis to test soils. First, MNP extraction and analysis methods should cover the size ranges of the particles in the test material. Since MNP extraction is often limited to the mesh size of the filter used, usually stainless steel with a mesh size of 10 μm, 20 μm or 25 μm [66], smaller MNP fractions may be filtered out. The same is true for analytical performance. Most commonly, μFTIR spectroscopy is limited to a particle detection size of 20 μm and μRaman spectroscopy to 1 μm [101,102], so smaller particles can be overlooked, but some equipment allows the detection down to 10 μm for μFTIR and 0.5 μm for μRaman spectroscopy [66]. Additionally, MNP detection should be performed on a sufficient number of subsamples and technical replicates to ensure accurate measurements. Therefore, careful consideration should be given to whether MNP analysis will yield a reliable indication of actual exposure levels.

As the detection of MNPs in soil is a challenging task for a standard ecotoxicological laboratory, and also considering the reasons above, we argue against making this analysis mandatory. Such analysis may, for example, not be needed if the MNPs in the test material are well characterized and measures are taken to ensure homogenous distribution of the MNPs in soil. However, it should at least be checked whether the control test soil or food itself are not contaminated with MNPs. This is not trivial since natural soils that are completely free from MNP contamination are likely to be scarce. This issue has not yet received much attention in the context of soil ecotoxicity testing. Also, the preparation of the soil or food (e.g., storage, drying or moistening) for testing should not introduce additional MNP contamination. Ideally, to evaluate the homogeneity of the MNP distribution in the test medium, the MNP concentration in the soil or food should be analyzed in the beginning and at the end of the test. However, due to time, technical and financial limitations this is not always feasible. Determining the size distribution of MNPs in each test concentration is important when preparing small concentrations using weighing methods, as MNP dosing by mass can result in large differences in particle number-based concentrations between treatments. Checking for possible changes in particle size and shape at the end of the test is recommended especially when testing particles composed of biodegradable plastic or where fragmentation of conventional MNPs may have occurred. This would provide information on the degradation of the particles.

As discussed in section 3.3, organisms can have different exposure routes and abilities to ingest MNPs due to anatomical differences of mouthparts, calling for an assessment of ingestion. In doing so, care should be taken to avoid errors that may occur as a result of MNP adsorption to the body. This can partly be solved by rinsing, but also by isolation/dissection of the digestive system. However, in many organisms MNP analysis is not feasible due to their small size. In case we are interested in the interaction of MNPs via bio-adhesion, i.e., how organisms are affected by MNPs adhering to their bodies, the attached MNPs must also be monitored, e.g., by analyzing weakly adhered particles in rinsing water or by chemical digestion of organisms with adhered MNPs [103]. Analysis of ingested and adhered MNPs is also important for assessing the potential trophic transfer of MNPs.

4.2. Analysis of plastic additives

Leaching of plastic additives (PAs) from MNPs and their analysis is a critical issue in ecotoxicological testing to distinguish whether the toxic effects are related to plastic particles or additives. Although monomers and other plastic-associated chemicals like contaminants may also potentially leach from plastics, here the focus will be on PAs. The development of accurate, sensitive and robust analytical methods for the determination of PAs in soil is a rather challenging task due to the diverse physicochemical properties of the various PA classes as well as the complexity of soil as a matrix [104]. To date, the majority of studies have focused on phthalates, flame retardants and bisphenols determination in environmental matrices; however, monitoring of PAs typical for agricultural plastics should also be considered when examining their potential hazards in soil [105]. Many PAs used in agriculture are not reported or they are reported only with the commercial trademarks, which leads to challenges associated with traceability and confidentiality, hindering the communication between academia and the market [37]. Thus, it is advised to confidently identify and monitor PAs in soils. The implementation of instrumental non-targeted analysis to identify PAs in the material of interest, for example pyrolysis gas chromatography mass spectrometry (Pyro GC-MS), can enhance the trust on literature findings as the available information is limited.

After identifying PAs in test MNP particles, it is important to determine whether and to what extent these PAs may leach out of materials and contaminate the surrounding soil or food used for testing. Subsequent target analysis can be performed for the leaching of PAs in test soils. Care should be taken with methods for extracting PAs from soil. Ideally, the detected amount should correspond to the PAs leached from the test material. Nevertheless, available extraction protocols cannot guarantee that because MNPs can still remain in the soil. In this way, the total extractable amount of PAs is monitored, which may be still used as an indicator of the leaching. To detect the bioavailable leached PAs in soil, the filtering of soil samples through metallic sieves may be an option. In this way, at least a certain particle size cut-off level corresponding to the sieve pores can be achieved. On the other hand, this is an additional pre-analytical step increasing the sample preparation time. Besides that, by adding steps, accuracy issues may arise, and the use of internal standards is highly recommended to face this challenge. Alternatively, in the case of controlled experiments, run-off water can be analyzed to check the bioavailable amount of polar or moderately polar PAs. Again, such an approach would have not considered the case of analytes being strongly bound to the soil organic matter, especially in the case of non-polar compounds. In any case, the limitations in the methods should be reported with the results.

The use of plastic consumables (e.g., centrifuge tubes, microfilters) and the purity of extraction solvents during sample extractions should be tested carefully to prevent false positive results. Last but not least, monitoring intervals for PA content in test soils depend on the test duration and the availability of analytical instrumentation. If frequent PA analysis is not feasible, we recommend testing for the leaching of PAs (discriminating between extractable and bioavailable PAs, if possible) at least at the end of the experiment. We propose a strategy for analyzing PAs in soils that may arise from the use of agricultural plastics (Fig. 3).

4.3. Analysis of physicochemical properties of soil

Soil properties, such as pH, organic matter content, texture and soil aggregate stability, moisture content and nutrient concentrations strongly influence biota (fitness, performance, sensitivity, tolerance, etc.), their exposure to toxic substances (e.g., chemical fate, behavior, sorption, degradation, bioavailability) [106] and were shown to be the key factors that could influence most of the soil functions and processes [109]. Besides that, they interfere with substantial effects on biota and may mask or change (increase/decrease) the observed effects and risks [107,108]. Therefore, key soil properties should be carefully measured during the tests.

There is evidence for changes in soil properties due to MNP contamination [22,23]; both for physical properties like water holding capacity (WHC), bulk density, soil aggregation and porosity, and for chemical properties such as pH, organic matter content, nutrient levels and migration of pollutants. Trends observed in the literature typically vary and may be positive, negative or neutral, dependent on the MNP properties and type of soil [25]. These inconsistencies stress the importance of measuring soil properties in soils contaminated by MNPs.

MNPs may indirectly affect the well-being of soil organisms by affecting soil properties. For example, at low pH, the reproduction of invertebrates and microbial activity may be decreased [110]. MNP addition may, for example, increase WHC [21], which makes the soil feel more "dry" for the organisms if the moistening is done according to the properties of uncontaminated soil. This may potentially affect soil organisms that prefer moist soil conditions (e.g., for nematodes soil moisture content should be adjusted to 80 %, for earthworms, enchytraeids and springtails to 40–60 % of the maximum WHC). Measurements of pH and WHC are relatively easy [72,111] which may provide a preliminary view of MNP impacts on these soil properties. We also recommend measuring organic carbon content, as MNPs contain a lot of carbon and their degradation (especially when testing biodegradable MNP) may serve as a potential source of carbon for microorganisms or change the fate of other contaminants [112].

In the standardized toxicity test guidelines according to ISO or OECD, soil texture (2 or 4 mm sieved) is standardized within a given range. Calcium carbonate ($CaCO_3$) or potassium hydroxide (KOH) is used to set the pH of artificial soil to 6.0 ± 0.5. A list of physicochemical soil properties that are essential to be measured or are recommended to be determined for effective interpretation of test results is usually recommended in these test guidelines [113]. This includes WHC, water content, electrical conductivity, cation exchange capacity (CEC), clay content and organic carbon content.

5. Study quality screening for soil microplastic hazard assessment

In this paper, we have provided several practical recommendations to ensure the sufficient methodological quality of soil ecotoxicity tests to assess the hazards of MNPs. A further step in ensuring the production of relevant and quality data is the use of quality assurance/quality control (QA/QC) tools to screen the quality of ecotoxicity studies. This allows for the identification of critical points in the design of experiments and provides guidance to increase their reliability and usability in risk assessment [114]. The general idea behind the use of QA/QC tools is to assess the data completeness, e.g., whether certain information is reported within a study. In the case of ecotoxicity studies, for example, basic categories such as reporting primary particle characteristics and their characteristics in test media experimental design, test organisms and endpoints, sample preparation and results are suggested to be reported [115].

Several quality criteria screening tools for ecotoxicity studies, like the "Klimisch score" and the CRED approach, have been developed for chemicals [116]. Different criteria have been proposed to address the particularities of other test materials, in particular particles. For example, for nanomaterials, which have very distinct physicochemical properties compared to soluble chemicals, the nanoCRED, DaNa criteria, and GUIDEnano framework were proposed [115]. With the development of MNP research, it became clear that similar approaches need to be implemented. Jemec Kokalj et al. [50,115] proposed a refined list of QA/QC criteria for ecotoxicity testing of nanoplastics. For microplastics, QA/QC criteria have been developed for their detection in biota [49], freshwater and drinking water [117], sediment [118] and soil [119], and for ecotoxicity tests with aquatic organisms [48,118], but not yet specifically for soil hazard assessment. We would thus suggest the development of such criteria which could be designed based on the recommendations set in Table 1. A further step would be the design of minimum reporting standards, which would facilitate the potential reuse of the data generated in hazard assessment for secondary analysis, meta-analyses or integration with other datasets [50].

6. Recommendations

This article drafted several recommendations for ecotoxicity testing for the hazard assessment of MNPs in soils. These recommendations along with the rationale of their application are presented in Table 1. Fig. 4 shows the main proposed elements for the hazard assessment of MNPs in agricultural soils.

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Table 1
Recommendations for the ecotoxicity testing of micro- and nanoplastics (MNPs) in soils arising from this paper. For each recommendation, a brief rationale is added that also tries to link to quality assurance and quality control (QA/QC) criteria; see text for further explanation of the background and arguments supporting the different recommendations.

Topic	Recommendations	Rationale and QA/QC considerations
MICRO- AND NANOPLASTIC TEST MATERIALS		
Production and characterization of MNPs	a Information on MNP test material, including origin, preparation method, chemical characterization, shape and size should always be provided.	a These parameters can help to link properties of MNPs to their ecotoxicological effect and increase the usability of toxicity data in risk assessment of particles with certain properties.
	b Stock of the material should be stored for potential further needs. Every batch of MNP is unique.	b Test material can then be further characterized or used in ecotoxicological tests.
	c More transparent information on the chemical additive composition of agricultural plastics is highly needed.	c This will enable better monitoring of potentially harmful chemical compounds contained in agricultural plastics.
	d Harmonized guidelines for MNP preparation and characterization are needed.	d Test materials can then be prepared and characterized in a similar way ensuring comparability.
	e Test MNPs should be produced by top-down approach by fragmentation of relevant plastic materials.	e In the environment MNPs are generated through fragmentation due to the various natural processes.
TESTING THE HAZARDS OF MICRO AND NANOPLASTICS		
Exposure concentrations	a MNP test concentrations should cover a range of relevant realistic (from 0.001 % to 0.05 %) and worst-case (up to 5 %) exposure levels.	a For proper hazard assessment and deriving reliable toxicity data, full dose-response curves are needed.
	b Exposures in toxicity tests should be expressed both on mass and on particle number concentrations.	b For comparison of toxicity data with MNP concentrations in field soils, the same concentration units are needed.
MNP spiking in soil and food	a Standardized procedures across all tests are needed.	a To ensure comparability of toxicity data, all test soils should be prepared in the same way.
	b Contamination control must be ensured at all stages of the experiment.	b Contamination of soil/food with other MNPs may cause combined effects that may be misinterpreted.
	c MNPs should be well mixed in with soil or food.	c Application of non-homogeneously mixed soil may lead to wrong interpretations of toxicity data.
	d Preferably MNPs should be applied on dry soils (<1 % moist) and soil should be moistened right after spiking.	d MNPs may form aggregates during mixing if applied to moist soils but are also easily segregated in dry soil if not moistened after spiking.
	e Care needs to be taken to prevent loss of statically charged MNPs.	e During mixing statically charged MNPs may attach to mixing equipment and be lost.
Mode of exposure	a The selection of exposure medium (soil, food) should follow the research question and the biology of the organism. For plants, air exposure scenario may also be of importance.	a Organisms have different interactions with MNPs. Mimicking different exposure routes in toxicity tests will provide relevant data for ecologically relevant risk assessment.
Test organisms and endpoints	a An array of test organisms should be used, going beyond the classic selection of earthworms and springtails in regulatory contexts of e.g., pesticides, covering different taxonomic groups, life histories, body size, feeding strategies or functional feeding groups (e.g. producers, consumers, decomposers).	a Test species selection should cover the whole spectra of MNP exposure routes and different organism groups to increase ecological relevance of the data, but often will also depend on practical issues, like ease of testing and culturing species.
	b Whole-organism endpoints should be supplemented with below-individual level effects (molecular, biochemical, physiological). These early changes and ecologically relevant endpoints should be linked.	b MNP effects start at below-individual level, providing tools for early warning and insight into modes of action.
	c Long-lasting, full-life cycle or multigenerational tests are needed.	c As MNPs are persistent in soil, short tests may not be sufficient to adequately determine their hazard.
	d Also, higher-tier approaches like mesocosm and field experiments should be performed.	d Environmental relevance of mesocosm or field experiments is higher compared to single-species testing; these experiments may account for impacts of MNPs due to biotic and abiotic interactions.
Interactions of MNP with other environmental stress factors	a Testing approaches should consider also co-exposure to other types of stress, like chemicals and other environmental stressors.	a MNPs are often present with other pollutants in soil and may affect their toxicity and fate. Other stressors, like climate change, may impact MNP toxicity and fate.
ANALYSES OF THE BIOTA AND SOIL		
MNP analytics	a Careful consideration should be given to whether analytical verification of MNP exposure levels in soil is required.	a/b/c Current MNP detection methods are limited by factors such as particle size, and only few methods to detect nanoplastics are known but not routinely used. Analytical costs and sample preparation time also limit sample size and number of replicates analyzed, leading to large uncertainties in calculated exposure concentrations.
	b Mass-specific MNP analytical methods should be used to control mass exposure.	
	c Spectroscopic analytical methods should be used to characterize MNPs in soil.	
	d Consideration should be given to the need for MNP analysis in test organisms/feces.	d In very small organisms, MNP analysis is not possible. The detection of nanoplastics may not be possible in most ecotoxicological laboratories.
Additive analytics	a Presence of additives in soil should be monitored.	a More emphasis needs to be paid to additives commonly occurring in agricultural plastics. However, there is a limitation of separating the particles from soil prior to analysis.
	b Leaching of plastic additives in test soils can be investigated by target analysis once the information of additives in MNPs is already known.	b Combining targeted and non-targeted approaches may provide comprehensive analytical information.
	c Efficient, robust and sensitive analytical workflows achieving acceptable quality performance characteristics are necessary.	c Matrix effects can highly vary depending on tested matrix, e.g., soil, food, biota, indicating the need to always apply optimized and validated analytical methods in each case.
Physicochemical properties of soil	a Water holding capacity, and pH should be measured in each ecotoxicity test and for each test concentration.	a/b MNPs may change the physicochemical properties of soil which may affect the outcome of toxicity tests.
	b Additionally, electrical conductivity, clay content, and organic carbon content would aid in the interpretation of the results.	c Using standardized methods will enable comparison of results of tests performed in different laboratories.
	c Standardized test soils should be used (e.g., artificial soil, LUFA standard soil) (e.g., ISO 11268, 2023).	
STUDY QUALITY SCREENING		
	a Development and use of minimum reporting standard would be beneficial.	a Minimum reporting standards are common for other types of ecotoxicity studies. They define essential information that should be provided when reporting findings.

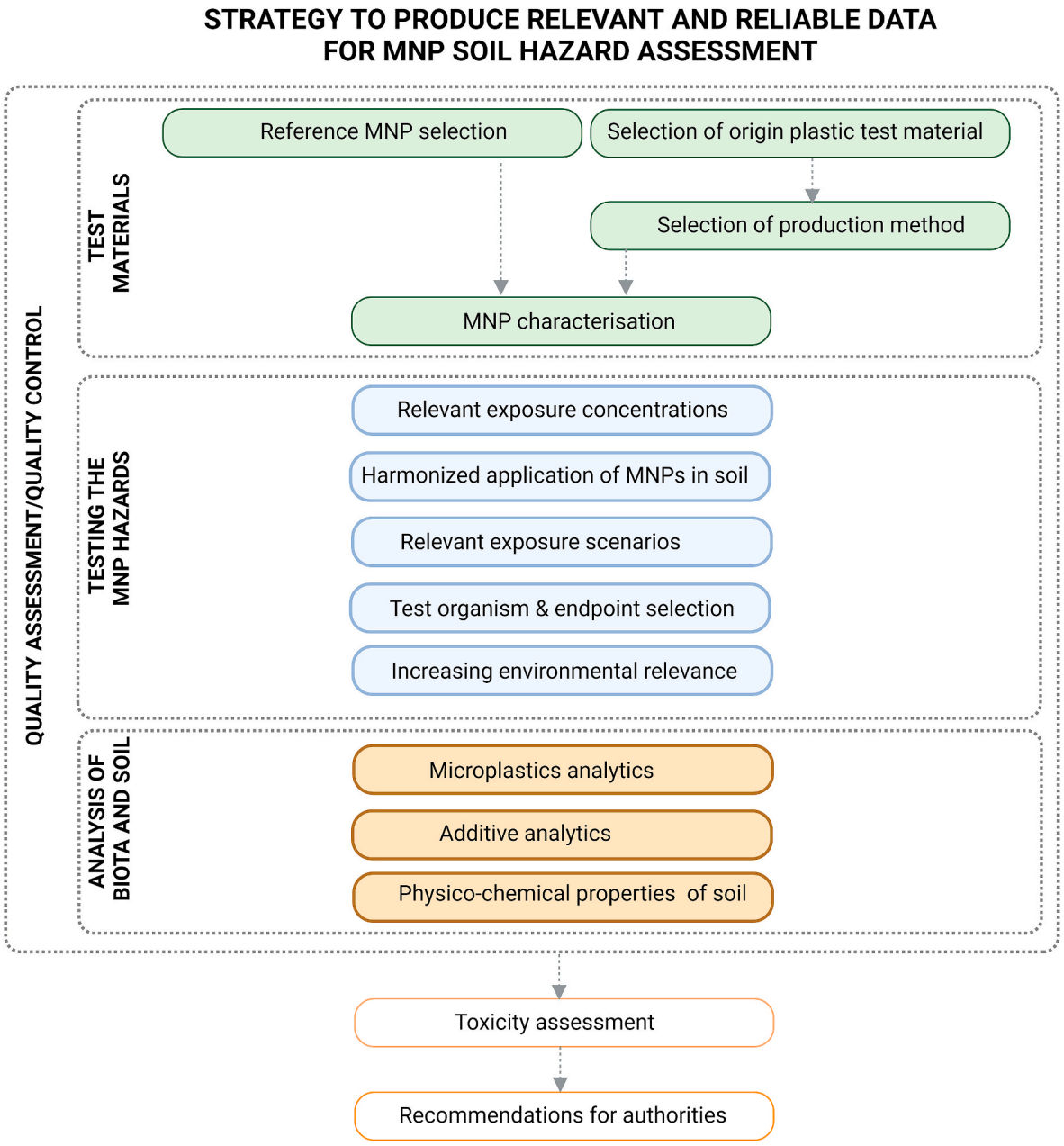

Fig. 4. Proposed elements of an environmentally relevant hazard assessment of micro- and nanoplastics (MNPs) in agricultural soils.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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