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## **Life in plastic, not always fantastic: Nano- and microplastics and their impact on terrestrial plants and the food chain**

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### **Citation**

Zantis, L. J. (2025, September 12). *Life in plastic, not always fantastic: Nano- and microplastics and their impact on terrestrial plants and the food chain.*

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## CHAPTER 7

# General Discussion

To date most research on the impact of plastic particles has focussed on aquatic environments. Yet, new research has highlighted that terrestrial ecosystems are equally vulnerable to plastic pollution. Soils are considered major sinks for nano- and microplastics (NMPs; B. Zhang et al., 2020; Sridharan et al., 2021). The widespread presence of NMPs raises important questions about their impact on terrestrial plants, and the potential transfer of plastics in the terrestrial food web. Despite the critical role plants play in terrestrial food webs and ecosystem health, research on the effects of NMPs on terrestrial plants only started in 2019 (Bosker et al., 2019). Moreover, biodegradable plastics are now often used and marketed as environmentally friendly alternatives. Nevertheless, the impact of these biodegradable plastics on plant health remains poorly understood. This thesis aims to help address this gap by investigating the effects of NMPs from conventional and biodegradable plastics on plant growth and health, as well as NMPs integration and transport within food webs. The overall objective of this thesis is to further the understanding of the following question:

**How and to what extent do nano- and microplastics impact different types of terrestrial plants and biota?**

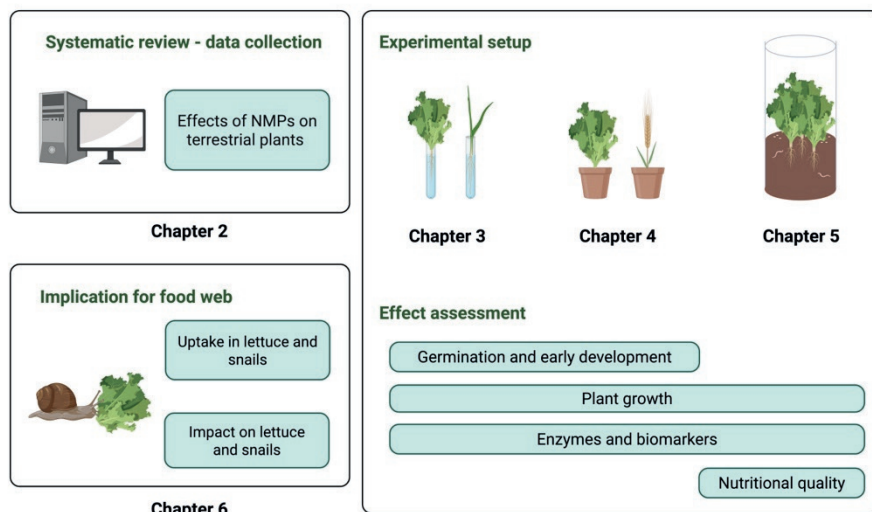
In pursuit of answering this research question, the following four sub-questions (SQ) were investigated:

1. What is the current knowledge of the effects of NMPs on terrestrial plants?
2. What are the impacts induced by different types of plastic particles on the growth and development of a variety of plant species?
3. What are the impacts of conventional and biodegradable microplastics (MPs) on lettuce in a complex environmental system?
4. To what extent are nanoplastics (NPs) being transferred along a terrestrial food chain of lettuce-snail and what are the associated impacts on these organisms?

**Section 7.1** compiles the findings of **chapters 2 to 6** to offer a general and holistic overview of the impact of NMPs on terrestrial plants (**Figure 7.1**). Rather than discussing this per sub-question, I will discuss the main findings of my thesis, which are:

- Plants are impacted by NMPs at environmentally relevant levels (SQ 1-3, *chapters 2-5*)
- Effects vary, dependent on plant species (SQ 2, *chapters 2-4*)
- Testing biodegradable plastic alternatives is important (SQ 2-3, *chapters 4-5*)
- Incorporating environmental relevance is essential when evaluating impacts (SQ 2-3, *chapters 2-5*)
- Plants can take up NPs, but transfer to snails is limited (SQ 4, *chapter 6*)

After this, in **section 7.2**, these findings will be contextualized within the framework of existing research, offering suggestions for future directions. Finally, **section 7.3** presents concluding remarks and insights derived from the overall thesis.



**Figure 7.1** Overview of the testing approaches used in the chapters in this thesis.

## 7.1 Synthesis of research questions

### 7.1.1 Plants are impacted by NMPs at environmentally relevant levels

This PhD trajectory was started by conducting a systematic review of the literature into the effects of NMPs on terrestrial plants. We showed in our review (**chapter 2**) that in all included studies adverse effects of NMPs on either germination, growth and/or biochemical markers were found. However, there was quite some variation in results, with effects ranging from positive, neutral, to negative.

Our review highlighted that seed germination of dicots is sensitive to NMP exposure, with NMPs either delaying or hindering the germination of the seed (**chapter 2**). This was also confirmed in **chapter 3** in our hydroponic setting. It is hypothesized that during germination these particles can alter water availability around seeds and create physical barriers that hinder the uptake of nutrients (Lozano et al., 2022; Srivastava et al., 2021). This in consequence could result in reduced or delayed germination rates, leading to weaker buds (Lozano et al., 2022; Srivastava et al., 2021). We also found impacts on early development of plants (**chapters 2, 3 and 4**) next to germination reductions.

Long-term effects were more diverse than those on germination and early development. For instance, plant growth responses to NMPs varied across species, ranging from positive to neutral to negative effects. Only seedling biomass and length showed consistent signs of adverse impacts (**chapter 2**). This variability in responses was also observed within our hydroponic setup (**chapter 3**) and the pot-plant setup in soil exposure (**chapter 4**). Interestingly, in **chapter 5**, where plants were exposed in more environmentally relevant settings with soil and multiple species inoculated within the test, no effect on physical growth traits of lettuce was observed. This highlights the variance in responses due to different experimental designs.

On a biochemical level, NMPs induced stress responses within the plant, impacting key biomarkers that are reflective of plant health. This was a highly consistent plant response, as observed in **chapters 2 to 5**. Plants under stress from NMPs often showed elevated levels of lipid peroxidation, and a high response in stress respondents, such as antioxidant enzymes (e.g., superoxide dismutase and catalase) which serve as defence against oxidative damage (Dumanović et al., 2021). The overproduction of these enzymes could emphasize that plants were experiencing stress. The oxidative stress can affect critical biochemical pathways, reducing photosynthesis, respiration, and growth efficiency (Dumanović et al., 2021; Ekner-Grzyb et al., 2022). Additionally, in **chapter 5**, the presence of NMPs also altered nutrient content, which further impedes quality and development.

Maybe most important, effects were commonly observed at environmentally relevant levels. Effects on germination, plant growth and enzyme and biomarker activities were commonly recorded at levels between 0.01% and 1% w/w (**chapter 2**), which are levels detected in the environment (Büks & Kaupenjohann, 2020; Vollertsen & Hansen, 2017). Especially germination and enzyme and biomarker activities were impacted at very low, realistic levels (below 0.01% w/w). This response by plants at low levels is quite unique as in most cases effects on other organisms are usually only seen at very high exposure levels (summarized for example by Science Advice for Policy by European Academies (SAPEA), 2019). Our results clearly indicated that NMPs have a wide range of adverse impacts on plants at environmentally relevant levels.

### 7.1.2 Plant species-dependent effects

Although plants are sensitive to NMPs exposure, differences between species are present. In this thesis, we were able to make this direct comparison as we studied the difference between plant species under

identical conditions allowing clear comparison, something that had been lacking in the literature. One main finding is that there was a difference between monocots and dicots for certain endpoints, such as germination. Physiological and structural differences in monocots and dicots could influence how NMPs affect growth and development. **Chapter 2** highlighted that, across most studies, seed germination and early development were more frequently adversely affected in dicots than in monocots. This was confirmed also within our hydroponic exposure setting in **chapter 3**. When exposed to polystyrene (PS) MPs, the germination rate was lowered for lettuce, while no effects were seen in carrot, wheat and barley. The most likely explanation for this difference between monocots and dicots, especially during germination, is related to seed size, since monocot seeds are usually larger than dicot seeds. Plant-contaminant interactions are thought to be facilitated by the higher surface-to-volume ratio of a small seeded species (Cañas et al., 2008). In addition, impacts on early development were more commonly noticed on dicots, with only lettuce and carrot being affected (**chapters 3 and 4**). In contrary to dicots, we noticed that the early development of monocots seemed to be less severely affected by the exposure to NMPs (**chapters 3 and 4**), which lines up with previous research (**chapter 2**).

Nevertheless, we also found variation between individual dicot species. For instance, germination of lettuce was negatively affected when exposed to PS MPs in a hydroponic setting (**chapter 3**) while no effects were observed when exposed to polyethylene (PE) or starch-polybutylene adipate terephthalate (PBAT) blend contaminated soil (**chapter 4**). In this case, plant species or exposure medium may have a moderating effect on how germination and development are impacted. Here responses to NMPs might be polymer-type dependent as different plastic types affected lettuce growth differently. Moreover, sensitivity to NMPs may be species-specific, for other reasons than species type or seed size, as lettuce was affected while carrot was not (**chapter 3**). Within and between species, the comparison between different polymers and different particle sizes remains difficult, making it hard to make generic statements across all plant species tested.

### 7.1.3 Effects induced by conventional plastic versus biodegradable plastic

Awareness towards the persistent and potential effects of conventional NMPs has led to the development of biodegradable substitutes, which are often perceived as more environmentally friendly. Biodegradable plastics are developed to break down into biodegradable NMPs and eventually dissolve via the activity of microorganisms in the natural environment

(Bandopadhyay et al., 2018; Serrano-Ruiz et al., 2021). Compared to conventional plastics, we noticed in our review that only a limited number of studies focussed on biodegradable polymers to assess their impacts on plant growth (**chapter 2**). Specifically, the direct comparison between impacts of conventional and biodegradable plastics on plant growth under identical experimental conditions remains possible only to a limited extent.

In my thesis I experimentally compared the impact of a commonly used conventional plastic mulch with a biodegradable alternative in **chapters 4 and 5**. Our findings showed that both types of plastics impacted the growth of tested crops in a pot-plant setup, but effects were more commonly observed and stronger when plants were exposed to biodegradable MPs (**chapter 4**). For example, during acute exposure, dicots were more severely impacted by the starch-PBAT blend through a reduction of the root length in lettuce and of the shoot length and bud biomass in carrot. We also observed this after long-term exposure, in which the shoot biomass of both lettuce and barley was significantly reduced by the starch-PBAT blend. In **chapter 4**, we also reviewed current studies that directly compared conventional and biodegradable plastics, noting that effects were more commonly observed when plants were exposed to starch-PBAT and/or polylactic acid (e.g., Sun et al., 2023; Yang et al., 2021) at similar concentrations as in case of conventional plastics. This highlights that adverse effects of biodegradable MPs on plant health were commonly observed under controlled conditions. Due to the limited research available, the mechanisms underlying these effects remain unclear. We hypothesize that they may result either from the presence of physical fragments that are not fully degraded or from the release and interaction of plastic-associated chemicals contained in these materials (see **section 7.2.1.1**).

Currently, we are lacking a holistic view on the effect of NMPs and especially their degradable substitutes on plant health and yield, as studies concentrate mainly on laboratory conditions. This is what we have addressed in **chapter 5**. In a mesocosm setup, we noticed over a three-month experiment that both plastic types affected the plant biochemistry. In contrast to the results from **chapter 4**, and the literature review we carried out, we saw a different response pattern in the mesocosm. The conventional PE MPs impacted biomarker endpoints more frequently, and their effects were more pronounced compared to the starch-PBAT blend MPs. Considering the characteristics of these two plastic types, effects induced by the starch-PBAT blend may be more subtle, as they might result from degraded by-products. In contrast, the effects caused by PE MPs may arise from their stable and unchanging material and

structure. Here both experimental design and exposure time seem to be important factors, suggesting that as conditions become more environmentally relevant, adverse effects may be less pronounced compared to single-species tests (see **section 7.1.4**).

There is a need to unravel the mechanisms behind the differences in impacts, especially for biodegradable plastics. For example, are these effects due to physical particles, or chemicals released during breakdown (see **section 7.2.1.1**). Overall, we require a better understanding of which components, physical or chemical, of biodegradable plastics persist compared to conventional plastics (see **Textbox II**). These comparative analyses are crucial to compare benefits and disadvantages of the biodegradable plastics versus conventional polymer-based materials.

### **Textbox II. The Unexpected Trade-offs of Biodegradable Plastics**

The debate surrounding biodegradable plastics stems from their perceived environmental benefits versus their actual impact. Biodegradable plastics offer advantages, such as reduced production costs and lower emissions compared to conventional plastics. For example, the production of polylactic acid (PLA) saves approximately two-thirds of the energy required for conventional plastics (Atiweh et al., 2021). Nevertheless, it is also important to highlight their environmental shortcomings. Their degradation often depends on specific conditions, such as certain temperatures and moisture levels, which, depending on the region, may not always be available (Chamas et al., 2020; Serrano-Ruiz et al., 2021). In natural environments like soil, water, or marine ecosystems, they may degrade more slowly or fragment into NMPs, contributing to pollution.

Additionally, the faster breakdown of biodegradable plastics can lead to the accelerated release of embedded chemicals, raising concerns about their effects on ecosystems and organisms (see **section 7.2.1.1**). The production of biodegradable plastics could also consume resources needed for food production and potentially compete with agricultural lands by redirecting land use from food crops to biodegradable plastics and bioplastic production (Atiweh et al., 2021; Nizamuddin et al., 2024). More work is therefore needed to assess the impacts of biodegradable plastics through a balanced approach, including improved materials, clearer guidelines for use and disposal, and waste management systems.

### 7.1.4 Need for environmental relevance and realism in studies

Ensuring environmental relevance in studies is essential for generating reliable data that could inform risk assessments. This relevance can be enhanced by selecting appropriate experimental designs, using relevant plastic materials, or testing relevant exposure concentrations. Through **chapter 2**, we noticed that most studies investigating the impacts of NMPs on terrestrial plants have been performed in the laboratory, while only a subset of studies was executed in greenhouse settings. This makes sense when doing initial screening, as these experiments are often small-scale, fast and relatively cheap. Nevertheless, the only field study identified in **chapter 2** highlights that essential factors, such as weather conditions or interaction with soil organisms, are important parameters which might influence the impacts. In addition, the most tested polymers in the laboratory tests were PS and PE particles, often in a pristine form. These do not accurately reflect the shapes and types of particles typically found in soils, nor do they allow for meaningful extrapolation to the effects of weathered particles with varying morphologies present in real-world conditions. This highlighted the need for more environmental relevant conditions and tested particles, which we addressed in **chapters 4 and 5**.

Across the research chapters, we addressed five key aspects to build a comprehensive understanding of NMP impacts on plants. Regarding the polymers tested, we shifted from pristine PS particles in **chapter 3** to MPs derived from plastic mulch commonly used in agriculture in **chapters 4 and 5**, which better reflect real-world conditions. Moreover, we directly compared conventional and biodegradable particles. We tested four plant species with physiological differences to capture species-specific responses as effects induced by NMPs might not be the same for all species due to differences in physiology and development (see **section 7.1.2**). To evaluate plant health and toxicity mechanisms, we assessed physical traits, biochemical markers, and nutritional indicators, which are essential for both plant health and nutritious quality within the food chain. Our experiments transitioned from hydroponic setups to pot-plant experiments in soil, incorporating agricultural soil conditions and interactions with soil invertebrates for greater environmental realism. Additionally, we examined both short-term (acute) and long-term (chronic) impacts, with experiments lasting up to three months, to capture immediate responses and cumulative effects on plant development.

Even though we noticed similar impacts induced by NMPs in **chapters 3, 4 and 5**, especially on the plant's biochemistry, striving for

environmentally realistic conditions remains crucial. Controlled laboratory experiments may provide insights into potential mechanisms, but they often simplify complex environmental interactions. Environmental realism ensures that findings account for factors such as interactions with other organisms, and long-term exposure (see **section 7.2.1.3**), which can influence the behaviour and degradation of NMPs. These conditions also improve the relevance of results for agricultural settings, enhancing the accuracy of risk assessments and the development of mitigation strategies that reflect actual environmental scenarios to ensure food safety and quality.

### 7.1.5 Uptake of NMPs in the food web and their impacts

Testing NMPs in agricultural settings and if they are prone to penetrate plants and further up into the food chain is a scientific and societal relevant question. As determined in **chapter 6**, lettuce seedlings exposed to NPs can absorb NPs through their roots. Translocation can occur upwards towards the leaves of the plant through their transport network. The uptake by plant roots and distribution across the shoots was also observed by other studies on lettuce (L. Li et al., 2020; Y. Li et al., 2023a), cucumber (Z. Li et al., 2021), mung bean (*Vigna radiata*; Chae & An, 2020) and wheat (L. Li et al., 2020). As plants are at the base of many food webs, the presence of NMPs in edible crops raises concerns about potential consumer exposure to these particles via their diet. Particularly, the particles accumulated in plant tissue could be passed on to herbivores and, subsequently, to higher trophic levels, including humans. This is why we created a food-chain study by feeding NPs-contaminated lettuce to snails (*Cantareus aspersus*; **chapter 6**). These snails were selected as they are also of culinary significance for humans. As indicated, we found uptake of the particles in the roots, and translocation to the shoots of lettuce. However, when we fed the leaves containing NPs to snails, we did not find trophic transfer from lettuce to snails, as NPs were found to be present only in the feces but not detected within the digestive gland. This finding differs from the findings of Y. Li et al. (2023b) who identified NPs within the digestive gland of snails at exposure concentrations 10 to 100 times higher than those used in our study. Nevertheless, Y. Li et al. (2023b) also observed that most of NPs were excreted in the feces, and only a small proportion was traced in the digestive gland and transported to the soft tissue. These results show that the transfer to organs is limited, even at high exposure levels. In lettuce, NPs can reach the roots without crossing biological barriers or membranes, whereas in snails, uptake occurs only through ingestion.

Even though we did not find any trophic transfer from the lettuce to snails in our study, we observed that the indirect exposure to NPs adversely affected the snails. The growth rate of the snail shell was lower in snails ingesting contaminated lettuce compared to snails fed with lettuce grown without NPs. The slower growth was observed with mung bean (Chae & An, 2020) or lettuce (Y. Li et al., 2023b) and the African giant snail (*Achatina fulica*). This shows that shell diameter might be a sensitive endpoint, though it remains unclear how the snails are affected. Two hypotheses might explain this difference in growth. The first is related to chemicals present in NPs that might leach from these NPs inside the snails and then interact with cells to induce effects on a molecular level (Rodrigues et al., 2023). Until now, very little is known about these chemicals leaching of, and their interaction with organisms, as it is a very diverse group of contaminants (Wagner et al., 2024; see **section 7.2.1.1**). A second hypothesis is that the NPs might change the nutritional value of lettuce. It is possible that the particles are interacting with the micro- and/or macronutrients in the lettuce, causing disruptions to physiological processes. As we showed in **chapter 5**, the nutritional value of lettuce was negatively reduced after exposure to NMPs. These two hypotheses still need to be tested in future experiments. Addressing the uptake and accumulation of NMPs in plants and food chains are critical for understanding and mitigating their potential impacts on organism health and food security.

## 7.2 Future perspectives and implications

### 7.2.1 Focus for future studies

#### *7.2.1.1 What about plastic chemical compounds?*

Plastics often contain a range of chemicals to enhance their properties, such as plasticizers, stabilizers, flame retardants, and colorants. In the latest report of the United Nations Environment Programme, over 13,000 substances have been linked to plastics, either as components in plastic production or as contaminants detected in plastic materials (United Nations Environment Programme, 2023). Of concern is that more than 4,200 of these plastic chemicals are hazardous to both the environment and human health (Wagner et al., 2024). Although these chemicals enhance the durability and functionality of plastics, they can also pose significant risks when they leach into the environment.

These chemicals are intentionally added to modify the physiochemical properties of plastics to enhance their functions (Costa et al., 2023). However, the degradation of plastics in the environment can result in the release of chemicals into ecosystems. For example, plastics additives have

already been recorded in agriculture soils where plastic mulch has been used (Y. Xu et al., 2024). Several studies have explored the effects of leached additives on plants. H. Wang et al. (2024) compared the effects of leachate, leached polyvinyl chloride (PVC) MPs and unleached PVC particles on wheat. The unleached MPs caused greater negative impacts on root structure than the leachate alone or the leached PVC MPs, indicating a synergistic effect between the chemical toxicity of the leachate and the physical damage from the particles (H. Wang et al., 2024). In addition, biodegradable plastic, seen as a more sustainable option, should be investigated as these also contain various added plastic chemicals (Aznar et al., 2019; Zimmermann et al., 2020). Notably Macan et al. (2024) found that biodegradable PBAT-based mulch films released a higher concentration of chemical compounds, while low-density PE films liberated a wider variety of chemicals. This could be attributed to the faster breakdown of biodegradable plastics into smaller particles, leading to a faster release of chemicals into the environment, highlighting a trade-off associated with these less persistent materials.

Addressing the issue of compounds leaching from NMPs is complex. We are currently investigating how chemicals leaching from both conventional and biodegradable plastics influence plant growth and performance within a pot-plant setup. It is also important to assess the kinetics of leaching, as these vary for different chemicals and are influenced by factors such as different polymer properties or their 3D structure (Y. Li et al., 2024; L. Liu et al., 2022). These are important parts to find missing pieces to the puzzle to get a full comprehensive understanding on how NMPs, physically and/or chemically, affect plants also by testing for example different plastics and/or plant species.

### *7.2.1.2 Combining stressors to simulate real world conditions*

The terrestrial environment is a complex and dynamic system where plants and organisms are exposed to multiple and interacting stressors, including pollutants, temperature fluctuations, drought, and others. Within **chapter 2**, we noted only limited numbers of studies on joint toxicity of NMPs with other stressors, ranging from a combination of several different pollutants to the combined impacts of NMPs and environmental stress (e.g., drought or heat). In the environment, plants rarely face single stressors, as these stressors hardly act in isolation. Instead, these stressors interact in ways that can amplify or mitigate their individual effects, creating unique responses that differ from those seen under single-stressor conditions (Orr et al., 2020).

To improve the relevance of experimental studies, it is essential to replicate these complex conditions by incorporating combinations of stressors. Here we can differentiate between chemical stress on stress (e.g., pesticides, metals), and environmental stressors (e.g., drought, flooding). On the one hand, agriculture fields are exposed to a range of pollutants beyond plastics, such as pesticides, herbicides, fertilizers or heavy metals (Angon et al., 2024). Focusing specifically on heavy metals, recent studies have for instance observed the interactive effect of NMPs and cadmium, showing that combined exposure can cause more severe harm to plants than exposure to each pollutant individually (Iqbal et al., 2023; F. Wang et al., 2020a). Wheat seedlings exposed to both MPs and cadmium were observed to have a lower root area and also a reduced leaf gas exchange compared to the single treatment of each stressor (Iqbal et al., 2023). Interestingly, the single treatment of MPs resulted in the least negative impacts compared to cadmium and the combination of both stressors (Iqbal et al., 2023). Next to pollutants, agricultural fields are also exposed to numerous environmental conditions, such as drought, flooding, extreme temperatures, or soil erosion, which can reduce crop resilience and yield (Kumar et al., 2022). For example, Khan et al. (2024) investigated individual and combined effects of MPs and drought on rice. They observed that the combination of both resulted in the lowest grain yield and in negative impacts on physiological processes. Investigating these combined stressors is crucial, especially as changing environmental conditions from climate change intensify stressors on ecosystems. Another option is to first expose the plant to one stressor, followed by the exposure to a second, allowing for the evaluation of cumulative or sequential effects on plant health. The asynchronous stressors can result in more severe effects on organisms' health and community (e.g., García-Astillero et al., 2024). Overall, by mimicking these multifaceted interactions, studies can offer insights into cumulative impacts, as well as threshold levels and adaptation strategies of plants.

### *7.2.1.3 Linking laboratory studies to field studies*

Bridging the gap between laboratory studies and field research is crucial for understanding how controlled findings translate to real-world agricultural conditions (**Figure 7.2**). Our review (**chapter 2**), along with findings from **chapters 3 to 5**, revealed significant variation in results due to the diverse approaches used across studies. These studies assessed NMP impacts using a wide range of endpoints and methodologies, with substantial differences in study duration and exposure media. Furthermore, no clear dose-response relationship was evident from our findings from **chapters 3 to 5**. Within individual studies, effects were sometimes observed only at specific concentrations, or responses varied

inconsistently across different concentrations. These observations underscore the challenge of identifying consistent trends across studies and emphasize the need to address this variability in future research (see **Textbox III**).

### **Textbox III. Addressing High Variation in Responses**

Within **chapters 3 to 5**, we observed a high variance in results depending on the type of plastic tested, the species used, or the exposure medium. Our systematic review (**chapter 2**) highlighted that even under highly controlled experimental conditions, significant variability in responses persists. This pattern is also evident in our H2020 PAPILLONS project, not only with plants but also with soil properties and invertebrates, prompting the question of how to effectively address this variation.

For example, within mesocosm studies, we tested the same concentration of starch-PBAT blend MPs (0.8% w/w) on lettuce (**chapter 5**) in the same CLIMECS facility as Adamczyk et al. (2024). In contrast to our study in which we did not find any effects on growth parameters, Adamczyk et al. (2024) identified a reduction in shoot height and biomass of lettuce plants. This emphasizes that variations in responses can arise even under identical experimental settings and conditions.

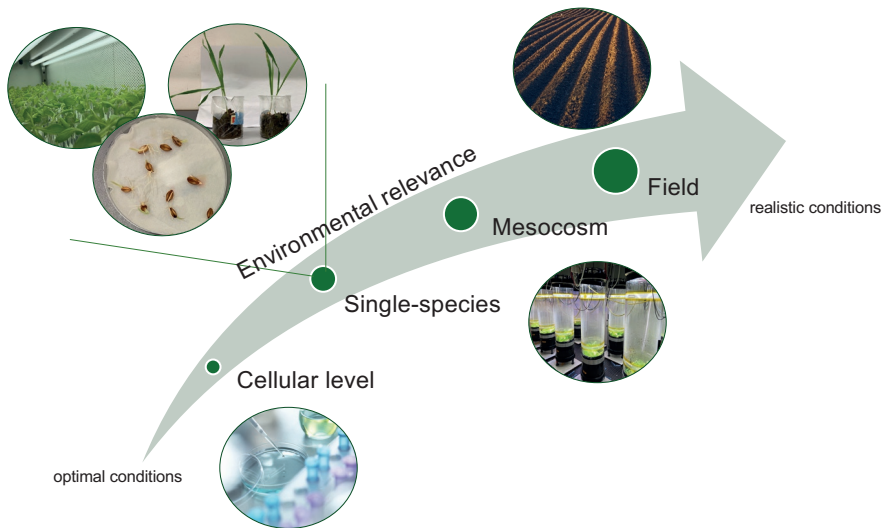
One potential approach is to examine general trends through a systematic literature review, as we did in **chapter 2**. This method allows for the identification and integration of relevant studies to grasp a comprehensive understanding of the broader topic. However, merging information in this way may risk overlooking specific responses and patterns. Interestingly, such variation in responses does not appear to be as prevalent with other contaminants, like pesticides or heavy metals (Alengebawy et al., 2021; Geiger et al., 2010), needing deeper exploration into the factors underlying these differences.

In conclusion, this remains a challenging topic that needs to be addressed in future studies to develop a more comprehensive understanding on NMP impacts on plants.

To address these challenges, we propose several directions for future research. First, increased standardization of testing protocols and more realistic exposure conditions are needed to improve consistency and enable the detection of effect sizes. Many experiments lack testing under realistic environmental conditions. Laboratory experiments, while

valuable for isolating specific variables like NMPs, provide only limited insights into how plants respond to complex, multi-factorial stresses. Mesocosm studies, such as those conducted in **chapter 5**, serve as an intermediate step, simulating real-world conditions within controlled environments (Auffan et al., 2019). However, field experiments remain the most relevant for capturing the complexity of natural conditions, where plants face interacting stressors such as variable weather conditions and diverse soil properties. Second, using environmentally relevant NMPs is crucial, as these particles prevail in natural settings and typically consist of mixed polymer types. Expanding the physico-chemical diversity of particles tested will better reflect real-world scenarios. Third, more research is needed on the impacts of leached chemicals and combined stressors on plant performance, an area we aimed to address in **sections 7.2.1.1 and 7.2.1.2** as this remains a significant gap in the field.

Overall, most studies to date have been conducted under highly controlled conditions. The key question is whether these findings can be extrapolated to environmentally realistic conditions and, if so, how to translate them to inform field-relevant scenarios effectively.



**Figure 7.2** Ascending environmental relevance in different study designs going from optimal, controlled to realistic, un-controlled settings.

## 7.2.2 Evaluating risks and navigating decisions

Another challenge that needs to be navigated is comparing potential adverse effects from the use of mulching films, be it biodegradable or conventional, with other management techniques. To illustrate the challenge of decision-making, I will present a case study featuring three different crop management scenarios, all intended to manage weeds: the use of conventional plastic mulch (1), the use of biodegradable plastic mulch (2), and the application of pesticides without plastic mulch (3) in agricultural practices (**Figure 7.3**). Each of these approaches have their own advantages and disadvantages, which I will compare below. Importantly, this is not an exhaustive exercise, but just to illustrate the complexity of decision making.



**Figure 7.3** Scenario-based case study of 1) the use of conventional plastic mulch, 2) the use of biodegradable plastic mulch and 3) the use of pesticides instead of plastic mulch in an agricultural setting.

The use of conventional plastic mulch, usually PE, is highly effective for reducing and eliminating weeds and controlling moisture in the soil, especially within colder climates (Khalid et al., 2023; McIntosh et al., 2022). This mulch is designed for outdoor use, ensuring durability over multiple seasons. However, it poses concerns, as it creates more plastic waste, especially when it breaks down into NMPs, requires labour intensive disposal, and as we saw in **chapters 4 and 5** has potential adverse impacts on crop development and quality. Biodegradable plastics compared to PE mulch, offer comparable benefits in weed control and moisture retention while eliminating the need to be removed at the end of the season, as they are expected to degrade naturally (Khalid et al., 2023). Yet, biodegradable plastics are often more expensive than conventional plastics (Khalid et al., 2023). In addition, degradation of these materials does not always or completely occur in certain regions as there are discrepancies between tested and real-life conditions (Miles et al., 2017; Serrano-Ruiz et al., 2021). Moreover, as we saw in **chapter 4**

**and 5**, biodegradable MPs induce potential impacts on plant health and development. Finally, in scenario 3, choosing to use no plastic mulch and solely relying on pesticides, reduces plastic waste and material costs. However, this approach results in costs for weed management and a greater water loss in the soil due to the absence of plastic mulch. In addition, the increased use of pesticides and herbicides can affect other soil organisms and impact crop growth and quality (Gunstone et al., 2021; W. Zhou et al., 2024).

Overall, each approach has trade-offs, with the best choice depending on the environmental and economic priorities. There are good tools available to determine the best approaches, such as Life Cycle Assessment (LCA), which can help analyse the production chains of different types of plastics (e.g., Schwarz et al., 2024). However, weighing all the different impact categories requires a lot of data. Studies like ours contribute to this effort by providing valuable data (**chapters 4 and 5**) and examining the balance between short- and long-term impacts (e.g., **chapters 3 and 4**; Steinmetz et al., 2016).

### 7.3 Conclusions

Within this thesis, I aimed to address how NMPs influence plants, and in addition how NMPs can move into our food basket. Throughout our research, we demonstrated that plants are impacted by NMPs at different growth stages, and that effects by NMPs might be species-dependent, often with dicot species being more sensitive. However, the impact on a molecular level, particularly in terms of stress responses, remains consistent across plant species. Importantly, effects are often seen at environmentally relevant concentrations. Additionally, both conventional plastic and biodegradable alternatives showed to induce adverse impacts on plants. We found that experimental design and exposure duration are key factors influencing the observed effects. Across the chapters, the importance of considering environmental relevance and long-term ecological impacts became evident. Alongside this, the uptake of NPs by plants raises concern but trophic transfer from plants to organisms seems to be limited. Overall, these findings open new avenues for future research, as uncertainties persist regarding the effects of plastic chemicals and joint toxicity in the environment.