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Nano- and microplastics commonly cause adverse impacts on plants at environmentally relevant levels: A systematic review

Zantis, L.J.; Borch, C.; Vijver, M.G.; Peijnenburg, W.J.G.M.; Di Lonardo, S.; Bosker, T.

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Review

Nano- and microplastics commonly cause adverse impacts on plants at environmentally relevant levels: A systematic review



Laura J. Zantis^{a,*}, Caterina Borchi^{a,b}, Martina G. Vijver^a, Willie Peijnenburg^{a,c}, Sara Di Lonardo^{d,e}, Thijs Bosker^{a,f}

^a Institute of Environmental Sciences, Leiden University, P.O. Box 9518, 2300 RA Leiden, the Netherlands

^b Department of Civil and Environmental Engineering, University of Florence, Via di S. Marta 3, 50139 Firenze, Italy

^c National Institute of Public Health and the Environment (RIVM), Center for Safety of Substances and Products, P.O. Box 1, Bilthoven, the Netherlands

^d Research Institute on Terrestrial Ecosystems (IRET), National Research Council (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy

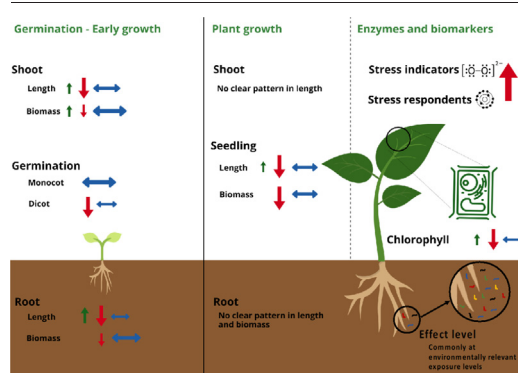
^e National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133 Palermo, Italy

^f Leiden University College, Leiden University, P.O. Box 13228, 2501 EE The Hague, the Netherlands

HIGHLIGHTS

- Effects of NMPs on terrestrial plants are ubiquitous.
- Effects on germination and growth were found with small differences among species.
- Stress levels within plants were frequently up-regulated due to exposure to NMPs.
- Effects occur at environmentally relevant exposure levels.
- Need for studies under field conditions to facilitate lab-field translation.

GRAPHICAL ABSTRACT



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ABSTRACT

Over the last years there has been significant research on the presence and effects of plastics in terrestrial systems. Here we summarize current research findings on the effects of nano- and microplastics (NMPs) on terrestrial plants, with the aim to determine patterns of response and sensitive endpoints. We conducted a systematic review (based on 78 studies) on the effects of NMPs on germination, plant growth and biochemical biomarkers. This review highlights that the majority of studies to date have used pristine polystyrene or polyethylene particles, either in a hydroponic or pot-plant setup. Based on these studies we found that effects on plants are widespread. We noted similar responses between and within monocots and dicots to NMPs, except for consistent lower germination seen in dicots exposed to NMPs. During early development, germination and root growth are more strongly affected compared to shoot growth. NMPs induced similar adverse growth effects on plant biomass and length in the most tested plant species (lettuce, wheat, corn, and rice) irrespective of the polymer type and size used. Moreover, biomarker responses were consistent across species; chlorophyll levels were commonly negatively affected, while stress indicators (e.g., ROS or free radicals) and stress respondents (e.g., antioxidant enzymes) were consistently upregulated. In addition, effects were commonly observed at environmentally relevant levels. These findings provide clear evidence that NMPs have wide-ranging impacts on plant performance. However, as most studies have been conducted under highly controlled conditions and with pristine plastics, there is an urgent need to test under more environmentally realistic conditions to ensure the lab-based studies can be extrapolated to the field.

* Corresponding author.

E-mail addresses: l.j.zantis@cml.leidenuniv.nl (L.J. Zantis), c.borchi@cml.leidenuniv.nl (C. Borchi), vijver@cml.leidenuniv.nl (M.G. Vijver), peijnenburg@cml.leidenuniv.nl (W. Peijnenburg), sara.dilonardo@cnr.it (S. Di Lonardo), t.bosker@luc.leidenuniv.nl (T. Bosker).

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1. Introduction

Nano- and microplastics (NMPs) are ubiquitous in the environment due to the rising use and improper end-of-life strategies of plastics (GRID Arendal, 2018; Kallenbach et al., 2022). Once released into the environment, plastics undergo continuous weathering processes resulting in smaller plastic particles, including nano- and microplastics (Abdolahpur Monikh et al., 2021). Microplastics (MPs) are most commonly defined as plastic particles with a size between 100 nm and 5 mm, while nanoplastics (NPs) are particles >100 nm (Hartmann et al., 2019; Hurley et al., 2020). NMPs are found in a variety of shapes, sizes, colours and are characterized by their small size, high longevity, and buoyancy (Kiran et al., 2022; Rochman et al., 2019). To date most research has focussed on the quantification and effects of NMPs in marine and freshwater ecosystems (Horton et al., 2017). Only recently attention has shifted towards plastic contamination of terrestrial ecosystems (Kallenbach et al., 2022), even though soil contamination is thought to be 4–23 fold higher than in aquatic environments (de Souza Machado et al., 2018; Sridharan et al., 2021). Reason for the relative late focus on soil systems is the challenge to quantify NMPs in complex soil systems (Abdolahpur Monikh et al., 2021), but with advancements in analytical chemistry this is now possible. The concentration detected in soil varies greatly among studies, and data are still very sparse (Okoffo et al., 2021). For instance, levels between 0.00014 % and 0.00044 % w/w MPs were found in agricultural soils in Chile (Corradini et al., 2019), 0.0915 % w/w were estimated in soils next to a road in Cologne in Germany (Dierkes et al., 2019), and up to 6.75 % w/w in the soil of an industrial area in Sydney (Fuller and Gautam, 2016).

Agricultural lands are considered a major sink of NMPs, and a key source of this pollution is the use of agricultural plastic products (Huang et al., 2020). It is estimated that 12.5 million tonnes of plastics are used globally in agricultural production, of which alone 310,000 t are used in crop production (FAO, 2021). Approximately 5400–23,500 t of MPs per

year are released by the agriculture and horticulture sector into the environment (UNEP, 2021; European Chemicals Agency, 2019). The application of treated sludge (biosolids) from wastewater treatment plants on agricultural lands is another major source of pollution with NMPs (Kallenbach et al., 2022; Xu et al., 2020). Nizzetto et al. (2016) estimated that in Europa 63,000 to 430,000 t of MPs are released from sewage sludge onto agricultural lands, while in North America this is calculated approximately between 44,000 and 300,000 tons. Besides agricultural activities, there is an increasing release into soils from non-agricultural sources, such as landfills, littering (e.g., tyres, disposals), and through atmospheric deposition (Bläsing and Amelung, 2018; Dris et al., 2015; Yadav et al., 2020).

The presence of NMPs in agricultural lands could potentially modify soil structure and subsequently affect organisms, including plants (de Souza Machado et al., 2019; Wang et al., 2022a). Research focussing on the effects of NMPs on plants is of great importance since they are at the bottom of the food chain, and crucial for food security. Research has shown that NMPs can be taken up by plant roots (Li et al., 2020a) and leaves (Lian et al., 2021; Sun et al., 2021). This in turn has raised concern about trophic transfer through the food chain, from soil to organisms and humans (Beriot et al., 2021; Huerta Lwanga et al., 2017). In addition, toxicological effects have been observed in plants exposed to NMPs, including impacts on growth performance (e.g., Colzi et al., 2022; Gong et al., 2021), and biochemical responses (e.g., Gao et al., 2021a; Pignattelli et al., 2020). However, responses vary greatly among studies. For example, NMPs negatively affected the germination percentage of cress (*Lepidium sativum* L.; Bosker et al., 2019), while no significant effect has been observed for wheat (*Triticum aestivum* L.) or corn (*Zea mays* L.; Gong et al., 2021). Next to species-specific responses, parameters like quantity, size, shape and type of NMPs can impact the effects, for example differences in plant growth depending on NMPs type (Pignattelli et al., 2020) and shape (de Souza Machado et al., 2019), as well as differences in enzyme activities depending on the size of NMPs (Li et al., 2021a).

Given the importance of understanding the effects of NMPs on terrestrial plants, the objective of this review is to summarize current research on the effects of NMPs on terrestrial plants. In addition, we will recommend possible avenues for future research in this relatively novel, but rapidly evolving field of study.

2. Methods and materials

2.1. Literature search parameters

2.1.1. Methodological justification

This systematic literature review follows the guidelines of Siddaway et al. (2019). This method was applied to summarize existing knowledge on the effects of NMPs on i) seed germination and early development, ii) plant growth, and iii) biochemical responses in terrestrial plants. The main search for literature was performed in December 2021, and an update was conducted in May 2022 with a cut-off date of May 2nd, 2022. Two online publication databases were used: Web of Sciences and PubMed. Both are high-quality search engines, ensuring an optimal chance of covering all relevant results (Falagas et al., 2008; Siddaway et al., 2019). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach was applied during the selection process of articles (Moher et al., 2009). Potentially relevant studies not found in online databases were explored and recorded from the bibliographies of peer-reviewed publications.

2.1.2. Search terms

During a first scoping exercise, the following search terms were utilized and resulted in a selection of relevant article:

- **Subject:** Microplastic*, "Plastic particle*", Microbead*, Microsphere*, Nanoplastic*
- **Target:** Crop*, Plant*, "Terrestrial plant*"
- **Outcome:** Effect*, Impact*, Response*
- **Exclusion:** Waste*, "Water treatment plant*"

We have combined the first three categories and decided to add the "exclusion" category as there was a high number of non-relevant hits during the search on both databases. The full search string thus reads as follows:

(Microplastic* OR "Plastic particle*" OR Microbead* OR Microsphere* OR Nanoplastic*) AND (Crop* OR Plant* OR "Terrestrial plant*") AND (effect* OR impact* OR response*) NOT (Waste* OR "Water treatment plant*")

2.2. Screening process

Articles found during the search were assessed for inclusion using a two-step screening process:

Step 1: Title and abstract screening

Articles not written in English and duplicates were immediately excluded. Next, the title and abstract of the remaining publications were screened for relevance using a number of inclusion criteria:

- o **Subject:** Investigates the impacts of NMPs on terrestrial plants.
- o **Type of study:** Empirical study published in a peer-reviewed journal.
- o **Results:** Present information on the effects of nano- and/or microplastic on terrestrial plants. For a detailed list of variables, we searched for and minimum requirements see Table 1.

It should be noted that meta-analyses studies on this topic were not found, neither systematic reviews. Moreover, studies on adjacent chemical leaching from plastics were not included in this review.

Table 1

Key information and data extracted (when reported) from papers on the effect of nano- & microplastics on terrestrial plants.

Characteristic	Categories/description
Study type ^a	Experimental; field based
Target species ^a	Species name and taxonomy
Method ^a	Hydroponic experiment; pot experiment; field experiment
Polymer characteristics ^a	Type of polymer; size; shape; qualitative description (e.g., pristine, weathered microplastics)
Treatment characteristics ^a	Microplastic concentration; exposure medium and time
Main findings	Description of effects of micro- and nanoplastics on seed germination and/or plant growth and/or biomarkers and enzyme activity on terrestrial plants

^a Highlights minimum information requirements studies need to contain.

If the relevance of the papers remained uncertain based on the abstract, it was included at this stage with the aim of maintaining the high sensitivity recommended by Siddaway et al. (2019).

Step 2: Full-text screening and data extraction

In the next step, the remaining papers were read in full length and data relevant for this review were extracted from the eligible papers (see Tables S2, S4 and S6). If the relevance of the papers remained uncertain based on the full text and whether it should be included in the review, it was added to the borderline cases. The decision on inclusion/exclusion of these borderline cases is recorded in Table S1.

2.3. Data analyses

Once the screening was finalized, the included papers were split into three categories: i) short-term studies focussed primarily on seed germination and early development (as early development, we defined studies that looked at the immediate effects of NMPs after germination), ii) impacts on plant growth, and iii) impacts on biochemical endpoints. If within a study two or more experiments were performed (e.g., using different plants or polymers) results were recorded as separate observations. All recorded data per observation (including species, characteristics of NMPs, number of replicates) can be found in Tables S2, S4 and S6.

If a statistically significant effect of NMPs on plant performance was reported, the nature of significance (increase or decrease) and at which exposure concentrations the significance was measured were recorded. Given the wide range of measurement variables, as well as the wide range of study approaches (e.g., exposure medium and study duration), we were not able to consistently and accurately determine effect size for comparison. To this end, we have used the Lowest Observable Effect Concentrations (LOEC: lowest concentration at which a statistically significant difference from control was observed) for each observation. In addition, if at a higher concentration the response differed (e.g., from a statistically significant adverse to a statistically significant positive effect), this was also noted, and included as a mixed response in the analysis.

3. Results and discussion

3.1. Results of systematic review

A combined total of 1462 results were found by using Web of Science and PubMed in December 2021, and an additional 176 papers were identified during an updated search in May 2022. In Fig. 1 descriptive numbers on publications found are given per selection criterium. From 1639 identified articles, a total of 78 articles met our inclusion criteria. Background details on all studies can be found in the Supplementary information (see Tables S2–S7). The majority focussed on the effects of NMPs on plant growth ($n = 56$ publications) and on biochemical and enzymatic activities ($n = 53$), while 18 studies focussed on seed germination and early development.

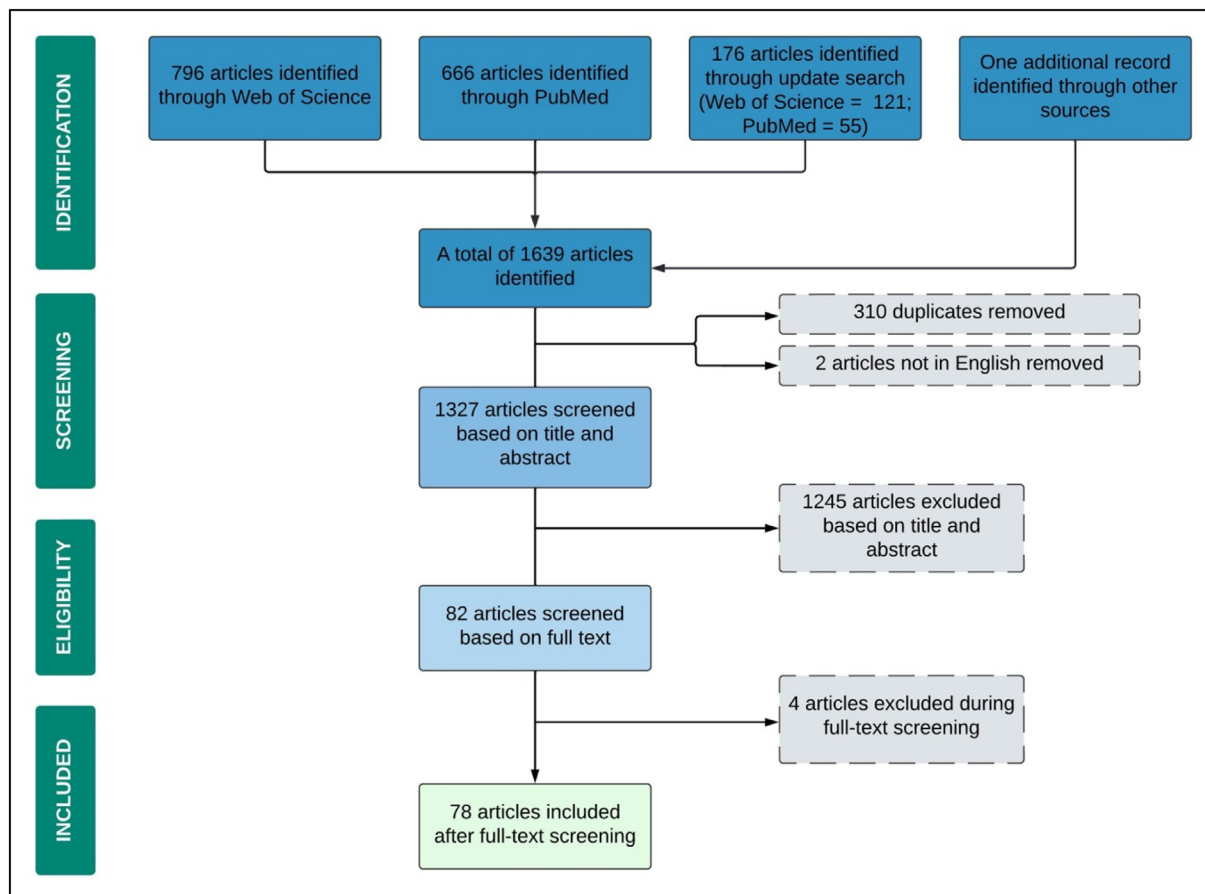


Fig. 1. Summary of the screening and inclusion of publications of the systematic review following the PRISMA statement (Moher et al., 2009) and Siddaway et al.'s (2019) guidelines. The number of publications included in each step is indicated.

3.2. Experimental design of studies

3.2.1. Types of terrestrial plants tested

In total, 54 studies performed toxicity tests on dicots, while 42 publications were on monocots (Fig. 2). Focussing on the studies on dicots, a total of 31 species were assessed, with most studies performed on lettuce (*Lactuca sativa* L., $n = 12$ studies; all varieties (e.g., green, purple, red and/or combined), followed by cress (*Lepidium sativum* L., $n = 5$) and Chinese cabbage (*Brassica rapa* L. subs. *chinensis*, $n = 3$). The strong focus on lettuce has the risk that species-specific responses might be missed or overemphasized. Having two additional well-studied dicots would already allow for a better comparison among species. A total of ten monocot species have been tested, with eleven studies on wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), and ten studies on rice (*Oryza sativa* L.). The more evenly distribution in monocots does allow for better comparison across species.

Of all plants tested, 61 % were agricultural crops ($n = 26$ species; either serving as food source or medical purposes), followed by grassland species ($n = 12$ species) and only three plants were herbaceous ornamental species. The strong focus on impacts of NMPs on crop and grassland species is understandable given their importance for food security, and the high levels of plastic pollution expected in agricultural soils (Kallenbach et al., 2022; Nizzetto et al., 2016). However, studies on NMPs levels due to industrial pollution can also be significant (Zhou et al., 2019). This means that also non-agricultural species are exposed to high levels of NMPs in the soil, while very limited knowledge on non-agricultural plants species is available.

3.2.2. Exposure conditions

Nearly two third of the studies have been performed in the laboratory ($n = 51$ publications), while 25 studies have been performed in a greenhouse setting (Fig. 2). The exposure to NMPs was performed mostly through pot

($n = 45$) and hydroponic ($n = 31$) experiments (Fig. 2). Often plants grow differently in water than in soil (Zabłudowska et al., 2009), which leads to a key issue of extrapolation of results from hydroponics studies to studies in soils (Dickinson et al., 2010). However, we noticed a shift away from hydroponic experiments in earlier studies to more realistic pot experiments which include soils in later experiments. Only one study (Hu et al., 2020) has conducted an experiment where NMPs were added to the soil under field conditions. This clearly highlights a lack of environmental realistic exposure conditions in the study design, which need to be addressed in future studies.

We only found two studies which performed foliar uptake by exposing leaves to NMPs. Foliar exposure is especially relevant as airborne NMPs have drawn increased attention from the scientific community (Amato-Lourenço et al., 2020; Liu et al., 2020). In addition, there is a rise of agrochemicals which are coated with plastics, for example pesticide and fertilizers (Katsumi et al., 2021). Some of these are sprayed on the leaves (Sohail et al., 2022), making foliar exposure to NMPs a relevant exposure route.

3.2.3. Types of polymers used

The most tested polymers were polystyrene (PS; $n = 35$ studies) and polyethylene (PE; $n = 17$) particles (Fig. 2). Fewer than ten studies used polyester (PES; $n = 7$), low-density polyethylene (LDPE; $n = 6$) and high-density polyethylene (HDPE; $n = 6$). The strong focus on PS particles has also been reported for experiments in other areas of study (de Sá et al., 2018), such as marine or freshwater organisms (Benson et al., 2022). Most toxicological experiments take PS spheres as a model compound, as they are easy to obtain and to analyse (Rozman and Kalčíková, 2022; Zhang et al., 2021). However, many combinations of polymers in different quantities are found in the environment (Yip et al., 2022). Research on NMPs in

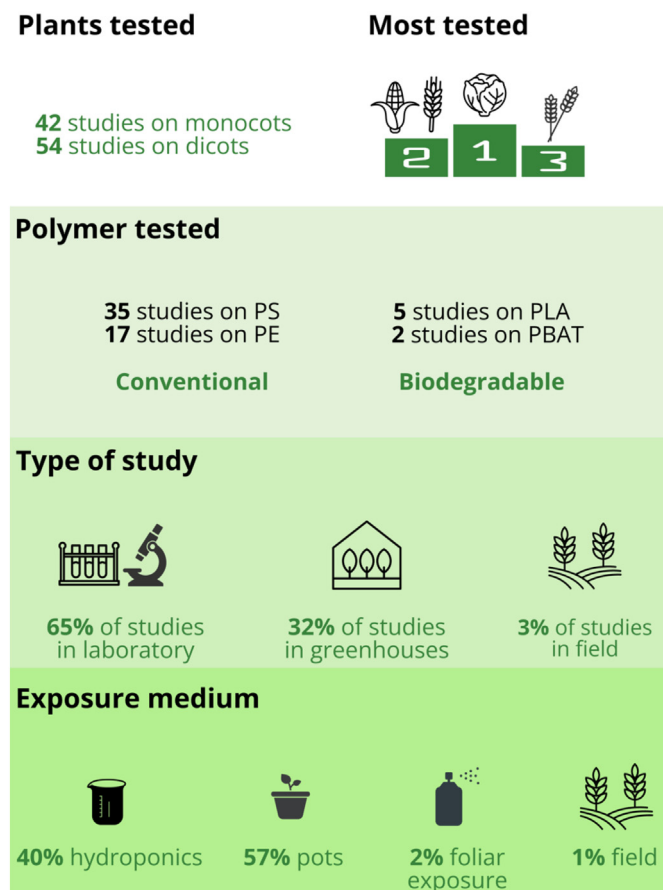


Fig. 2. Summary infographics on the most commonly studied plant species, plastic tested, and type of studies performed.

soils have shown that polyethylene (PE), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) are the most abundant polymers in the terrestrial ecosystem (Qi et al., 2020), which are also commonly found in agricultural plastic products (FAO, 2021). In addition, working with pristine PS spheres does not correspond to environmental realistic conditions, as weathering processes occur consequently leading to plastics of different sizes and shapes (Abdolahpur Monikh et al., 2021). Compared to non-biodegradable plastic, a limited number of studies has focussed on biodegradable polymers or bioplastics, mainly testing polylactic acid (PLA; $n = 5$ studies), polybutylene adipate terephthalate (PBAT-based; $n = 2$; Fig. 2) and a combination of PLA and PBAT ($n = 4$). Bioplastics have commonly been labelled as an environmentally friendly alternative to conventional plastics (Liwerska-Bizukojc, 2021). Nonetheless, adverse effects of nano- and micro-bioplastics (NMBPs) have been recorded on plant development (Qi et al., 2022; Serrano-Ruiz et al., 2021). Thus, there is an urgent need to better understand the impact of NMBPs on terrestrial systems, including on plants.

3.2.4. Additional characteristics of NMPs used

A range of NMPs sizes have been investigated. A total of 27 and 81 experiments were conducted using nanoplastics and microplastics, respectively. Within the microplastic size range, the number of studies was relatively equally distributed among size ranges: 0.1–10 μm ($n = 25$ observations), 10–100 μm ($n = 17$), 100–1000 μm ($n = 21$) and 1000–5000 μm ($n = 18$). Size is one of the key factors to include in studies as it plays an important role in uptake and fate in plants. A recent study showed that particles of size 0.2 μm are taken up by plants through the roots, while bigger particles of 2 μm were not taken up (Li et al., 2020a).

The shape of NMPs can also impact the fate and impact of plastics on organisms (e.g., Choi et al., 2018; Jaikumar et al., 2019). Even though the

most common shapes found in aquatic and terrestrial ecosystems are fibres, fragments and films (Burns and Boxall, 2018; Kiran et al., 2022), the most common shape of polymers found in this review were spherical ($n = 20$), followed by fragments ($n = 19$) and beads ($n = 10$).

More than one-third of studies have used weathered plastic particles ($n = 30$). Weathered particles are especially important to include as they are the most relevant and the most dominant types found in the environment due to the common process of degradation of larger pieces of plastics (Dissanayake et al., 2022; Guo et al., 2020). In addition, they often have a more rugged, irregular shape, which might influence toxicity (Choi et al., 2018; Simon et al., 2021; Xia et al., 2022).

3.2.5. Joint exposure

Only seven studies tested the effects of a combination of two or more polymer types on plants. The combined effects of different polymers are not only understudied in plant studies but is a general observation in microplastic research (Rozman and Kalčíková, 2022). Studying the combined effects of mixtures of polymer NM(B)Ps would provide more representative data on what happens in environmental conditions. For instance, Ren et al. (2022) showed that a combination of degradable mulching film and PS resulted in a lower diversity of soil microbes compared to single plastic treatments.

3.3. Effects of NMPs on terrestrial plants

3.3.1. Seed germination and early development

3.3.1.1. Seed germination. Seed germination was more often adversely impacting dicots compared to monocots (Table 2). For all five tested monocots species (totalling 11 observations; Table 2), no effect on germination was recorded, except for two observations of negative effects on perennial ryegrass (*Lolium perenne* L.) exposed to PLA and synthetic fibres (Boots et al., 2019). In contrast, 41 observations were reported on dicots, with 61 % of these observations demonstrating negative effects of NMPs on germination. These differences may be in part explained by the different sensitivities of plant species to NMPs. Gong et al. (2021) directly compared wheat, corn, lettuce, and radish under similar conditions and found the dicot lettuce to be more sensitive to PS-MPs exposure compared to the two monocots wheat and corn.

The most likely explanation for the difference in response between monocot and dicot species is related to seed size, since monocot seeds are usually much larger than dicot seeds. For example, 1000-grain weight of lettuce is about 0.7 g (Souza et al., 2019), while for rice it is around 25 g (Aslam et al., 2015). The higher surface-to-volume ratio of a small-seeded species can facilitate plant-contaminant interactions (Cañas et al., 2008), while larger seeds have a smaller surface-to-volume ratio, which provides greater protection against various contaminants (Cruz et al., 2013). In addition, the negative impact of NMPs on seed germination in dicots could be explained by the physical blocking of the seed pores. NMPs may deposit on the surface of seed pores, which hinders water and nutrient uptake due to physical blocking (Calero et al., 1981).

3.3.1.2. Bud length and biomass. Negative impacts on root length after germination are commonly observed for both monocots and dicots (Table 2). Although the response patterns were not as clear as with seed germination, 23 observations indicated that root growth was influenced by the presence of NMPs. For both monocots and dicots about half of the observations found effects on roots. The decrease in root length may be due to the accumulation of NMPs on the seed capsule and the root surface, which blocks the absorption and/or uptake of nutrients and water (Bosker et al., 2019; Jiang et al., 2019). Importantly, for a few plant species (rice, wheat, cress and mung bean) positive effects were observed (Table 2). However, this increase in root biomass could be a sign of stress, as the expansion of the root system is one strategy employed by plants to cope with stressful environments due to physical blockage by NMPs (Boots et al., 2019; Jiang et al., 2019). To overcome stress, plants grow larger and deeper root systems to increase

Table 3

Summary of effects of studies investigating NMP effects on plant growth, focussing on seedling length and biomass. A plus (+) and the green colour signifies increase in the endpoint, a minus (−) and the red colour signifies decrease. A mixed result (positive and negative) was shown as in orange, while blue signifies other changes/no clear trend; NS means not significant. The sum of the effects observed for each endpoint per plant species is reported in each coloured box.

	Length						Biomass											
	Seedling			Root			Seedling			Root			Shoot					
	+	−	NS	+	−	NS	+	−	NS	+	Mix	−	NS	+	Mix	−	NS	
Monocot																		
Bushgrass						1				1				1				
Corn	2		2		2		1	1		5	1	6	4	2	1	6	5	
Hard fescue						1				1							1	
Onion					1								1	1				
Rice	1	7		1	3	4	1	2				9	2			6	3	
Spring onion						6	2	4		2			4					
Wheat		7	9	2	4		4	1		2	2	4	9	2	2	2	12	
Yorkshire fog						1				1							1	
Total Monocot	3	14	11	3	10	13	0	8	8	12	3	19	20	6	3	14	22	
As%	11	50	39	12	38	50	0	50	50	22	6	35	37	13	7	31	49	
Dicot																		
Alfalfa						1										1		
Broad bean						2						2						
Broccoli						1											1	
Chinese cabbage									2	6								
Common bean						2					1		1			1	1	
Cucumber		1	3			4							4					
Flowering Chinese cabbage								2	3									
Gallant soldier	1													1				
Lettuce		5		3	5		1					8	1			5		
Lime tree	1	4	2				4	3										
Mouse-ear-hawkweed						1				1				1				
Mung bean	2	1	2	2		3												
Raddish		1															1	
Ribwort plantain						1				1							1	
Silver cinquefoil						1				1							1	
Soybean	1	1					2					2				2		
Spanish needles	1													1				
Strawberry		1				1	1					1						
Sweet potato								2										
Wild carrot										9			3	9			3	
Yarrow						1				1							1	
Zucchini												3	1			4		
<i>Glechoma longituba</i>						1											1	
<i>Plantago depressa</i>	1													1				
Total Dicot	7	14	10	7	7	12	2	10	14	13	1	16	10	14	0	12	10	
As%	23	45	32	27	27	46	8	38	54	33	3	40	25	39	0	33	28	

µm PS, while no effect was recorded when exposed to HDPE of the same size. For dicots, however, root and shoot biomass showed consistent adverse patterns to the endpoint seedling biomass (sum of root and shoot biomass; Table 3). Having root and shoot biomass as separate characteristics might be more specific to indicate adverse effects than overall seedling biomass. Seedling biomass of monocots was either negatively (50 %) or not (50 %) affected. In dicots, negative effects were only recorded for lettuce, soybean and strawberry (Table 3).

Root and shoot biomass were more commonly assessed, but responses varied (Table 3). For example, the root biomass of corn was decreased by PLA and PS at 0.1 % and 1 % w/w (Wang et al., 2020a,b) and PE at 0.2 % w/w (Fu et al., 2022), yet an increase was observed in different studies when exposed to PE and HDPE at 10 % w/w, and PS at 0.01 % w/w (Wang et al., 2020a,b; Zhang et al., 2022a). In dicots, root biomass was recorded in half of the studied species, and in 77 % of the observations (positive in 33 %; negative in 40 %) an effect was observed (Table 3). For instance, wild carrot showed an increase in root length when exposed to seven different polymer types (Lozano et al., 2021). On the other hand, lettuce root biomass was reduced significantly by PE and PS, and zucchini by PET, PP and PVC (Tables S4–S5). A similar pattern is seen in shoot biomass, where 51 % of observations in monocots showed a response (13 % positive;

31 % negative; 7 % mixed). Rice seems to be the most sensitive monocot as several studies showed a reduction in shoot biomass when exposed to LDPE, PBAT, PS and PVC (Table 3). In dicots, 72 % of observations found an impact of plastics on shoot growth, with 39 % positive and 33 % negative effect (Table 3).

3.3.2.3. Other characteristics. Two additional endpoints might be promising to investigate in future research (Tables S4, S5). For dicots, 23 observations were made on root characteristics of which two were positive and 11 negative affected. For lettuce, root characteristics, including root volume and diameter, were negatively affected when exposed to PS, PE and PS with different surface charges, while PVC showed positive effects on root characteristics (Table S5). The impact on stem characteristics could also be an interesting endpoint in future studies. In both monocots and dicots negative impacts were observed. For instance, stem biomass of corn and rice were negatively affected by NMP, and a reduction in stem diameter was reported in wheat (Tables S4–S5). In dicots, the stem diameter of strawberry was negatively impacted, while in soybean, stem diameter was reduced when exposed to conventional plastics (LDPE) compared to biodegradable one (PLA and PBAT; Tables S4–S5).

3.3.3. Enzyme and biomarker activities

Overall, six monocots and 22 dicots were tested in this category (Table 4). Similar to studies done on plant growth, four crops have been tested more frequently. Nonetheless, trends observed in enzyme and biomarker activities in both monocots and dicots show general signs of increased stress levels *in vivo* (Tables S6–S7).

3.3.3.1. Plant pigments. Chlorophyll is a common endpoint that has been recorded across studies. In monocots, no clear pattern of impact on chlorophyll was observed, since out of 31 observations, eight recorded an increase while 12 showed a decrease (Table 4). Even within the same plant species, no clear trend was found. For example, rice and corn show mainly a negative impact ($n = 12$ observations) on chlorophyll levels, but also some positive ($n = 3$). In dicots, more than half of the observations (52 %) had a negative impact on chlorophyll, especially for lettuce and flowering Chinese cabbage (Table 4). The change in chlorophyll levels can possibly be explained by NMPs accumulating in the vascular bundles of the stems and leaf veins, which hinders the transpiration of nutrients and water through the plant (Dong et al., 2020). Consequently, the adverse effects on plant pigments may have an impact on the development and growth of the plant, as seen in the previous section.

3.3.3.2. Stress indicators. Stress indicators (including oxidative stress and free radicals) showed a very consistent response to NMPs, with upregulation recorded in 65 % and 71 % of the observations for monocots and dicots, respectively (Table 4). Oxidative stress indicators (reactive oxygen species (ROS), MDA, and TBARS) were increased in 65 % of the tested monocots and 62 % of tested dicots (Tables S6–S7). Rice was the only species tested that showed a decrease in oxidative stress indicators. Usually, levels of oxidative stress indicators increase when plants are exposed to stress (e.g., Wang et al., 2022b; Wu et al., 2021). However, the decrease

in levels of oxidative stress indicators in rice could mean that oxidative stress is beyond the scavenging ability of the enzymes, leading to a decrease of these indicators (Wu et al., 2020).

There was also an increase of free radicals (hydrogen peroxide or hydroxyl radical) in 64 % of the observations on monocots and in 79 % on dicots (Tables S6–S7). An increase in free radicals can result in oxidative damage, which is caused by an increase of active oxygen in the plant (Tan et al., 2022; Zhang et al., 2022a). In addition, high oxidative stress could also have negative consequences for secondary metabolites, such as the production of amino acids and lipids (Wu et al., 2020). Both oxidative stress and free radicals were predominantly increased in plants exposed to NMPs, highlighting that these endpoints are sensitive endpoints.

3.3.3.3. Defence against stress. Defence mechanisms against stress (antioxidants enzymes as well as the AsA-GSH cycle) were detected to be upregulated in 56 % and 73 % of the observations for monocots and dicots, respectively (Table 4). Antioxidant enzymes are important indicators of stress, as they are active in the protection of the plant cells and can eliminate stress-induced overproduction of ROS (Tan et al., 2022). Out of 39 observations in monocots, 21 showed an increase in antioxidant enzymes (Tables S6–S7). Nonetheless, eight observations also showed a decrease in antioxidant enzymes. As mentioned before, oxidative stress levels may be beyond the scavenging capacity of the antioxidant system, which may inhibit the production of antioxidant enzymes (Wu et al., 2020). In dicots, the pattern of response is more pronounced compared to monocots. For 74 % of the observations, an increase in antioxidant enzymes was recorded, while only 5 % showed a decrease (Tables S6–S7). Of these 74 %, nearly half were recorded in lettuce.

Another defence mechanism against stress is the AsA-GSH cycle: the main antioxidant defence pathway of plants. The overall trend in monocots and dicots showed a clear upregulation (Tables S6–S7). These results show

Table 4

Summary of effects of studies investigating effects of NMP on enzymes and biochemical indicators, focussing on plant pigment, stress indicators and defence mechanisms against stress. For plant pigment, the green colour indicates an increase in the endpoint and the red one denotes a decrease; NS means not significant. For stress indicators and responders, the orange colour indicates an up-regulation, while the red one a down-regulation. The mixed positive and negative result is reported in yellow, while blue indicates other changes/no clear trend. The sum of the effects observed for each endpoint per plant species is reported in each coloured box.

		Plant pigment								Stress indicators					Stress responders					
		Chlorophyll			Caretinoid				Up	Mix	Down	NS	≠ parts	Up	Mix	Down	NS	≠ parts		
		Inc	Dec	NS	Inc	Mix	Dec	NS												
Monocot	Barley									1						2		1	3	
	Corn	1	6	5	1					6						8				2
	Onion									3	1									
	Perennial ryegrass	3																		
	Rice	2	6	1						17	1	8	2	Y	6	4	6	3	3	Y
	Wheat	2		5	1	1				6			4		12	1	1	1	1	Y
	Total Monocot	8	12	11	2	1	0	1	33	2	8	8		28	5	8	9			
	As%	26	39	35	50	25	0	25	65	4	16	16		56	10	16	18			
Dicot	Alfalfa	1																		
	Balsam									2					2					
	Broad bean										1	1				1	1			
	Broccoli									1			1							
	Chinese cabbage		10					2												
	Common bean		2																	
	Cress	5		4	5			1		9		1	5		9		6	1	1	
	Cucumber		2	2				2	2	4			8		2	1	1	4	4	
	Dandelion									6					10					1
	Dutch clover									2					2					
	Flowering Chinese cabbage	2		3																
	Gallant soldier			1																
	Lettuce		10	3	1		1	2		26			4		27					2
	Mung bean		2	2																
	Raddish									1			2							2
	Rape		1							1					1					
	Soybean									4					3	1				
	Spanish needles																			
	Sweet potato									1			1		3					1
	Zucchini		2	2																
	<i>Glechoma longituba</i>			1																
	<i>Plantago depressa</i>			1																
Total Dicot	8	29	20	6	0	5	5	57	1	1	21		59	3	8	11				
As%	14	51	35	38	0	31	31	71	1	1	26		73	4	10	14				

that the plants are trying to counteract the excess in ROS production by secreting more antioxidants enzymes and activating their defence mechanisms (Wang et al., 2022b). For future studies, indicators within the AsA-GSH cycle, such as APX, AsA, GSH, and antioxidant enzymes, such as CAT, are sensitive endpoints to quantify stress caused by NMPs (Table 4).

3.3.4. General patterns of response

For the four most commonly tested plant species (lettuce, corn, wheat and rice), we combined observations of NMPs effects per endpoint to look at general patterns of response (Table 5). As there was not a species with more than five studies for germination and early development studies, we only combined this for the plant growth and biomarker studies.

In 77 % of observations on plant growth performance of these four most studied plants an effect was detected, of which 59 % were negative, 17 % positive and 1 % mixed (Table 5). Observations on both length and biomass reported mostly negative effects. Lettuce, the most studied dicot, was the most sensitive crop as approximately 85 % of the observations were adverse, such as a reduction in root length and biomass. These general patterns show a clear indication that impacts on plant growth are common for the most tested species, regardless of NMPs type and size used. Moreover, 82 % of recorded observations showed adverse effects on enzyme and biochemical activities, of which 59 % showed an upregulation, 19 % a downregulation and 4 % an up- and down-regulation of stress indicators and responses (Table 5). This again indicates that NMPs exposure, regardless of polymer or size of NMPs used, causes stress levels within the plant to increase, and sometimes even to make the defence mechanisms of the plant to fail. For both stress indicators and defence mechanisms against stress, observations showed a clear trend of an upregulation of these endpoints. For stress indicators, free radicals and oxidative stress were highly sensitive to NMPs (Tables S6–S7). In addition, antioxidant enzymes were also particularly sensitive. All three endpoints are susceptible characteristics to show stress levels encountered by plants due to NMPs. Similar as observed in plant growth studies, lettuce seems to be the most sensitive crop as approximately 71 % of observations showed an upregulation in stress level and stress responses, followed by wheat (62 %) and corn (52 %). For rice, observations were split between an up (45 %) and downregulation (35 %) in stress indicators. A change in plant pigments was a less sensitive biomarker, nonetheless, nearly 50 % of observations showed a decrease in plant pigment production, most commonly a reduction in chlorophyll levels (Table 5).

3.4. Environmental relevance of exposure levels

For experiments that used mass (w/w) as the basis for expressing the exposure concentration, we categorized levels tested at which an effect (LOEC) was observed to assess the environmental relevance of the exposure levels. Studies were grouped in one of five categories: <0.01 %, 0.01 %–0.1 %, 0.1 %–1 %, 1 %–2 % and >2 % w/w. NMPs levels detected in the environment differ significantly between studies (reviewed by Büks and Kaupenjohann, 2020). Median NMPs levels detected ranged between

0 mg/kg and 2400 mg/kg (which translates to 0 % and 0.24 % w/w), and were lower in agricultural soils compared to soils near industry or roads (Büks and Kaupenjohann, 2020). For example, a maximum level of 67500 mg/kg (6.75 % w/w) was detected in an industrial area in Sydney, while a maximum level of 224 mg/kg (0.0224 % w/w) was detected in an agricultural soil in Denmark (Büks and Kaupenjohann, 2020). However, research on NMPs is a rapidly expanding field, and with advances in analytical techniques these levels can be adjusted up- or downwards. In addition, NMPs will continue to accumulate in the environment, which combined with their persistence, likely will increase the levels in soils (Lebreton and Andradý, 2019).

First, in the vast majority of cases when effects on seed germination and early development were detected, effects were found at a concentration of 0.01 % to 0.1 % w/w (Fig. 3). This was the case in 88 % of observations in germination, 82 % in length and 80 % in biomass (Fig. 3). For example, the germination rate of perennial ryegrass was significantly decreased when exposed to 0.1 % w/w of PLA and 0.001 % w/w of synthetic (nylon and acrylic) fibres. Moreover, the germination of cress was significantly inhibited by PE, PP and a combination of PE and PVC at a concentration of 0.2 % w/w and PET at 0.02 % w/w (Pignattelli et al., 2020, 2021). Secondly, for plant growth the majority of effects were observed at concentrations between 0.1 % and 1 % w/w for both length (63 %) and biomass (56 %, Fig. 3). These levels have been recorded in highly contaminated regions, such as industrial areas (Fuller and Gautam, 2016). Furthermore, 33 % of effects observed on biomass were made between 0.01 % and 0.1 % w/w. Thirdly, effects on biochemical endpoints were recorded at a wider range of exposure levels. Plant pigments are shown to be affected mostly at exposure levels between 0.01 % and 0.1 % w/w (50 %) and between 0.1 % and 1 % w/w (38 %, Fig. 3). For stress indicators and respondents, effects are mainly observed at exposure levels between 0.01 % and 0.1 % w/w (52 % for indicators, 43 % for respondents), while effects are also recorded at <0.01 % w/w (15 % for indicators, 19 % for respondents), between 0.1 % and 1 % w/w (15 % for indicators, 24 % for respondents) and between 1 % and 2 % w/w (15 % for indicators, 14 % for respondents; Fig. 3). This shows that enzyme and biomarker activities are impacted at very low, realistic but also at high, unrealistic exposure levels. Overall, these results show that effects on germination, plant growth and enzyme and biomarker activities are commonly recorded at levels detected in the environment.

3.5. More complex study approaches

3.5.1. Field experiment

In our review we only found a single study which was performed in the field. Hu et al. (2020) investigated the effects of residual plastic films (RPFs) on corn growth parameters during a two-year experiment using predicted environmentally relevant concentrations (18 years of continuously application of plastic-film mulch: 50 kg/ha, 45 years: 300 kg/ha; 100 years: 600 kg/ha; Yan et al., 2014). Both root and shoot dry biomass were significantly reduced for all treatments. Additionally, RPFs had severe negative impacts

Table 5

Summary of effects on combined endpoints of studies investigating NMP effects on plant growth and biochemical activity. For plant growth, plus (+) and the green colour indicates an increase in the endpoint, a minus (–) and the red colour denotes a decrease. The mixed positive and negative result is reported in yellow, while blue indicates other changes with no clear trend. For plant pigment, the green colour indicates an increase in the endpoint and the red one denotes a decrease. For stress indicators and responders, orange indicates an up-regulation, while red a down-regulation. The mixed positive and negative result is reported in yellow, while blue indicates other changes with no clear trend. The sum of the effects observed for each endpoint per plant species is reported in each coloured box. NS means not significant.

		Plant growth												Biochemical stress																
		Length			Biomass			Other charac.			Total			Plant pigments				Stress indicators				Stress respondents								
		+	–	NS	+	–	NS	+	Mix	–	NS	+	Mix	–	NS	Inc	Mix	Dec	NS	Up	Mix	Down	NS	Up	Mix	Down	NS			
Monocot	Corn	2	2	2	7	3	1	1	1	4	3	10	1	9	6	2		6	6	6		1	8	8		2				
	Rice	2	10	4	4	8	6			4	3	2		18	13	2		6	1			2	17	1	8	2	6	4	6	3
	Wheat	2	11	9	4	8	1			9	4	6		28	14	3	1		5			4	6		12	1	1	1	1	
Dicot	Lettuce	3	10		6			2		11		5		27		1		11	5			4	26			27			2	2
	Total	9	33	15	11	21	8	3	1	28	10	23	1	82	33	8	1	23	17			11	55	1	8	11	53	5	7	8
	As%	16	58	26	28	53	20	7	2	67	24	17	1	59	24	16	2	47	35			15	73	1	11	15	73	7	10	11

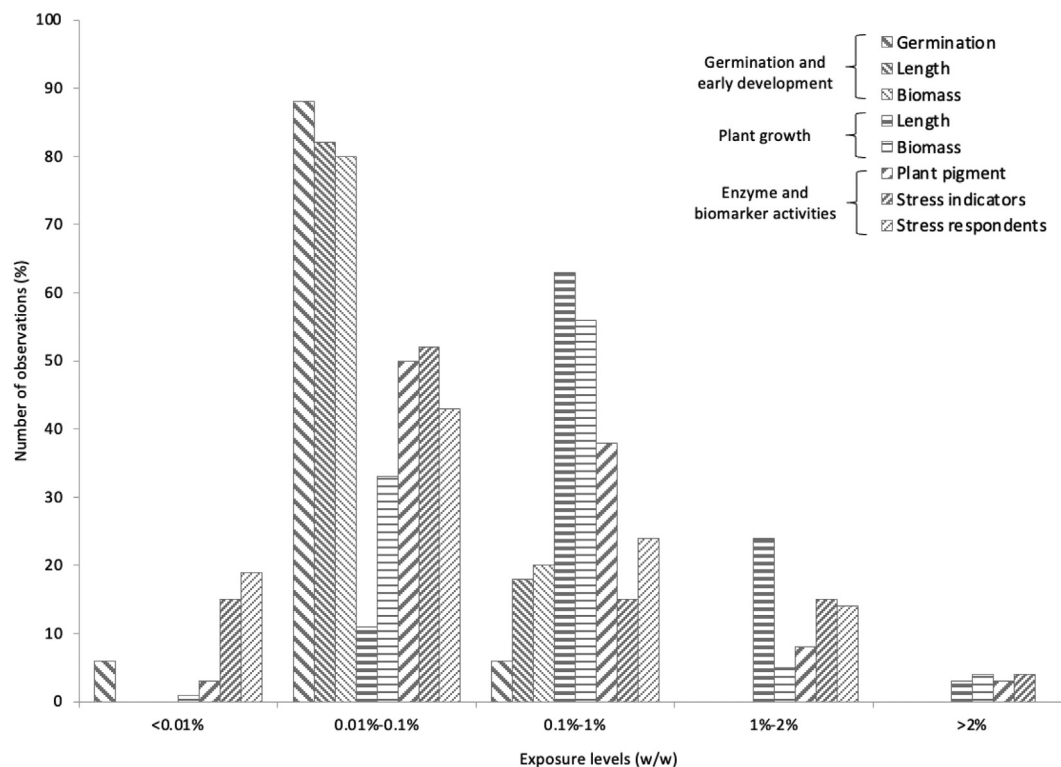


Fig. 3. Number of observations at which LOEC was recorded per endpoint. Whenever an effect by NMP was recorded, the lowest concentration at which this effect was seen was noted. The endpoints were divided across three categories: i) seed germination and early development, ii) plant growth, and iii) biochemical responses in terrestrial plants.

on root characteristics, such as a root length reduction. In particular, the amount of very coarse roots decreased during the different growth stages, while the number of finer roots increased. This is of particular interest as fine roots are usually very important in absorbing nutrients and water from the soil.

This study shows the effects of NMPs on crops in environmental realistic conditions and could be used as a baseline for future studies. In addition, it confirms the general patterns we have observed under more controlled conditions. Nevertheless, there is an urgent need for more of these large-scale experiments as numerous and correlated interactions are taking place in ecosystems (Carpenter, 1998). After all, laboratory generated results might not always translate to results generated in the field. Therefore, the tiered study or mesocosm approaches have been developed (OECD, 2003), aiming to stepwise add more ecological relevance to effect results as identified in the laboratory stage.

3.5.2. Joint toxicity and stress-on-stress

In farmlands, a range of other contaminants, including pesticide residues, metals and/or nanoparticles, are also present in the soil. These result in joint exposure of plants to a range of stressors. In fact, organisms are rarely exposed to a single stressor, but rather to a combination of different stressors and therefore exert joint toxicity to organisms (Wang et al., 2014). Out of the 78 studies, 27 publications investigated the joint toxicity of NMPs with another stressor, ranging from a combination of several different polymers to the combined impacts of NMPs and environmental stress (e.g., drought or heat). Studies on the joint toxicity of multiple pollutants were most commonly performed on NMPs and metals ($n = 8$), plastic additives ($n = 7$), and nanoparticles ($n = 3$). For instance, the combined mix of PS and DBP (plasticizer) further decreased photosynthesis of lettuce compared to single stress treatments (Dong et al., 2021).

Other studies investigated the impacts of NMPs and changing environmental conditions ($n = 5$), such as drought, low temperature and acid rain. For example, the fresh biomass of cress seedlings was further reduced

when exposed to PET and acid rain together compared with the PET treatment only (Pignattelli et al., 2021). Combining plastic pollution with other environmental stressors is important as environmental conditions are changing due to climate change (e.g., latest IPCC report, 2022).

4. Conclusions, open questions and avenues for future research

Here we present a detailed systematic review on the impacts of NMPs on plant germination and early development, plant growth and biochemical responses. We combined data from 78 studies on the effects of NMPs on plants, highlighting that a multitude of endpoints are currently used to assess effects (summarized in Fig. 4). Overall, we noted limited differences between monocots and dicots, except for significant lower germination in dicots compared to monocots (Fig. 4). Importantly, some endpoints are more sensitive indicators of exposure to NMPs than others. For example, during early growth, root growth is more strongly affected compared to shoot growth (Fig. 4). Plant growth showed most varied responses when comparing all species tested, with only seedling biomass and length showing some indications on adverse impacts by NMPs. In contrast, biomarker responses were consistent across species, regardless of NMP type or size. Chlorophyll levels were negatively affected, while stress indicators (e.g., ROS or free radicals) and stress respondents (e.g., antioxidant enzymes) were consistently upregulated across the tested species (Fig. 4). When focussing on the growth performance and biomarker response of the four most common crops (lettuce, wheat, corn, and rice) an overall adverse impact of NMPs was noted, regardless of polymer or size of NMPs used (Fig. 4).

We also show that these effects commonly occur at environmentally realistic exposure levels. These results clearly indicate that NMPs have a wide range of adverse impacts on plants. Yet, due to the large variety in study approaches, there are some open questions that could not be answered in this review, but which warrant further attention. We identified two key points of attention. Firstly, we did not look at the size of the impact, as there

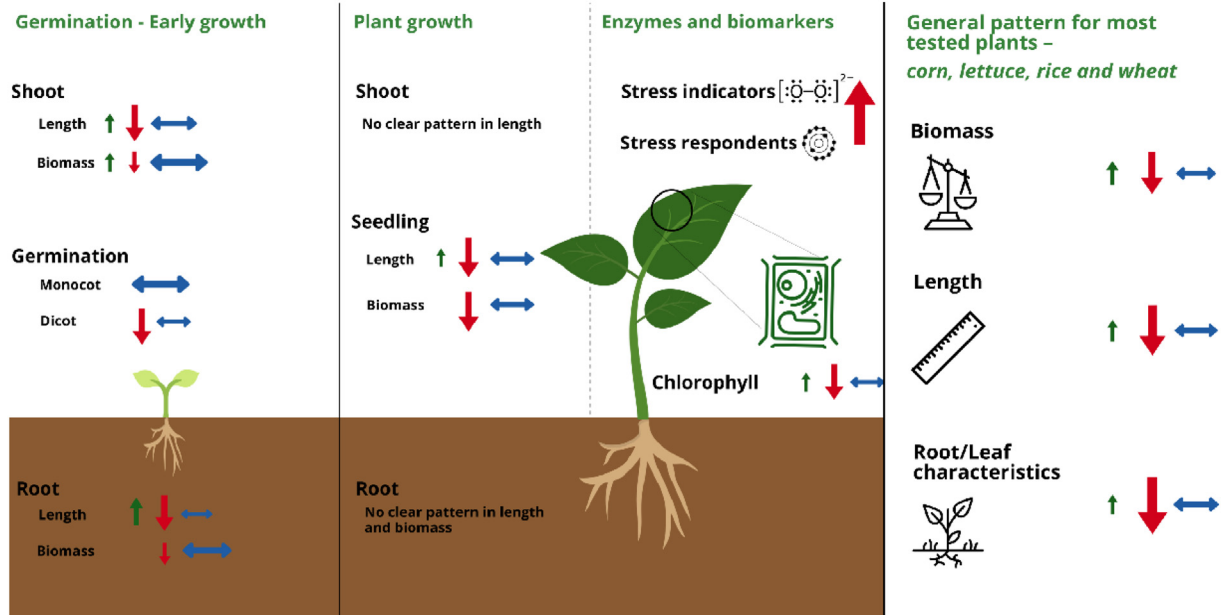


Fig. 4. Summary of impacts of nano- and microplastics on seed germination and early growth, growth and biomarker responses in plants based on a systematic review of 78 studies.

was a wide range of different endpoints used as well as study approaches (with great differences in study length and exposure medium). With increased standardization in these testing protocols, and more realistic exposure conditions, it should be possible to detect effect sizes in future studies. It is important in these cases to also determine how long these effects last, as some work has highlighted transient effects of NMPs on plants (e.g., Bosker et al., 2019). Secondly, we have noticed that there was sometimes not a clear dose-response relationship observed in the studies. For example, within a single study, effects sometimes could be seen at only one concentration, or mixed responses were observed between different concentrations.

Based on our findings, we suggest the following key avenues for future research.

- Firstly, there is a lack of testing under realistic environmental conditions. An important remaining question therefore is whether and how the results from the lab-based studies can be extrapolated to the field. For example, only one study was conducted under field conditions (Hu et al., 2020). In addition, only limited studies investigated the impacts of NMPs in combination with additional stressors (e.g., drought or acid rain). Most studies have been performed under highly controlled conditions. We need to verify whether these results can be translated to more environmentally realistic conditions, and if so, how we can extrapolate these results to make interpretations for field realistic scenarios.
- Secondly, the majority of studies still use pristine NMPs, most commonly PS and PE particles. Given that under field conditions weathered NMPs are dominant in a cocktail of different polymers, there is an urgent need to diversify the physico-chemical characteristics of NMPs used in plant studies.
- Thirdly, the impacts of leached additives on plant performance needs to be investigated. This fell outside the scope of this literature review, but it remains an important topic of research within the field of study. For example, leachates from plastics bags induced developmental abnormalities and a growth reduction in cress (*Lepidium sativum* L.) seedlings (Balestri et al., 2019).
- Finally, this work needs to be placed in a broader context and linked to studies on uptake and fate of NMPs in plants (Azeem et al., 2021; Zhang et al., 2022b), but also movement in the food chain. The associated ecological and health effects of NMPs throughout the food chain also warrants future investigation.

CRediT authorship contribution statement

Laura J. Zantis: Conceptualization, Methodology, Investigation, Writing - Original draft preparation, Visualization.

Caterina Borchi: Conceptualization, Methodology, Investigation, Writing - (Reviewing and Editing).

Martina G. Vijver: Writing - (Reviewing and Editing).

Willie Peijnenburg: Writing - (Reviewing and Editing).

Sara Di Lonardo: Methodology, Writing - (Reviewing and Editing).

Thijs Bosker: Conceptualization, Methodology, Writing - (Reviewing and Editing), Supervision.

Data availability

Data will be made available on request.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.161211>.

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