RESEARCH ARTICLE

Micro- and nano-plastics in hydroponic environment are critical for plants: A meta-analysis

Shi-Peng Li, Yu-Xi Feng, Xiao-Zhang Yu*
College of Environmental Science & Engineering, Guilin University of Technology, Guilin 541004, China

Abstract: The presence of microplastics (MPs) and nanoplastics (NPs) in the environment is ubiquitous, and as such, the toxicity of these plastics, exposure scenarios, and mechanisms of plant response are to be determined. Hereby, a meta-analysis is performed to investigate the effects of MPs and/or NPs on different plant species under hydroponics and soil conditions to assess the current scenario. We examined the response level of root system, photosynthetic parameters, and antioxidant system of plants against MPs/NPs. Root response level in soil condition against various concentrations and types of MPs was higher than in hydroponics however, this response was opposite for the types of MPs. Photosynthetic parameters, including chlorophyll a and b, carotenoids, total chlorophyll, and maximum quantum efficiency of PSII were higher in soil conditions than in hydroponics. Antioxidant parameters, such as malondialdehyde (MDA) and H_2O_2 content were higher in hydroponics plants, while, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) showed a mix trend of response level. In addition, proline (Pro) content was significantly higher in soil and ascorbic acid (ASA) in hydroponic cultured plants under different types, sizes, and concentrations of MPs. These three systems i.e., root, photosynthesis, and antioxidant parameters were also compared between different species, however, the results are generally consistent with the above mentioned one. Overall, these analyses suggest that plants grown in hydroponics are more sensitive to the plastic pollution than in the soil environment.
Keywords: Hydroponic, microplastic, nanoplastic, oxidative stress

Correspondence to: Xiao-Zhang Yu, College of Environmental Science & Engineering, Guilin University of Technology, Guilin 541004, China; E-mail: xzyu@glut.edu.cn

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1 Introduction

Plastic contamination became a serious ecological threat due to its persistence in nature, toxic to living organisms, and large scale global production. It is estimated that 368 million tons of plastic were produced globally in 2019 only, while it is regularly increasing per annum due to its durability, lightness, and low price. Currently, its use is very common in every sector of life, however, its use in agriculture, fisheries, packaging, construction, transportation, and several industries are directly associated with soil and water pollution (Li et al., 2022). Soil and water bodies are the major sinks of plastics due to the discharge of industrial effluents, municipal waste, and agricultural practices, including plastic mulch, addition of municipal sludge, and landfill sites. These activities add million tons of macroplastic (> 5 mm), microplastic (1 μm-5 mm), and nanoplastic (< 1 μm) regularly (Luo et al., 2021, Shao et al., 2020). In addition, several physical, chemical, and biological processes such as mechanical wear and tear, ultraviolet radiation, microbial degradation, and other decomposition processes convert macroplastic into microplastics (MPs) and nanoplastics (NPs). Introduction of this large scale MPs and NPs into the environmental matrixes i.e., water and soil affects both plants and animals (Chen et al., 2022, Huang et al., 2022).

A number of studies in the last decade have worked on the effects of MPs/NPs in agricultural systems, specifically since 2016 (Souza Machado et al., 2019; Rillig et al., 2020; Zantis et al., 2023). Previous studies indicated that MPs/NPs not only change the physical and chemical properties and microbial communities of soils, but also accumulate a wide range of harmful chemicals in soils (Yu et al., 2021; Hu et al., 2022). For example, the addition of MPs significantly promoted the emission of soil carbon dioxide, soil pH, dissolved organic carbon, ammonia nitrogen, and the phospholipid fatty acid (Gao et al., 2021b). Another study revealed that exposure of MPs to soil resulted in highly negative effects on soil bulk density and aggregate stability (Qiu et al., 2022). Accumulation of MPs in soil damaged the physical and chemical properties of soil including water permeability, air permeability, soil organic carbon, and nitrogen cycling (Gao et al., 2022). In addition, plants are also vulnerable to changes in the environment such as MPs/NPs pollutants. A recent study showed that MPs can accumulate in root crown cells observed by the fluorescence labeling method (Hartmann et al., 2022). In fact, accumulation of MPs in plants inhibits their growth and development through affecting root system, photosynthesis, and antioxidant system. For example, polystyrene MPs had significant inhibition effect on seed germination rate, germination percentage, and physiological and biochemical indexes of three herbaceous ornamental plants in a hydroponic experiment (Guo et al., 2022). Similarly, polystyrene MPs also reduced plant biomass and induced oxidative stress damage significantly in leaves and roots of lettuce (Gao et al., 2021b). In another study of lettuce, microfibers had adverse effects on plant height, photosynthesis, and chlorophyll content (Zeb et al., 2022). In a pot experiment of soil culture, the root activity, malondialdehyde (MDA) and proline (Pro) content of cucumber were significantly increased under the stress of polystyrene MPs/NPs (Li et al., 2022). Despite these studies, the effect of MPs/NPs on phytotoxicity and their potential mechanisms are currently elusive. It is necessary to do a comprehensive assessment regarding their toxicity and risk in the ecosystem to provide a platform for future research on this crucial issue.

Meta-analysis is a statistical method used to integrate the results of several studies, analyze it, and generate overall results on the same scientific problem. Due to its large scale application, the number of studies on meta-analysis also increased rapidly, and meta-analysis has been widely used in the environmental field (Roy et al., 2023; Shi et al., 2023). Although, reports are available on the meta-analysis of MPs/NPs effects on plants and soils, however, the comparative toxicity of MPs/NPs to plants cultured in hydroponics and soil conditions is still unknown. It is also very important to find the effects of MPs/NPs on plants in hydroponics, because several plants grow in water, while a large amount of plastic is discarded into the water. On the other hand, water culture is an important experimental method in the study of plant toxicology, especially in the aspect of bioavailability. Hereby, we hypothesized that the physiological response of plants is different in hydroponics and soil-contained pot experiments. Therefore, the meta-analysis aimed to analyze the effects of MPs/NPs types, concentrations, and sizes on plants in hydroponics and pot experiments. Overall, this study provides a new insight into the effect of MPs/NPs on plant growth in soil and hydroponic environment, and considers their toxicity of exposure to plants.

2 Methodology

2.1 Literature retrieval

All studies (till October 2022) collected from the Google Scholar, China National Knowledge Infrastructure, ResearchGate, PubMed, and Web of Science were investigated in the interaction of MPs/NPs and plants in the two different cultural conditions i.e., soil and hydroponics. We used a few keywords including “Microplastic”, “Macroplastic”, “Nanoplastic”, “Plastic”, “Plant”, “Physiological response of plants to MPs/NPs” and “Biochemical response of plants to MPs/NPs” to find studies for meta-analysis. We developed a strict rule to search and collect the most novel, relative, and reliable data sets. Initially, we searched more than 400 research articles, but we chose 42 articles for further analysis by implementing the following criteria in our search: (1) The effects of MPs/NPs on plants. If the MPs/NPs and other contaminants are taken together in a study, there must be a separate study of MPs/NPs on plants, otherwise it was excluded if they used mix; (2) The study contained at least one plant that responded to MPs/NPs treatment; (3) The report contained at least one indicator that measured plants, such as root length, oxidative stress, antioxidant substances, chlorophyll, etc.; (4) The control treatment was included, and the samples must contain the mean value and sample sizes of variables, or at least three experiments had repeated. The studies who met these four criteria were included in the study.

2.2 Data collection and pretreatment

Data were collected from the 42 studies following the above mentioned criteria. We recovered data of MPs/NPs sizes, concentrations, and types, including different polymers (polystyrene (PS), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polylactic acid (PLA)), plant species, exposure regimes, sample sizes, average, standard error, and control group data (mean $\pm$ SD). We used the Plot Digitizer software to digitize data from the graphs present in the selected articles.

The collected data was classified into two main groups on the basis of exposure regimes i.e., soil and hydroponics to facilitate subsequent data analysis. Further, these two types of experiments were classified into three sub-categories, such as root system, photosynthesis, and oxidative stress. Moreover, the effects of MPs/NPs concentrations ($\leq$ 100 (low), 100-500 (medium), 500-1000 (high), mg/L or mg/kg), sizes (μm, nm), and types of exposure regimes were considered. The indexes with significant differences were selected on the basis of these comparisons, and the specificity of different plants on this index was studied.

2.3 Meta-analysis

Data from the selected research articles can not be used directly to compare, analyze, and build graphical plots because of the differences in the units among the selected research data and the difference between variables is too high. In order to avoid miscalculation, all experimental data were normalized (*NED*) using the following formula (Shi et al., 2014):

$$NED = \frac{\frac{1}{n} \cdot \sum_{i=1}^{n} I_i}{\frac{1}{m} \cdot \sum_{j=1}^{m} I_j}$$

Where, I_i and I_j are the data from the treatment and control, respectively; i is the replicate 1, 2, ..., n, and j is the control 1, 2, ..., m. The following formula is used to calculate the standardized *SD* value:

$$SD_{I_i} = \frac{SD}{I} \times I_i$$

Where, *SD* is the data from the treatment and control, respectively; *I* is the treatment and control. This treatment can map different variables to the same dimension level, and facilitate the analysis and comparison of data in different articles under different quantitative levels.

2.4 Statistical analysis

To analyze the effects of MPs/NPs on plant growth in hydroponics and soil environment, all the data were classified, and then Microsoft Excel 2016 was used for data processing, statistical analysis, and graph presentation. The assessed polled effect was formed by first-order meta-analysis with 95% confidence intervals. Negative and positive values indicate negative and positive effects on plant growth parameters, respectively.

3 Results and Discussion

3.1 Response of plant root in different cultivated conditions

Overall, 42 research articles were identified from 2012 to October 2022 that contained experiments of plant interactions with MPs/NPs and met the criteria mentioned in the methodology section were subjected into meta-analysis and studied the effects of MPs/NPs concentrations, sizes, and types on plant root, photosynthesis, and oxidative system in soil and hydroponic cultures.

3.1.1 Response to different concentrations of MPs/NPs

The concentration of plastic in soil and water is directly proportional to the plant growth as higher concentration of plastic affects plant growth more severely (Huo et al., 2022). The overall results related to the concentration of MPs/NPs revealed that the root structure of plants was almost similarly affected in both hydroponics and soil culture. However, relatively lower level of response was showed by plant roots in hydroponics rather than the soil culture experiments (Figure 1A). Li et al. (2018) studied that the root length of lettuce was significantly longer (46.51%) in hydroponic culture than subsoil conditions without any stress. In this way, Lei and Engeseth (2021) also had the similar observation, i.e., the root length of lettuce grown under hydroponic conditions was significantly longer than that grown under subsoil conditions. So, it shows that the negative effects on plant root were directly related to the MPs/NPs. In addition, Gong et al. (2021) found that root length of wheat was decreased by 13.08% when grown in hydroponics with low concentrations of MPs, while in the study of Liao et al. (2019), the root length of wheat decreased by only 0.58% under MPs stress in soil culture environments. In accordance with these reports the meta-analysis findings showed that MPs/NPs have more severe effect on root growth of plants grown in hydroponic culture.

3.1.2 Response to various sizes of MPs/NPs

There are three major types of plastics according to their sizes i.e., macro-, micro- and nano-plastic (Roy et al., 2023). Exposure of plants to different sizes of MPs/NPs affects root growth in both soil and hydroponic cultures. Similar to the concentration factor, no significant difference in plant roots was observed between different sizes of MPs/NPs. However, the relative response level of plant roots in soil condition was higher than hydroponic culture (Figure 1B). One of the reasons behind higher response level of roots in soil condition is that the roots in soil are block by MPs mechanically which affect plants growth, and produce stronger response than the hydroponic culture (Kalčíková et al., 2017).

3.1.3 Response to different types of MPs/NPs

Among different types of plastics, PE and PS are the most common species which affect plant growth negatively (Roy et al., 2023). Comparative results of our meta-analysis exhibited that the response of plant root treated with PE was

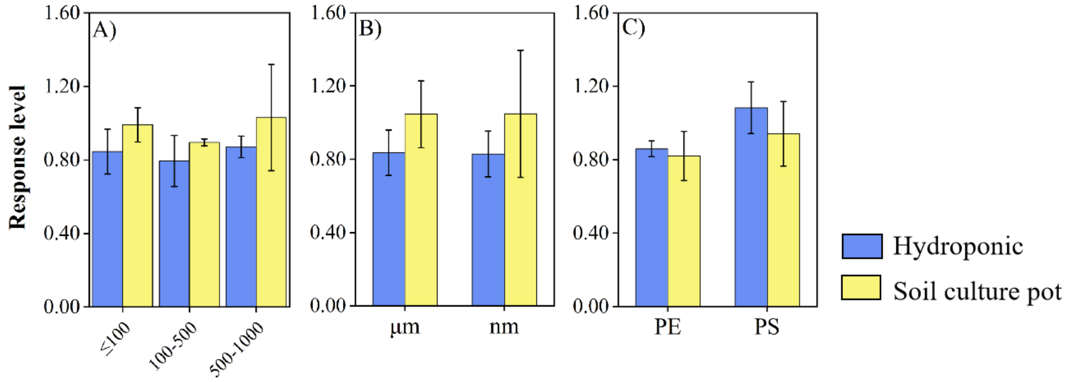

Figure 1. Response of root length in different cultivated conditions

significantly lower than that of plants treated with PS. In addition, the response level of root length in hydroponic environment was stronger than that in the soil environment (Figure 1C). These results may be due to the adsorption potential for different types of MPs/NPs, or the difference in degradation capacities of MPs/NPS and the toxicity of degradation products, or the shape of MPs (Lian et al., 2022).

3.2 Response of photosynthetic parameters in different cultivated conditions

3.2.1 Response to concentrations of MPs/NPs

Plastic (MPs/NPs) concentrations affect photosynthetic pigments (chlorophyll a and b, and carotenoids) and several enzymes that eventually affect overall photosynthesis and plant growth (Shi et al., 2023). Herein, we analyzed the response levels of chlorophyll a and b, carotenoid, and maximum quantum efficiency of PSII (Fv/Fm) in plants under soil and hydroponic conditions. The response level of these photosynthetic pigments was found slightly higher in pot experiments than that of hydroponics. However, no significant difference in the response level of photosynthetic pigments was observed between low and high concentrations of MPs/NPs. In the case of culture conditions, there was no significant difference in the responses level between hydroponics and soil environments. However, the response level of chlorophyll a and b under low concentration of MPs showed a lower response tendency in hydroponics than that in the soil, and the total chlorophyll also showed a similar responding trend (Figure 2A-E). These results are in line with the study of Lei and Engeseth (2021) who found that the content of chlorophyll a, chlorophyll b, and carotenoids in lettuce plants grown in soil conditions was higher than in hydroponics. At low concentrations, the plants in soil culture environment can efficiently tackle the effects of contaminants on plants, so the content of chlorophyll and carotenoids in plants was increased. Similarly, the total chlorophyll of rice and broad bean was reduced by 9% and 5.7%, respectively at 100 mg/L in the soil environment, showing that the response may depend on different types and sizes of MPs or different species of plants (Dong et al., 2020, Ye et al., 2021). On the other hand, response of Fv/Fm to moderate level of MPs to plants was detectable; however, there was no significant difference between the hydroponics and soil conditions (Figure 2E). Fv/Fm represents maximum quantum efficiency of PSII, reflecting the conversion efficiency of the intrinsic light energy in the PSII reaction center (Li et al., 2020a).

3.2.2 Response to various sizes of MPs/NPs

The size of MPs/NPs is a crucial response parameter to the plant photosystem (Azeem et al., 2022). Hereby, the effect of different sizes of plastics on photosynthetic pigments was an-

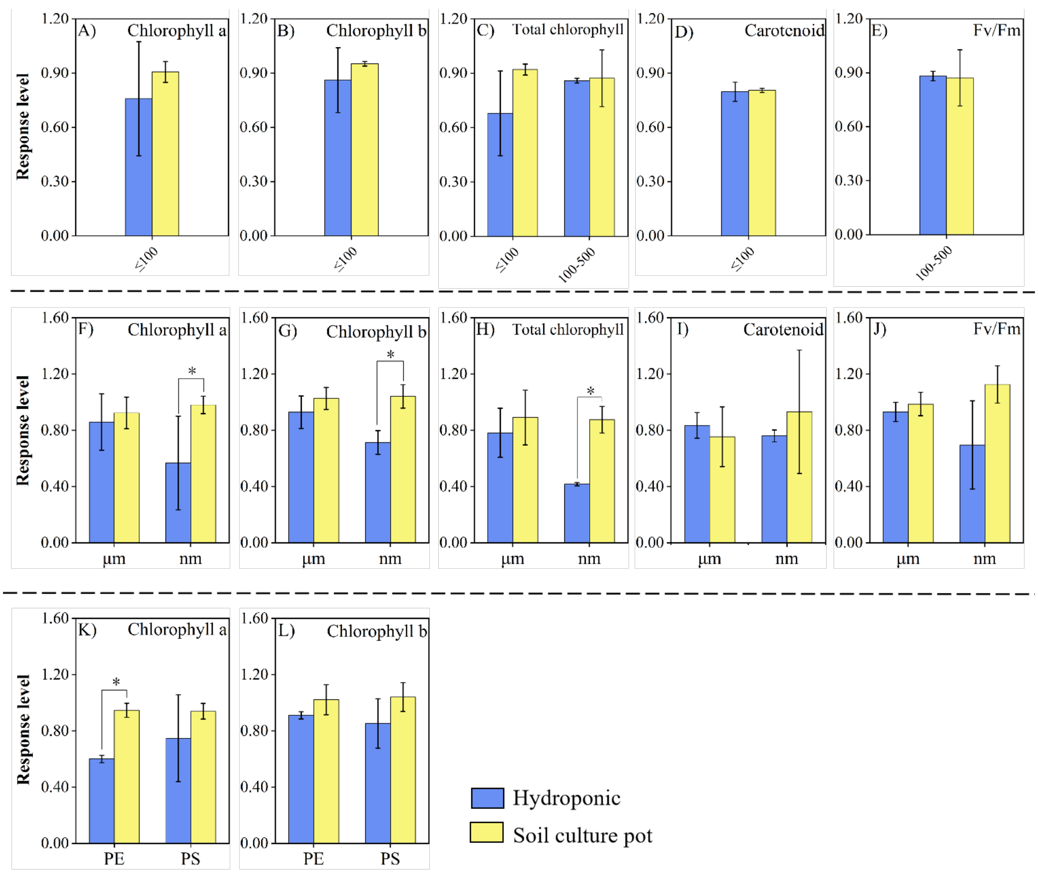

Figure 2. Response of photosynthetic parameters in different cultivated conditions

alyzed, and chlorophyll a, chlorophyll b, and Fv/Fm showed significantly stronger response to NPs in both hydroponics and soil culture than MPs (Figure 2F and G). Nanoplastics can be easily taken up by plant root and subsequently translocated into other tissues which alter several biochemical processes, while macro-plastics and MPs only obstruct root growth (Roy et al., 2023). Carotenoids also showed a measurable response to both MPs and NPs, but no significant difference was detected. While, plant response level in term of all these photosynthetic parameters was overall higher in soil condition compared to the hydroponics (Figure 2F-J). These results are in accordance with Li et al. (2020b) who found no significant effect of MPs on chlorophyll a and chlorophyll b in cucumber leaves. In another hydroponic experiment, NPs treated cucumber leaves showed much lower content of chlorophyll a and chlorophyll b (Li et al., 2020c), which reveals that NPs are more toxic compared with MPs, specifically in soil conditions. These results suggest that smaller sizes of NPs are more easily taken up, and translocated by plants which subsequently affect photosynthetic parameters compared to the MPs.

3.2.3 Response to different types of MPs/NPs

Response level of plant photosynthetic parameters under PE and PS were analyzed, the response level of chlorophyll a in hydroponic environment was significantly lower than in soil conditions. Although the response level of chlorophyll b also followed the same trend as chlorophyll a, but the difference between the two exposure regimes was not significantly different. It was noticed that the response level of both chlorophyll a and b to both PE and PS plastic was almost similar (Figure 2K and L). PVC induced a decrease in chlorophyll content and photosynthesis in marine and freshwater algae, (Wang et al., 2020) and a decrease of 11.79% in chlorophyll content and photosynthetic rate in lettuce under PE stress was found under hydroponic conditions (Gao et al., 2019). In another study, the chlorophyll content of lettuce decreased by 0.13% under PEF stress (Zeb et al., 2022). The Meta-analysis showed that PE and PS have a smaller effect on photosynthesis of plants grown in soil environment than in hydroponics.

3.3 Response of antioxidant systems in different cultivated conditions

3.3.1 Response to the concentrations of MPs/NPs

The increase in concentrations of MPs/NPs in the external environment disrupts the plant homeostasis system and produces reactive oxygen species (ROS) which subsequently induces MDA accumulation in plants. In response to ROS accumulation, plant antioxidant system activates several enzymes and non-enzyme substances, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbic acid (AsA), amino acids, and sugar to cope with the oxidative stress (Ben et al., 2014; Boublin et al., 2022). Both low and high concentrations of MPs induced higher content of MDA and H_2O_2 in plants grown in hydroponics than in soil conditions. On the other hand, higher concentrations of MPs induced higher content of MDA and H_2O_2 compared with the low concentration in hydroponics. However, no significant difference in MDA and H_2O_2 content was detected between different concentrations of MPs under soil conditions (Figure 3A and B). These analyses are consistent with the study by Zong et al. (2022), who found that MDA content

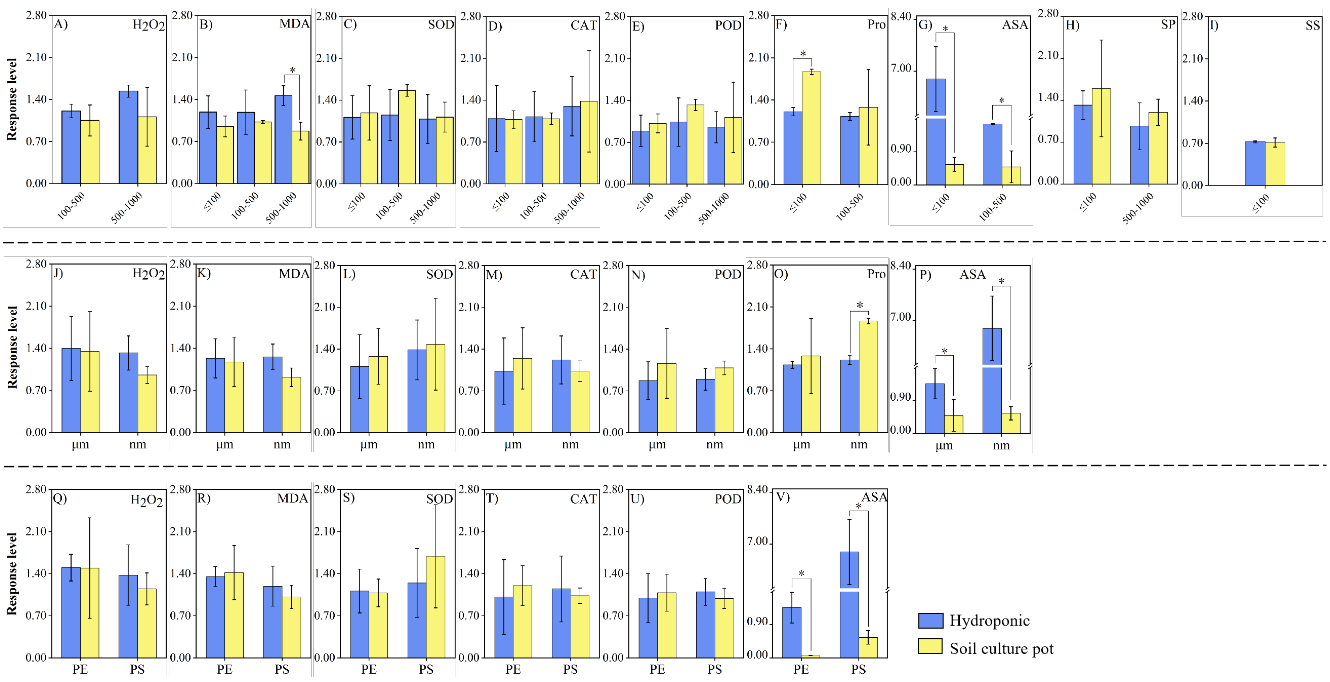

Figure 3. Response of antioxidant systems in different cultivated conditions

in peanut seedlings increased with the MPs concentrations. Similarly, Li et al. (2020a) found that the H_2O_2 content in cucumber increased under MPs stress in hydroponic environment. It reveals that an increase in MPs concentration is directly proportional to the production of ROS and subsequently increases the content of MDA and H_2O_2 in plants grown in hydroponics.

Scavenging substances of ROS i.e., Pro, SOD, POD, CAT, AsA, soluble sugar (SS), and soluble proteins (SP) were analyzed here in both soil and hydroponic conditions. Proline acts as an osmoprotectant for plants and is also responsible for scavenging of ROS. Meta-analysis showed that the Pro content in plants grown in soil environment was higher than in hydroponics (Figure 3C). Similarly, the activities of SOD, POD, and CAT in hydroponic growing plants under different concentrations of MPs were lower than in soil environment (Figure 3D-F). In addition, the content of AsA was significantly higher in hydroponic cultures at both low and higher concentrations than in soil environment (Figure 3G). The content of SP decreased with the increase in MPs concentration, and the content in the soil environment was higher than in hydroponics. However, there was no significant difference in the level of SS in plants between soil environment and hydroponics (Figure 3H and I). In the study of Sun et al. (2021), the content of H_2O_2 and MDA in plants grown in hydroponics under MPs stress was higher than that in soil environment, while the levels of SOD, POD, CAT, and SP were higher in soil condition than in hydroponics, revealing that MPs in hydroponics is more toxic to plants than in the soil, and the internal function of the ROS scavenging system in soil culture is more efficient than in hydroponics.

3.3.2 Response to the sizes of MPs/NPs

Overall, the sizes of MPs and NPs had no significant effect on MDA, H_2O_2, the antioxidant enzymes, and osmoprotectants in both culture environments (Figure 3J-P). Meta-analysis showed that both MPs and NPs induced MDA, H_2O_2, SOD, POD, and CAT in a mix trend under hydroponics and soil environments. Gao et al. (2021a) found that MPs stress induced MDA and H_2O_2 content in lettuce plants at the same rate. On the other hand, plants activated antioxidant enzymes to reduce the oxidative damage caused by MPs/NPs, under different exposure regimes, different plant species used, and types of MPs/NPs, etc., (Azeem et al., 2022). However, Pro and AsA contents increased under NPs compared with MPs, but their relation to the culture condition was antagonist i.e., Pro content was higher in soil but AsA content was higher in hydroponic culture (Figure 3J and P). It reveals that the NPs are more toxic compared with NPs due to their smaller (nano) size that can easily be taken up by plants and disrupted several biological functions, meanwhile plants produce more antioxidant substance to rescue plants from NPs toxicity (Roy et al., 2023).

3.3.3 Response to the types of MPs/NPs

The responses of MDA and H_2O_2 to PE stress were slightly higher than PS in both soil and hydroponic environments. In addition, the response of MDA and H_2O_2 to PS stress in hydroponics was stronger than in soil environment, but their response to PE stress was almost similar in both exposure regimes (Figure 3Q and R). Similarly, Pignattelli et al. (2020) observed that plants exposed to acute level of PE showed higher content of H_2O_2 than other plastics. On the other hand, our analysis showed that the responses of antioxidant enzymes, i.e., SOD, POD, and CAT were significant, in which the response of SOD to PS stress was stronger than PE treatments, and response level of POD and CAT was almost similar (Figure 3S-U). Moreover, the response level of AsA was significantly higher in hydroponic condition than the soil, wherein the response level of AsA to PS stress was stronger than PE stress under both exposure conditions (Figure 3V). As mentioned earlier, uptake and translocation of MPs/NPs by plants grown in hydroponics are higher than in soil environment, which induced high rate of oxidative stress and consequently increased the level of AsA. It is evident that AsA act as an enzyme cofactor, a radical scavenger and a donor/acceptor in electron transport mechanism, so it may function in minimizing oxidative stress in hydroponics more efficiently (Pignattelli et al., 2021).

3.4 Identification of sensitive parameters under different cultivated conditions

To investigate the specific response of plants to MPs under different cultural environments, we analyzed the factors that showed significant differences in the meta-analysis. The heat map (Figure 4) was used to show the specific performance of different plants under different environments, because the data used are not from the same article, it is difficult to compare the same plants in the same row, but rather in a separate row.

At high concentrations of MPs/NPs, the response level of MDA in lettuce is different between hydroponic and soil cultures. Although, MDA is the common product of lipid peroxidation in plant membrane, rice and sweet potato showed higher MDA content in the soil environment than soybean and lettuce, which may be due to the different types and sizes of MPs applied, and/or different gene traits of plants (Ekner-Grzyb et al., 2022). Under the NPs stress, the response level of both chlorophyll a and b in cucumber and lettuce plants was higher in soil culture than in hydroponics, showing that plants grown in hydroponics are more sensitive to NPs stress due to its easier uptake and translocation potential (Roy et al., 2023). Under low concentration of MPs, the Pro response in cucumber was significantly higher in soil culture than in hydroponics. In addition, Pro content in cucumber grown in soil environment responded to NPs stress is higher than in hydroponics. The lower level of Pro under hydroponic conditions may be due to the fact that the plants grown in

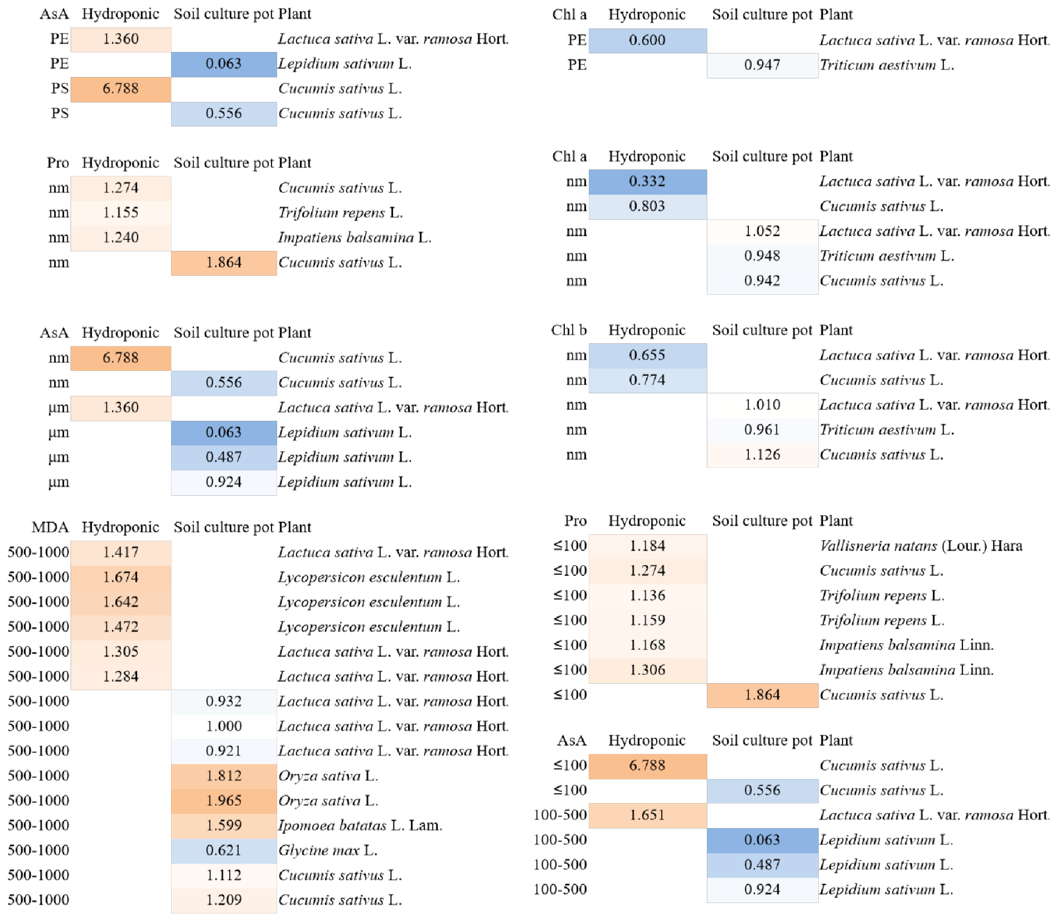

Figure 4. Identification of sensitive parameters in different cultivated conditions

hydroponics affected severely under MPs/NPs than in soil environment, and the oxidative scavenging system has been disturbed severely (Ma et al., 2022). On the other hand, response level of AsA in cucumber under PE stress in soil environment was much lower than in hydroponics. Similarly, the response level of AsA in cucumber grown in soil culture was also lower under NPs stress than that in hydroponics. Moreover, the AsA content in cucumber exposed to NPs was higher than that in lettuce and garden cress exposed to MPs. Finally, the response level of AsA was significantly higher in cucumber under low treatment of MPs compared with lettuce and garden cress (Figure 4). AsA could be directly involved in mitigating the adverse effects of MPs/NPs exposure in plants, and it is more responsive in hydroponics than soil culture environment (Khalid et al., 2020). These analyses indicate that the toxicity of MPs to plants under hydroponic environment is more severe than the soil environment, most likely due to the sizes and types of MPs/NPs used.

4 Conclusion and Future Perspectives

The plant response to MPs/NPs stress in hydroponics and soil culture was studied by meta-analysis. Although, the current analysis does not explain specific pathways and mechanism of plant response to MPs/NPs stress, however meta-analysis from previous studies related to the same research area provide valuable information, which direct future research. Results from the current meta-analysis indicated that a) different types of MPs had different effects on plants; b) NPs are more toxic than MPs; c) the response level of photosynthetic parameters was higher in soil environment; d) AsA concentration in hydroponic plants was significantly higher than soil environment; e) MPs/NPs stress is more toxic to plants grown in hydroponics than in soil environment. These conclusions give the readers a clear evidence to conduct further investigations.

Author Contributions

Writing-original draft preparation, and Data analysis Shi-Peng Li; Conceptualization, Data analysis, and Visualization Yu-Xi Feng; Supervision, Writing-reviewing and Editing, and Funding acquisition, Xiao-Zhang Yu. All of the authors contributed to the final review of the manuscript.

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Data Availability

The datasets supporting the conclusions of this article can be obtained from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no competing interests.

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