Knowledge gaps in microplastics research
- wastewater treatment plant sludge and application on farm lands

This work is done for

The Danish Environmental Protection Agency, Ministry of Environment and Food of Denmark
(Miljøstyrelsen, Miljø- og Fødevareministeriet)
During November and December 2019

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This report is written as the output of the project:

*Kortlægning af videnshuller omkring mikroplast, udarbejdelse af handlingsplan for videre arbejde med opbygning af viden omkring mikroplasts forekomst, skæbne og effekt i spildevandsslam i forbindelse med udspredning på landbrugsjorde, samt nabotjek vedrørende status på vidensopbygning.*

By Jes Vollertsen, Alvise Vianello, and Claudia Lorenz, Aalborg University

And finalized the 20th of December 2019

The authors want to thank Nanna B. Hartmann from DTU Environment for her kind support and great feedback on the work.
Mapping of knowledge gaps

Background

The awareness of plastic pollution is quite recent, and was initially focused on plastics in the oceans. The beginning of this awareness is commonly dated back to the 2004 Science article of Richard C. Thompson et al. ‘Lost at Sea: Where Is All the Plastic?’. It was also in this paper that the term ‘microplastics’ was coined. A search on scientific articles containing the term ‘microplastics’ in the title or abstract illustrates that not much happened for quite some years after Thompson et al. published their article. The topic was quite dormant till around 2013-2015, where the interest in this topic took off – both with respect to scientific publications (Figure 1) and the public interest in the topic (Figure 2).

Figure 1. Scientific publications with the search term ‘microplastics’ in the title, abstract, or keywords. The search is done using the Web Of Science website.

Figure 2. Google web searches for the term ‘microplastics’. Note that this search engine was improved in 2016 and that data before this is somewhat inaccurate. “Numbers represent search interest relative to the highest point on the chart for the given region and time. A value of 100 is the peak popularity for the term” (Google Trends, 2019)

1 A minor fraction of the publications found by searching for the term ‘microplastics’ is unrelated to what we today understand by this term, hence the publication intensity in the last decade is slightly over-estimated
2 Web of Science (previously known as Web of Knowledge) is a website which provides subscription-based access to multiple databases that provide comprehensive citation data for many different academic disciplines. It is widely recognized as the leading database for international peer-reviewed scientific publications of high scientific quality.
**General knowledge gaps in microplastics**

In the period from 2004 to 2013, the vast majority of published articles had a marine focus. The main research areas addressed were ecology, biology, toxicology and oceanography. This trend has continued into the ‘bloom’ of microplastics research in the second half of the current decade, albeit with some widening of the addressed topics (Table 1). Out of the 2226 publications published in 2014-2019, three quarters (1581 publications) addressed marine microplastics either fully or to some degree.

Table 1. Scientific articles and the research areas in which they were published. The search is done using Web Of Science.

<table>
<thead>
<tr>
<th>Research Areas</th>
<th>2004-2013 records</th>
<th>% of 75</th>
<th>2014-2019 records</th>
<th>% of 2229</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENVIRONMENTAL SCIENCES ECOLOGY</td>
<td>52</td>
<td>69.3</td>
<td>1635</td>
<td>73.4</td>
</tr>
<tr>
<td>MARINE FRESHWATER BIOLOGY</td>
<td>38</td>
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<td>543</td>
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<tr>
<td>ENGINEERING</td>
<td>9</td>
<td>12.0</td>
<td>303</td>
<td>13.6</td>
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<tr>
<td>CHEMISTRY</td>
<td>8</td>
<td>10.7</td>
<td>216</td>
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</tr>
<tr>
<td>SCIENCE TECHNOLOGY OTHER TOPICS</td>
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<td>4.0</td>
<td>186</td>
<td>8.3</td>
</tr>
<tr>
<td>TOXICOLOGY</td>
<td>7</td>
<td>9.3</td>
<td>151</td>
<td>6.8</td>
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<tr>
<td>WATER RESOURCES</td>
<td>2</td>
<td>2.7</td>
<td>112</td>
<td>5.0</td>
</tr>
<tr>
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<tr>
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<td>52</td>
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</tr>
<tr>
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<td>5.3</td>
<td>46</td>
<td>2.1</td>
</tr>
<tr>
<td>SPECTROSCOPY</td>
<td>34</td>
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<td>1.5</td>
<td></td>
</tr>
<tr>
<td>BIOCHEMISTRY MOLECULAR BIOLOGY</td>
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<td></td>
<td>33</td>
<td>1.5</td>
</tr>
<tr>
<td>FISHERIES</td>
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<td>1.3</td>
<td>25</td>
<td>1.1</td>
</tr>
<tr>
<td>MATERIALS SCIENCE</td>
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<td>2.7</td>
<td>25</td>
<td>1.1</td>
</tr>
<tr>
<td>METEOROLOGY ATMOSPHERIC SCIENCES</td>
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<td>2.7</td>
<td>21</td>
<td>0.9</td>
</tr>
<tr>
<td>GEOLOGY</td>
<td></td>
<td></td>
<td>20</td>
<td>0.9</td>
</tr>
<tr>
<td>MICROBIOLOGY</td>
<td>1</td>
<td>1.3</td>
<td>19</td>
<td>0.9</td>
</tr>
<tr>
<td>BIOTECHNOLOGY APPLIED MICROBIOLOGY</td>
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<td>1.3</td>
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</tr>
<tr>
<td>POLYMER SCIENCE</td>
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<td>1.3</td>
<td>16</td>
<td>0.7</td>
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<tr>
<td>PHYSICS</td>
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<td></td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>ENERGY FUELS</td>
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<td></td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>INTERNATIONAL RELATIONS</td>
<td></td>
<td></td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>ZOOLOGY</td>
<td>4</td>
<td>5.3</td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>AGRICULTURE</td>
<td></td>
<td></td>
<td>9</td>
<td>0.4</td>
</tr>
<tr>
<td>PHARMACOLOGY PHARMACY</td>
<td>9</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

Note that only 9 articles fall into the research area category of ‘agriculture’. Looking closer at these, one sees that the first was published in January 2018 and addressed interactions between microplastics and a species of springtails (*Collembola*). Two articles were rejoinders to that article, two were not really addressing agriculture but seem to be incorrectly categorized. One was a discussion paper, not presenting new data. One of the remaining articles addressed biological impacts on springtails, one addressed the distribution of microplastics on coastal beaches, and the last addressed interactions between glyphosate and microplastics. All in all this highlights that experimental studies on microplastics in soils and agriculture are very few compared to other fields in which microplastics have been studied.

The observation presented above is further corroborated by for example the review of Akdogan and Guven (2019) who went through over 200 papers involving microplastic pollution, published between 2006 and 2018. They concluded that ‘**whilst marine microplastics have received substantial scientific research, the**

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3 Searches were done by end of November 2019. Each day new articles are published on microplastics, and the actual numbers will hence change continuously.
4 Web Of Science search for the keywords ‘microplastics’ and ‘marine’
5 The incorrect categorization is caused by it not being the articles which are categorized individually by Web of Science, but the journals which are assigned a category. Hence, when an article on water goes into a ‘soil journal’, it will be miscategorized.
The extent of microplastic pollution in continental environments, such as rivers, lakes, soil and air, and environmental interactions, remains poorly understood. They found that 54% of the studies addressed the marine environment, 18% addressed estuaries, rivers and lakes were the focus of 11% each, while the atmosphere was addressed by 2%, and soil systems were addressed by 4% of the papers. Of the papers, more than half addressed occurrence and characterization of microplastic particles, followed by papers addressing ecotoxicity, and finally fate and transport.

There are quite some scientific publications addressing which are the knowledge gaps in the field. A Web Of Science search containing the keywords ‘microplastics knowledge gaps’ revealed 70 such publications, hereof 33 review articles. All recent articles (since 2017) were checked for their opinion on knowledge gaps. The opinions on what are knowledge gaps varies, probably based on the field of interest of the researchers stating the gaps. In general, the consensus is that there are substantial knowledge gaps related to:

<table>
<thead>
<tr>
<th>Area</th>
<th>Knowledge Gaps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysing and reporting of microplastics</td>
<td>Accurate and cheap methods to collect, process and analyse microplastics in natural samples.</td>
</tr>
<tr>
<td></td>
<td>The smaller microplastics sizes (below roughly 300-500 µm) cause problems, and quantitative analysis of microplastics below 10 µm is still not possible in natural matrixes.</td>
</tr>
<tr>
<td></td>
<td>Car tire wear particles cause severe analytical problems.</td>
</tr>
<tr>
<td></td>
<td>Recognized quality control of analytical methods, here amongst standards for inter-laboratory comparison are lacking</td>
</tr>
<tr>
<td></td>
<td>Standardization of analytical methods are lacking</td>
</tr>
<tr>
<td></td>
<td>Standardized classification and quantification systems for macro- and microplastics with respect to composition, size, shape and other parameters.</td>
</tr>
<tr>
<td></td>
<td>No methods exist on quantification of nanoplastics in environmental matrixes.</td>
</tr>
<tr>
<td>Microplastics in the marine environment</td>
<td>Vertical distribution of microplastics in the water column</td>
</tr>
<tr>
<td></td>
<td>Processes leading to sedimentation of microplastics, and the related sedimentation rates. Which processes cause low density plastics to sink?</td>
</tr>
<tr>
<td></td>
<td>Interactions between biota and microplastics at environmentally realistic concentrations</td>
</tr>
<tr>
<td></td>
<td>Weathering of plastics, and the related change in distribution</td>
</tr>
<tr>
<td>Microplastics in the freshwater environment</td>
<td>Occurrence and fate of microplastics in freshwater bodies</td>
</tr>
<tr>
<td></td>
<td>Transport from land to sea via rivers – water and sediments</td>
</tr>
<tr>
<td>Microplastics in the terrestrial environment</td>
<td>Occurrence of microplastics in the terrestrial environments</td>
</tr>
<tr>
<td></td>
<td>Vertical transport of microplastics in soils – the pathway to the groundwater</td>
</tr>
<tr>
<td></td>
<td>Microplastics in rural surface runoff</td>
</tr>
<tr>
<td></td>
<td>What parameters determine the mobility of microplastics (and nanoplastics) in soil?</td>
</tr>
<tr>
<td>Microplastics in the atmospheric environment</td>
<td>Sources for microplastics in the air</td>
</tr>
<tr>
<td></td>
<td>Dry, occult and wet atmospheric deposition under different conditions and at different locations</td>
</tr>
<tr>
<td></td>
<td>Long-distance spread of microplastics via the atmosphere</td>
</tr>
<tr>
<td>Microplastics in groundwater</td>
<td>Is microplastics (or nanoplastics) present in the groundwater?</td>
</tr>
<tr>
<td></td>
<td>Can microplastics (or nanoplastics) reach the groundwater?</td>
</tr>
<tr>
<td>Fragmentation and degradation of microplastics</td>
<td>Fragmentation and degradation of macroplastics to microplastics in aquatic systems – rates and processes</td>
</tr>
<tr>
<td></td>
<td>Fragmentation and degradation of macroplastics to microplastics in terrestrial systems – rates and processes</td>
</tr>
<tr>
<td></td>
<td>Fragmentation and degradation of macroplastics to microplastics in technical systems – rates and processes</td>
</tr>
<tr>
<td></td>
<td>Chemical, biological and physical decomposition of microplastics</td>
</tr>
<tr>
<td>Environmental toxicity of microplastics</td>
<td>Uptake and excretion of microplastics by biota in water, sediment and soil</td>
</tr>
<tr>
<td></td>
<td>Microplastics as a vector for toxic chemicals originally contained in the plastics</td>
</tr>
</tbody>
</table>
Microplastics in soils
In general there is very little research on microplastics in soils, be it in terms of quantification methods, occurrence in soils, or the impacts on terrestrial ecosystems. This has been pointed out in numerous publications, for example Chae and An (2018). Many of these publications have also pointed out that agricultural sewage sludge application is one pathway for microplastics to the soil systems. However, actual quantification here of is very limited.

1. Occurrence of microplastics in agricultural soil
Literature on microplastics from wastewater sludge on agricultural soil is quite limited, and all relevant studies which the authors are aware of are hence discussed below.

Ljung et al. (2018) reports the occurrence of microplastics in the sludge of a Swedish wastewater treatment plant (WWTP) (Sjölunda, Malmö) and the microplastics in the soils of an agricultural test field ‘Fältförsök Petersborg, Malmö’, which had received sludge from Sjölunda since 1981. One set of fields had received 3 ton dry matter per hectare and year, one had received 1 ton, and one had not received any sludge. The amount of sludge that today is brought out on agricultural fields in Sweden is about 0.5 ton per year. All
microplastics sampling and analysis was carried out by Aalborg University and reported as both particle number and particle mass down to 10 µm using state-of-the-art µFTIR imaging.

An elevated amount of microplastics was found in the fields with the highest load of sludge. The fields that had received the lower load had concentrations similar to the fields that had not received sludge. Concentrations were 0.3 mg/kg TS (total solids) for fields without sludge, 0.34 mg/kg TS for fields with 1 ton/year and 3.4 mg/kg TS for fields with 3 ton/year. Even though the fields with the lower sludge load of 1 ton/year had similar concentrations than the fields without sludge, a clear ‘fingerprint’ in terms of a more varied polymer composition compared to the fields without sludge was observed. In addition it was attempted to close the mass balance for the test fields by assuming that the sludge sent to the field since 1981 contained the same amount of microplastics as was measured in the Sjölunda sludge during the year of the investigations. This showed that only a small fraction of the microplastics brought out over the years could actually be recovered from the soil of the fields. Where the missing microplastics went is unknown, but could be fragmentation into smaller particles which could not be detected by the applied method, it could be degradation and mineralisation, it could be a loss of the smaller particles into the soil column and ultimately to the groundwater, or it could be that the analytical methods were too inaccurate to yield a trustworthy mass balance.

Corradini et al. (2019) investigated microplastic accumulation in agricultural soils from sewage sludge disposal. They evaluated 31 agricultural fields with different sludge application records and similar soil and land use conditions. The sludge application records covered a period of 10 years, where the fields received different numbers of sludge application. One application was in all cases 40 ton dry matter per hectare. The analytical method was simple stereo microscopy and manual sorting. No verification of the identified particles was made to ensure that particles were really of plastics. The applied method is not very sophisticated nor precise, but will still allow comparison of similar systems for microplastics down to roughly 300-500 µm. Masses were estimated from particle sizes. The results in terms of particle numbers and masses found in the fields showed that the content of recovered plastics increased with the number of applications the fields had received and that microplastics hence seemed to accumulate in the studied fields. In terms of microplastic mass the increase was however not proportional to the amount of sludge applied. A 5-times higher application of sludge only gave rise to a doubling in microplastic mass.

While this study was rather comprehensive in terms of the fields investigated, the analytical methods applied were rather unsophisticated, and the increase in concentrations not that large. It is hence somewhat uncertain how valid this study actually is.

Piehl et al. (2018) studied the amount of plastic particles down to 2 mm on a field that had not received any sludge, and where microplastic-containing fertilizers and agricultural plastic applications were never used. They found around 200 particles >2 mm per hectare and concluded that even fields under conventional agricultural use contained plastic particles.

Concluding remarks on the occurrence of microplastics in agricultural soil
Wrapping up the above discussion it is quite clear that we know very little indeed about the occurrence of microplastics in agricultural soils and how it is affected by spreading of wastewater sludge, or for that matter, other forms of biological waste products. Many research gaps exist in this context, the most basic of which is that we do not actually know how much microplastics is generally present in agricultural soils. We do not know if the microplastics concentration levels are affected by the spreading of wastewater treatment plant sludge. We do not know to what degree microplastics are broken down in agricultural fields. We do not know how the microplastics get to the soils – is it mainly agricultural activities, is it a related to long-distance atmospheric deposition, or is it mainly wind-borne transport of macroplastics.
which then degrades to microplastics? Another major knowledge gap is that we do not know the spatial variability of microplastics in a field. While this in some ways may sound trivial, it is crucial knowledge for designing monitoring campaigns as it governs where, how and how much to sample for microplastics.

2. Microplastics in sludge from wastewater treatment plants

The study of Ljung et al. (2018) discussed above also reports the occurrence of microplastics in the inlet and outlet of Sjölunda wastewater treatment plant (WWTP) and in the sludge from this plant. The sludge was a composite sample covering one year. The wastewater inlet and outlet was sampled during two campaigns in 2017. Assuming that the difference in microplastics in the inlet and outlet would have entered the anaerobic digester tank, the study showed that around 60% of the microplastics was unaccounted for. It could not be recovered in the digested sludge. Whether this discrepancy was due to degradation and breakdown of microplastics in the digester, an artefact of statistical uncertainty, or due to the fact that it is more difficult to measure microplastics in digested sludge than in wastewater is unknown.

In terms of concentrations, the Sjölunda digested sludge contained 420 mg/kg TS of microplastics. At a volatile solids content of 62.6% this corresponded to 0.07% of the organic matter in the sludge was microplastics.

Dierkes et al. (2019) quantified microplastics in sludge and other samples via pressurized liquid extraction and pyrolysis gas chromatography mass spectrometry (pyrolysis GC-MS). They were able to analyse for three common polymer types, polyethylene (PE), polypropylene (PP), and polystyrene (PS) and found around 4 g/kg TS from two wastewater treatment plants. This is higher than what Ljung et al. (2018) found with µFTIR imaging. Here it needs to be mentioned, that the sum of PP, PE, and PS in Ljung et al. accounted for approx. half of all plastics in the samples. This taken into account, Dierkes et al. found around 20 times higher plastics concentrations than did Ljung et al. What the reason is for this difference is unknown, but most likely leads back to differences in the analytical approaches.

Li et al. (2018) analysed microplastics in 79 sewage sludge samples from 28 Chinese treatment plants. They found 23,000 particles per kilogram of dry sludge based on a manual sorting approach using a stereo microscope followed by FTIR-based quantification of the polymer type of the identified particles. They did this down to sizes of 37 µm, however, realistically speaking this approach only gives reliable results down to roughly 300-500 µm. Below this the approach of manual sorting is highly biased towards underestimating the number of particles. They also reported other studies analysing for microplastics in sludge applying similar but not identical approaches and reported particle number concentrations to vary some two orders of magnitude between these studies. All in all it is hence rather difficult to say how much microplastics is actually in sludge based on these studies.

Simon et al. (2018) investigated 10 Danish wastewater treatment plants, together treating around one quarter of all Danish wastewater. They found that the treatment plants were very efficient in retaining microplastics. The microplastics in the raw wastewater constituted 0.055-0.13% of the wastewater suspended solids. They estimated that Danish treatment plants on an annual basis received 191 ton of plastics of which the 3 ton were released to the environment, and 188 ton captured in the sludge.

Numerous other studies have quantified microplastics removal efficiency in wastewater treatment plants, for example Xu et al. (2019) and Blair et al. (2019). The latter study also lists other studies conducted on microplastics in wastewater treatment plants. Removal rates are typically found to be in the upper 90% range. However, the efficiencies have been calculated based on removed number of particles, of various size intervals, and with various methods of various analytical quality, which makes it quite difficult to
estimate how much actually had ended up in the sludge. But it still seems prudent to assume that the vast majority of the microplastics entering a treatment plant would have ended up in its sludge.

The sludge which is spread on agricultural soil originates from various treatment plant steps. Typically the sludge has passed through an anaerobic digester which is either operated at mesophilic (=35-37°C) or thermophilic (=55-60°C) conditions. In some cases the sludge is treated before digestion, for example applying high temperature and pressure (thermal hydrolysis) or mechanical disintegration. How these different operational conditions affect the microplastics originally entering the wastewater treatment plant is unknown. The study reported by Ljung et al. (2019) indicates that microplastics might be lost or transformed during digestion. That study was however not sufficiently precise to draw such conclusion with certainty.

The sludge entering the digester might furthermore come from primary sedimentation, secondary sedimentation, and tertiary treatment steps (Hartmann et al., 2018). It can furthermore be supplemented by fat and grease from the grease separation step of the treatment plant, and often the digester also receives some sort of external organic matter, for example fat and grease from separators at industries and restaurants. How these streams affect the load on the digester is largely unknown.

Concluding remarks on microplastics in sludge from wastewater treatment plants
Wrapping up the above discussion it is clear that the present knowledge on amounts and types of microplastics in wastewater treatment plant sludge is quite limited. Very fundamental understandings, such as what is the variability in microplastic concentration in sludge from a treatment plant is also unknown, making it difficult to conduct monitoring of this matrix. Another significant knowledge gap is how processes and loads related to treatment steps such as anaerobic digestion systems affect the microplastics that are ultimately applied on agricultural soil. What is probably not needed is a further focusing on mapping the efficiency of wastewater treatment plants as this has been studied to quite some degree, and it is generally accepted that treatment plants are highly efficient in retaining microplastics – also the smallest size fractions.

3. Fate of microplastics in the terrestrial environment
The terrestrial environment, more specifically farmlands, are considered one of the largest environmental sinks of plastics, with emission rates equal or higher than for surface waters (Nizzetto et al., 2016). Very little data exists on the fate of microplastics in the terrestrial environment. Many researchers have touched on what could happen to microplastics based on what basically are educated guesses, but in-depth studies of the fate of microplastics in the terrestrial environment are missing. Studies that to some extent address this are the previously discussed study on Swedish test fields (Ljung et al., 2018) and the study by Corradini et al. (2019) on agricultural fields. However, while these studies seem to point towards microplastics ‘going missing’ in the fields, they are not precise enough to state this with certainty nor to cast light on the mechanisms behind this.

Several processes have been speculated to be of relevance for where microplastics end up. In general, soil will likely act as an efficient filter and barrier for microplastics, especially for those bigger than 1 µm. (Mackevica & Hartmann, 2018). It has been argued that microplastics with higher density are more likely to be retained in soils and transported to deeper soil layers, whereas microplastics with lower density are more susceptible to wind and surface runoffs (Wu et al., 2019). Local geological conditions (e.g. cracks) may facilitate increased transportation of microplastics, also those >1 µm (Mackevica & Hartmann, 2018). However, the proof in terms of actual experimental studies is missing and information on plastic particles in
the nano-size range are limited. With respect to transport mechanisms, it has though been shown that earthworms can be an agent for transporting microplastics in soils, causing vertical transfer of microplastics in soil profiles (Lwanga et al., 2017; Rillig et al., 2017). Lwanga et al. (2017) exposed earthworms to soil surface litter with PE microplastics at concentrations of 0%, 7%, 28%, 45% and 60%. While these concentrations are many orders of magnitudes above environmentally realistic concentrations, the study still shows that earthworms can be an active transport mechanism for microplastics into the deeper soil layers. A similar result was reported by Rillig et al. (2017) operating at conditions closer to what is environmentally realistic (300 mg of PE microbeads per kg soil). Maaß et al. (2017) found that also springtails can aid in transporting microplastics through the soil matrix.

Breakdown and degradation processes are well-known to occur for plastics in for example the marine environment, such as physical abrasion, photooxidation, oxidative degradation, hydrolytic degradation, and biodegradation. However, while these processes are likely to also occur in the terrestrial environment, they have only been experimentally investigated to a rather limited degree. For example by Lwanga et al. (2018) who extracted bacteria from the gut of earthworms and showed that particles of low-density polyethylene decreased in size when incubated with such bacteria. Yang et al. (2014) showed that bacterial strains from the gut of waxworms also seemed able to degrade polyethylene. Janczak et al. (2018) showed that biofilm forming rhizosphere microorganisms with high metabolic activity, together with plants, could form systems that accelerate the biodegradation of polylactide (PLA) and polyethylene terephthalate (PET) in pot experiments.

**Concluding remarks on the fate of microplastics in the terrestrial environment**

Wrapping up the above examples of fate studies, it shall be mentioned that there are very few lab-based studies on the breakdown mechanisms, and that field-based experiments are absent. Those studies that do exist point in the direction that plastics are broken down in terrestrial systems. Numerous detailed questions do however remain open, for example which plastic types are more readily broken down, which conditions enhance this breakdown, which mechanisms are involved, whether the breakdown is primarily a fragmentation to smaller particles or a true mineralization, and whether or not the very fine microplastics are mobile in the soil matrix and finally potentially can reach the groundwater.

4. **Biological impacts on the terrestrial environment**

Some studies have in the recent years been published on the impacts of microplastics on soil organisms, plants as well as animals. However, the knowledge of effects of microplastics on soil organisms is still very limited. In the following a number of these studies are discussed to give an overview of the state of research on this topic.

Ingestion of microplastics has been shown for earthworms (Cao et al., 2017; Rilling et al., 2017; Lwanga et al., 2016; 2017) with the worms having reduced growth rates when exposure to PE microplastics. Effects were shown at concentrations of 0.2 - 1.2% of microplastics in dry soil. Other studies have shown that microplastics can affect important aspects of the soil environment, including effects on plant development (Qi et al., 2018). They added 1% of plastic residuals from plastic mulch\(^6\) to sandy soil and planted these with wheat. In addition hereto, Rillig et al. (2019) discussed probable mechanisms which can lead to effects of microplastics on plants.

\(^6\) The plastic coverings used to suppress weeds for certain crops
Boots et al. (2019) addressed the impacts of microplastics on soil ecosystems above and below ground. They added biodegradable polyactic acid (PLA), polyethylene (PE), and microplastic clothing fibres (from a standard household washing machine) to soil. Earthworms were also added to the microcosms. The authors observed impacts on the germination of ryegrass for the fibres and the PLA and impacts on the earthworms for the PE. The microplastics were added to concentrations of 1 g/kg of PE and PLA and 0.01 g/kg of clothing fibres, respectively.

Prendergast-Miller et al. (2019) exposed earthworms to polyester microfibers at concentrations of 0, 0.1 and 1% w/w of fibers to soil. They found that the worms did not actively avoid the fibres. At the highest concentration the amount of worm casts (worm faeces) decreased and the worms expressed stress biomarkers. It was further concluded that it was not a possible metal content of the fibres which caused this.

Zhu et al. (2018) fed Enchytraeus crypticus – a worm used as model species in soil ecotoxicology – with a mix of oatmeal and nano-particles of polystyrene (50-100 nm). Concentrations in the oatmeal were 0, 0.025, 0.5, and 10% on a dry weight basis. At the highest concentration, 10%, a significant reduction of the weight of the animals were observed, while those fed at 0.025% showed increased growth rates. For those animals fed with 10% nano-particles in the oatmeal they observed a shift in the gut microbiome.

A study by Judy et al. (2019) points, however, in the other direction, namely that addition of PE, PET (polyethylene terephthalate), and PVC (polyvinyl chloride) added to soils did not affect the soil ecosystem. The authors concluded that “addition of microplastics had no significant negative effect on wheat seedling emergence, wheat biomass production, earthworm growth, mortality or avoidance behaviour and nematode mortality or reproduction compared to controls. There was also little evidence that the microplastics affected microbial community diversity, although measurements of microbial community structure were highly variable with no clear trends”. In the study soil was enriched with a compost-like product from municipal mixed wastes (1% w/w). The compost was enriched with plastics so that the final added plastic concentration in the soil was 10 - 100 mg of each plastic type per kg of soil.

A study by Wang et al. (2019) showed that microplastics in soil could have beneficial impacts in the presence of arsenic. They concluded that “microplastics alleviated the effect of arsenic on the gut microbiota possibly via adsorbing/binding As(V) and lowering arsenic bioavailability, thus prevented the reduction of As(V) and accumulation of total arsenic in the gut which resulted in a lower toxicity on the earthworm”.

**Concluding remarks on biological impacts on the terrestrial environment**

Wrapping up, the above examples of impact studies shows that the amount of microplastics that was added in most cases exceeded environmentally realistic concentrations by many orders of magnitude. This is similar to what has been frequently criticized for aquatic ecotoxicity studies. In no cases have studies addressed concentrations as low as what was reported for the soils of an agricultural test field by Ljung et al. (2018). At the same time, results do point in different directions even at such elevated concentrations. All in all it hence seems quite fair to say that we do not know whether or not microplastics from sewage sludge cause impacts on terrestrial soil organisms and/or ecosystems. What does, however, seem clear is that the processes and biological systems involved are quite complex, and that it probably will be quite challenging to proof or disproof actual impacts under environmentally realistic conditions.
5. Chemical impacts and interactions on the terrestrial environment
Several types of microplastics are known to have a high affinity towards sorbing dissolved substances such as organic and inorganic micropollutants. This has been studied to quite some extent in the aquatic environment, but no experimental study hereof has been published for the terrestrial environment (Tourinho et al., 2019). Tourinho et al. (2019) conducted a theoretical comparison of sorption of model chemicals in soil environments and aquatic environments and concluded that overall a higher partitioning of chemical contaminants to microplastics can be expected in soils compared to aquatic environments.

Hüffer et al. (2019) conducted a study on how PE particles influence the transport of two pesticides and concluded that PE did increase the mobility of these organic contaminants. However, they applied quite high PE concentrations, 10% w/w, which is very far from realistic for soils receiving wastewater treatment sludge.

Liu et al. (2019b) investigated the interactive effects of glyphosate and microplastics on dynamics of soil dissolved organic matter, phosphorous and nitrogen. They concluded that interactive effects could be seen. However, the microplastics concentrations used were rather high, namely 7% and 28% of the weight of the soil.

Concluding remarks on chemical impacts and interactions on the terrestrial environment
Wrapping up the above discussion it is clear that not too much is known of how microplastics affect other chemicals in the terrestrial environment, and that such studies as have been conducted were done so at concentrations many orders of magnitude above what is environmentally realistic for Danish agricultural soils. This does leave a knowledge gap on whether or not chemical impacts, either from chemicals conveyed by the plastics or by sorption/desorption processes, play an important role at environmentally realistic concentrations.

6. Methods for analysing microplastics in the terrestrial environment
Analysis for microplastics in soil and sludge from wastewater treatment plants consists of sampling, microplastics extraction, analysis of the extracted material, and reporting. The sampling for soil and sludge is technically simple, basically boiling down to grab sampling using shovels, spoons, and such. However, the inhomogeneity of microplastics in soil and sludge might be high, leading to uncertainties with respect to collecting a representative sample. How large the microplastics inhomogeneity is, and consequently how to sample to compensate here for is basically unknown and has not been addressed thoroughly.

The second step of the analysis is the microplastics extraction. This step is quite challenging and more so for soil and sludge than for most other matrices. A major issue with soil is that it contains large amounts of inorganic particles (sand, silt, clay) as well as difficult to degrade organic fibres and particulates. To separate the inorganic particles from the matrix, the matrix is commonly suspended in a liquid of high density where the organic material containing the microplastics float while the inorganic material sinks. The most common separation fluid is a zinc chloride solution, but other solutions based on for example sodium iodide and sodium polytungstate are also used. In some cases researchers have used sodium chloride and other salts with low solubility, however, an efficient separation cannot be achieved with such chemicals (Li et al., 2019). The microplastics might be strongly embedded in the structure of the matrix, which has to be opened before separation and without damaging the plastics. For this purpose, some researchers have lately introduced a pre-oxidation for soils and sediments (Liu et al., 2019a; Li et al., 2019) as this allows opening up the matrix before separation.
The density separation has been the focus of quite some research because it is the same approach that is used for sediments from the aquatic environment, and there has also been some studies including recovery of particles spiked to the matrix. An example is Han et al. (2019) who developed a new separation method and spiked 6 different plastic types. One thing is however characteristic for these recovery study: They have been done with rather large plastic particles. Han et al. (2019) used for example particles of shredded plastics between 100 and 6100 µm, while Liu et al. (2019a) used 100 µm polystyrene beads. How the separation performs at lower particle sizes and for microplastics closer to shapes and ages found in the environment is largely unknown (Nguyen et al., 2019).

Two-phase separation is another approach for separation of plastics (and other organic materials) from soils that has been suggested and tried. In this approach the matrix is first dissolved in water upon which a nonpolar liquid is added. Upon mixing, the organic materials (including the plastics) will tend to accumulate in the nonpolar liquid. Mani et al. (2019) used for example castor oil for separating 0.3-1 mm large plastic fragments from three aquatic sediments and one agricultural soil. They achieved quite good recovery rates, indicating that two-phase separation worked quite well for the studied matrixes. The benefit of this method is that it is simpler and faster than the density separation, however, it is not nearly as well documented, especially for lower particle sizes. Furthermore, the oil will leave a film on the particles which has to be removed prior to analysis by FTIR, Raman or pyrolysis GC-MS as the oil otherwise will hamper the detection of the microplastics.

Following the reduction of the sample matrix by removing the inorganic material, the natural organic material must be removed without damaging the microplastics (too much). This step has received quite some attention as it is also used in other areas of microplastics research. However, the actual method for reducing the organic part of the sample matrix depends strongly on the matrix in question. The most common approach today is based on a combination of enzymatic degradation of specific substances (Löder et al., 2017) and oxidation with peroxide (Hurley et al, 2018). There have been some studies that compared different extraction protocols, for example Lares et al. (2019) who compared six protocols with wastewater and sludge spiked with plastics of sizes 300-6700 µm. They concluded that it is quite important to avoid harsh chemical treatment, high temperatures and multistep procedures. While the first two goals can be achieved by using combinations of enzymatic treatment with peroxide oxidation, the use of multistep procedures is more problematic to avoid, as the reduction of the organic matter sample matrix for sure requires such multiple steps to identify small microplastics. All in all, there is today a quite a good grip on how to reduce the organic matter of a sample without damaging larger microplastics (>100 µm). However, there is quite little knowledge on how the smaller microplastics are affected.

The next step in the analysis is the quantification of microplastics in the concentrated sample. Here there have been applied and proposed a variety of methods which can be divided into the following main groups:

- Identification based on visual inspection
  - Light microscopy where the human eye and judgement are used to identify what is microplastics and what not.
  - Fluorescence microscopy where particles are stained by a stain that has some preference to common plastics. Fluorescence is then used to identify what is microplastics and what is not.
- Identification based on single point spectroscopy
  - ATR-FTIR where particles are sorted out of an assembly and identified one by one by placing the particles on the ATR-crystal of the FTIR machine.
  - Single point µFTIR where particles either are sorted out of an assembly and identified one by one or analysed one by one in the assembly (for example on a filter). Samples are analysed in transmission or reflection mode.
Single point Raman where particles either are sorted out of an assembly and identified one by one or analysed one by one in the assembly (for example on a filter).

- Identification based on mapping spectroscopy
  - µFTIR imaging creating continuous maps of IR spectra which then allow mapping of microplastics. This method allows fully automated microplastics analysis.
  - µRaman imaging creating continuous maps of IR spectra which then allow mapping of microplastics. This method allows fully automated microplastics analysis, albeit at rather low scanning speeds.
  - Scanning single point µFTIR where particles first are identified in the assembly by various means (for example particles on a filter or a window) and single FTIR spectra then obtained from each particle
  - Scanning single point µRaman where particles first are identified in the assembly by various means (for example particles on a filter or a window) and single Raman spectra then obtained from each particle

- Identification based on pyrolysis/thermal desorption GC-MS
  - Pyrolyzation of particles where particle concentrates undergo thermal desorption (for example in a pyrolyzer) and the decomposition product then analysed on GC-MS
  - Dissolving particles and pyrolyze the dissolved material, which then is analysed on a GC-MS

It is beyond the scope of this report to go through all different methods and discuss their strengths and weaknesses. The different research groups have different preferences, partly depending on what equipment they have available. Furthermore, no method can detect all polymers and it is hence prudent to apply more than one method if all polymer types are to be analysed, as has been shown for FTIR and Raman spectroscopy by Käppler et al. (2016) as well as for µ-ATR-FTIR and pyrolysis-GC-MS by Käppler et al. (2018).

A lot of research has been put into developing the different methods. Nevertheless, it is still quite much debated which method gives the more precise result. This is mainly caused by a lack of intercalibration between the methods. Part of the explanation of this lack is that most research laboratories have only one method in-house. However, the authors of this report are aware of research underway which will compare µFTIR imaging and pyrolysis GC-MS. Another substantial hindrance in the comparison of methods is that attempts to create homogeneous materials for proficiency testing have not yet succeeded.

Finally the data have to be reported. Traditionally the content of microplastics has been reported as particle numbers within certain size ranges. Which size ranges, and how particles sizes have been defined has varied from study to study, making comparison problematic. First lately have studies started to report microplastics also in terms of mass. For the spectroscopic methods addressing the small particle size ranges, such approach was first presented by Simon et al. (2018) for microplastics in wastewater. This approach has since then been applied in subsequent studies and will become more widely spread as it is included in the software commonly used for interpretation of µFTIR and µRaman imaging. For the GC-MS based methods the mass quantification is an inherent aspect of the output. However, these methods cannot quantify particle numbers.

Concluding remarks on methods for analysing microplastics in the terrestrial environment

Wrapping up the discussion on analytical methods from sampling, microplastic extraction, analysis of the extracted material, to reporting, it seems that quite some effort has been and still is being put into

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7 siMPle, see www.simple-plastics.eu
improving this. What is however lacking is validation of the various methods and approaches for especially the small particle sizes below 300 µm with most focus on the very small particles below 100 µm. In case the validation of the methods, for example in terms of recovery, turns out to be poor, further research must be invested to improve them. Furthermore, there is a lack of suitable standard particles and proficiency materials for ensuring analytical quality.

A final comment on microplastics analytics is that the lower size limit of quantification today is around 10 µm in the particles major dimension. This can be pushed down to probably 5 µm or even a bit lower. Below this size it still is possible to detect particles, also in the nano-range below 1 µm. However, quantification is at the moment not possible, and it is the opinion of the authors that we still are quite far from being able to quantify nanoplastics in environmental matrixes.

Ongoing research in other countries

There is quite some ongoing research on microplastics, and the funding allocated for its continuance is substantial. Microplastics are studied all over the world, with Europe being the main engine due to the substantial focus on this emerging issue. Europe as a whole accounts for 67% of all the published scientific articles on this topic during the last five years (Figure 3).

![Figure 3. Scientific articles on microplastics in the period 2014-2019. The search is done using the Web Of Science website.](image)

The published research is of course of highly varying quality, as is always the case in science. Furthermore, what is of high quality and what is not is naturally a matter of debate between researchers. Nevertheless, it seems safe to say that especially in the early days of microplastics research (meaning a few years ago), all what held the word ‘microplastics’ in its title was quite easy to get published, even though the underlying methods were less rigorous and the study less well-conducted than in more established research fields. This is slowly shifting as microplastic research gets into a more mature state. Another interesting observation in the field of microplastic publications is that a quite substantial part, one out of eight publications, is not original research but review articles or editorials. In other words, there is quite a lot of work done on writing about what we know and do not know, compared to the actual doing of the research.
Looking at the research groups around the world and the level of quality and cutting edge that their products have, the authors of this report are of the opinion that especially Germany comes across as a hotspot in terms of high-quality research. This is an opinion and not a solid fact based on for example citations of articles. It is furthermore not the intention to say that researchers in other countries do not also produce high quality science. However, again and again we see that German research is just a bit in front of that of other groups.

While the ‘who is the best and strongest research groups’ is not an answerable question, there are indices that Europe, and especially some countries in Europe, are leading the research. In 2019 till date, German papers (97 in total) were cited 1.98 times per paper8, while the country producing most papers, China, had a citation rate of 0.85 (213 papers in total). The country producing the second largest number of papers, USA had a citation rate of 1.06 (117 papers in total). On the other hand, countries like Italy and the UK had citation rates similar to Germany based on 78 and 77 papers, respectively. Another interesting point is that Denmark – although being a quite small country compared to those listed above – still published 20 articles on microplastics this year, and had a citation rate of 2.65.9

There are quite some funding allocated to microplastics research in Europe. Table 2 gives an overview of open or just closed European calls addressing various aspects of microplastic pollution. In addition to these calls, there are quite some calls that address other aspects related to sustainable plastics, recycling and clean-up of plastics.

Table 2. Recent and ongoing European research calls related to microplastics

| Horizon 2020, Work Programme 2018-2020 | Health, demographic change and wellbeing | The following research priorities on micro- and/or nano-plastics, inter alia, can be considered:
| Health, demographic change and wellbeing | SC1-BHC-36-2020: Micro- and nano-plastics in our environment: Understanding exposures and impacts on human health | – Environmental/food/water sources for micro- and/or nano-plastics and transmission to humans;
– Methods for identification and quantification of micro and/or nano-plastics in foods, environmental media and tissues;
– Exposure levels of humans to micro- and/or nano-plastics and methods for human biomonitoring;
– Analytical methods for detection of micro- and/or nano-plastics and particles and contaminants;
– Microbial colonisation of micro- and/or nano-plastics as vectors for potential pathogens;
– Micro- and/or nano-plastics as condensation nuclei and/or carriers for airborne particulate matter and chemicals harmful to health;
– Toxicology and uptake of micro- and/or nano-plastics and additives/adsorbed contaminants;
– Fate of micro- and/or nano-plastics in the gastro-intestinal or respiratory tracts and secondary organs;
– Effects and transport of micro- and/or nano-plastics across biological barriers, and bioaccumulation and cell uptake of micro- and/or nano-plastics, including studies at the cellular and molecular levels;
– Consideration of the effect of shape (as well as size) of micro- and/or nano-plastics, and comparison with the behaviour and effects of non-synthetic homologues, e.g. wool fibres; |

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8 Excluding self-citations, as this biases the citation index substantially
9 This quite high number is mainly due to the fact that the most hot and highly cited paper in microplastics this year was published by Nanna B. Hartmann et al. (2019) from DTU.
| Food security, sustainable agriculture and forestry, marine, maritime and inland water research and the bioeconomy | SFS-21-2020: Emerging challenges for soil management. B. [2020]: Emerging challenges for soil management: use of plastic in agriculture (RIA) | Proposals shall cover analysis of the use of plastic in agricultural production and its impact on soil. The particular focus of the proposals should be on the micro-plastic after harvest and its fate in the environment. The potential future impact of micro-plastic on soil biodiversity and its potential transfer to other parts of the environment and beyond should be analysed. Activities shall also analyse the impact of micro- and nano-plastics on soil properties and its ecosystem services function. In addition the focus of this analysis should be concentrated on the use of plastic during agricultural production at the field level but also at the farm level. It is expected that these projects lead to: Understand the impact of micro- and nano-plastics on soil biodiversity and ecosystem services Understand the impact of micro- and nano-plastics and other stressors in soil on agricultural productivity and ecosystem services; Understand and assess the chemical changes and disaggregation of micro- and nanoplastics in soils, their impacts and further behaviour in soils (including soil physics); |
| BG-07-2019-2020: The Future of Seas and Oceans Flagship Initiative. [C] 2020 - Technologies for observations | Proposals shall address: Sensors to measure variables for aquaculture, fisheries, micro and nanoplastics, and marine litter and micro-litter. It shall contribute to determining the distribution and fate of marine litter and microplastics |
| Climate action, environment, resource efficiency and raw materials | CE-SCS-29-2020: A common European framework to harmonise procedures for plastics pollution monitoring and assessments | This action should ensure adequate flexibility for taking into account all relevant aspects prior to formal standardisation procedures and provide: a) harmonised methods for sampling, sample preparation and analytical detection of different kind of plastics in different environmental compartments and connected matrices, including realistic matrix reference materials; b) methods for monitoring to enable a comprehensive inventory to be carried out to classify the occurrence, to identify emission and pollution priorities and to determine changes in the occurrence by means of subsequent investigations; c) methods for identification and analysis of plastics in the environment; d) proposals as a basis for international and European standards (ISO / CEN); e) recommendations for future relevant EU policy and legislation; e) increased knowledge on the occurrence of plastics in the environment with respect to related questions, such as physical and chemical adverse effects on biota. |
| Climate action, environment, resource efficiency and raw materials | CE-SCS-30-2020: Plastics in the environment: understanding the sources, transport, | Scope: The aim of this action is to gain a better understanding on the sources, transport, distribution and impact of plastic pollution. The main areas for research activities should include: |
### Distribution and Impacts of Plastics Pollution

| Distribution and Impacts of Plastics Pollution | a) Sources of plastic pollution to different environmental compartments; b) Transport and pathways of plastics into and through different environmental compartments; b) Occurrence and distribution of plastic across all environmental compartments; c) Accumulation, including in soil and the food chain; d) Degradation mechanisms for different plastic materials under range of environmentally conditions; e) f) Physical and chemical effects of plastic pollution on different biotic and abiotic environments. |

| JPI-Ocean | Joint call for proposals on microplastics in the marine environment | This call comprises four main themes:  
- Identification, characterisation and quantification of the major microplastic sources, especially mechanisms and time scales of macroplastic fragmentation  
- New sampling and analytical methodologies - focusing on the smaller (nano-)particles and in situ measurement methods for all matrices (water, sediment, biota)  
- Monitoring and mapping of microplastics in the marine environment including its effects on the marine environment  
- Concepts to reduce inputs of plastics into the marine environment including through new recycling methods, raising public awareness, promoting behavioural change, socio-economic analyses. |

In addition to the above projects, there are a number of ongoing activities in the EU as well as on national basis. For example, the European Commission’s Joint Research Centre (JRC) on standardizing and validation methods is conducting and coordinating a number of initiatives on the plastic issue. Here especially the initiative ‘Micro and nano-plastics: Towards a more reliable assessment of exposure and biological effects’ should be mentioned. Here the JRC works on supporting:

- **Analytical methods to detect/quantifying microplastics in marine and fresh water, sediments, food, soil, wastewater treatment sludge to support harmonise existing methods for "larger" microplastics (>10 µm); develop fit-for-purpose methods for "smaller" micro(nano)plastics (<10µm).**
  - With respect to standardization of analytical methods there are quite some researchers and organization interested in this. However, it is the opinion of the authors of this report that we still are quite far from such standardization as standardization of methods grows out of a consensus on how to measure. While we today are closer to such consensus than we were just a year or two ago, we are still not at the point where the majority of researchers agree on the best method(s) to analyse for microplastics.
  - Another point is that there till date do not exist any (proven) method that is able to yield a quantitative and documented analysis of microplastics in natural matrixes below 10 µm in size, not to speak of nanoplastics, which still totally eludes our analytical capabilities.

- **Development of test and reference materials, as this is a critical step in providing fit-for-purpose analytical tools to reduce knowledge gaps**
  - In this context, the JRC has invited microplastics laboratories to participate in an inter-laboratory comparison (proficiency testing) on microplastics in drinking water and sediments. This activity has been somewhat delayed and postponed to 2020 as the JRC still is analysing the
(water) batch, which is to be sent to the participating labs, for its homogeneity. Sediments are planned to be shipped out at later date.

- Micro(nano)plastics in the transfer of chemical contaminants to identify robust analytical methods to quantify selected contaminants released from microplastics (e.g. heavy metals, persistent organic pollutants)
- Micro(nano)plastic interaction with microorganisms and cells to evaluate micro(nano) plastic uptake by selected microbiota and cells at realistic exposure levels, and to explore the effects on microorganisms following the uptake of micro(nano) plastics with and without additives or environment pollutants

The JRC also coordinates an action on ‘Environmental Impacts of Plastics – Marine Litter’. With respect to microplastics research this shall support harmonisation of methodologies for the quantification of litter in the marine environment (including microplastics), beach, sea-surface, seafloor and biota and provide scientifically sound marine litter data. The JRC furthermore works on water quality supporting monitoring and onsite studies on microplastics in wastewater.

Action plan

The mapping of knowledge gaps has identified quite large gaps with respect to microplastics in treatment plant sludge, soils and the impact of sludge on soils. Few of these are being addressed by research in neighbouring countries and ongoing research. Table 3 lists research actions, their initiating problem and their dimensioning.

Notes on cost and time estimates

All costs estimated in Table 3 are given as costs for research collaboration, that is incurred direct costs plus 44% overhead. If the projects are given out as tenders, overheads are higher, typically around 100% of the incurred direct costs. In such case a larger sum must be allocated.

For several of the actions, the cost of the action depends on how many samples are analysed. Soil analysis is quite demanding and a price of roughly 30.000 kr per sample has been used as a rough estimate for dimensioning costs for the analytics. This price covers the work done as a research collaboration, that is, including 44% overhead on direct costs. In case the project is given out as a tender, the corresponding cost would be around 42.000 kr per sample. On top of this comes planning, sampling, reporting, and other activities related to the work. Sludge samples are also demanding to analyse but a bit easier than soil and a price of roughly 25.000 kr per sample has been used as a rough estimate for dimensioning costs for the analytics. Also this cost is estimated based on the assumption that the work is done as a research collaboration, that is, including 44% overhead on direct costs. In case the project is given out as a tender, the corresponding cost would be around 35.000 kr per sample.

In terms of dimensioning the time for analysis, it is important to be aware that microplastics analysis is a slow and resource demanding process, where a soil sample will take around 3 months to analyse. Sludge samples take a bit less time, but not much.

The reason why microplastics analysis is so tedious and resource demanding is that the microplastics must be extracted from a matrix containing a lot of particulate and colloidal matter before analysis can be done to identify which particles are of plastic. In soil, for example, there are millions of natural particles for each

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10 Creating homogeneous batches of matrixes for microplastics proficiency tests has been attempted earlier, namely in the JPI-Ocean Baseman project concluded in 2018 – without success as adequate homogeneity could not be achieved. This illustrates some of the issues in the analysis as reference material (homogeneous batches of sample with known content of microplastics) is an absolute necessity for standardization and inter-laboratory quality control.
microplastic particle. First one hence must get rid of those other materials and concentrate the plastics in a smaller volume. This requires a number of purification steps as illustrated in Figure 4. First the matrix consisting of sand, silt, clay, and organic particles is opened up, meaning that plastic particles are not any longer baked together in an agglomerate of inorganic and organic material. Then the heavy sand, silt and clay must be separated from the lighter organic materials by a density separation. After having gotten rid of most of the heavy stuff, which makes up maybe 90-99% of the soil, the sample still has all the soil organic matter mixed in with a bit of microplastics. One must get rid of this natural organic matter without destroying the plastics. For this a series of gentle degradation steps are applied, which attack the natural materials, but not the plastics. For example enzymes and hydrogen peroxide are used here for. One then has to do a final density separation to get rid of the last bit of fine clay and silt in the sample. The final product is a concentrate holding the microplastics with a minimum of interfering material (Figure 4).

**Figure 4. The steps of sample preparation when analyzing a soil – from kilograms of soils to a fine concentrate containing the microplastic particles**

This final concentrate has to be analysed chemically. This is commonly done on a µFTIR imaging system equipped with a focal plane array (Figure 5). A sub-sample is deposited on an IR-transmissive window or filter, which then is scanned at high resolution. With the equipment in Aalborg, which has the highest resolution on the market, an area of 10 x 10 millimetre is typically scanned at a resolution of 5.5 µm, meaning that there is generated more than 3.2 million individual spectra by one scan. This huge dataset then needs to be interpreted to find the microplastic particles.
In the very beginning, the interpretation of the huge datasets was done manually. However, the work
involved was prohibitive, and the manual analysis introduced a substantial human bias. Hence an
automated data interpretation tool was developed, which allows a much faster and more secure analysis
(Figure 6). Even though this was a quantum leap in terms of analysing these huge datasets, it still is not a
‘push the button and out comes the results’ technique, as samples differ and each new type of sample
needs a thorough calibration of the interpretation tool and validation of the results.

National versus international actions
Some actions might be better embedded in an European context than in a purely national context. In
principle, most of the suggested actions could be European actions. This even holds true for the actions
that address the mapping of Danish soils and sludge, as it can be argued that such concentrations would be
similar to what is found in comparable countries, such as Germany, Sweden, and so on. Allocating the
actions to a purely European context would basically mean that Denmark would follow the lead of other
countries, as Denmark would not contribute with knowledge and hereby directly and indirectly affect the
development in a direction, which is desirable based on specific Danish interests and environmental
targets.

On the other hand, it does not make much sense if Denmark would conduct the described actions without
recognizing that other important work is done all around Europe. What the authors of this report suggest is
to initiate the actions described in a Danish context, and at the same time to ensure that the conducted work acknowledges, supplements, and strengthens ongoing work in Europe. By constantly being aware of the developments in the field, collaborating across Europe on such tasks, and contributing to filling the huge knowledge gaps in this field, Denmark ensures the best possible implementation of the actions and that specific Danish interests and environmental targets are taken into account.
<table>
<thead>
<tr>
<th>Research action</th>
<th>Initiating problem</th>
<th>Dimensioning of action</th>
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<tbody>
<tr>
<td><strong>1.1 Variability of microplastics in soil</strong></td>
<td>A proper sampling of microplastics in soils requires that the variability of microplastics in the soil is known. Spatial variability must be known over short ranges, for example a few meters, as well as longer ranges, for example for a whole field. Typical size distributions must also be known to ensure that sample sizes are adequate to cover the particle size range which is envisioned. Here is the rule that large particles are comparatively less abundant and hence requires larger sample volumes than smaller particles.</td>
<td>One or several test fields are selected. Each field is thoroughly sampled and the samples analysed. For example by collecting and analysing a number of small patches of soil across the field by multiple samples at each patch. A minimum dimensioning of such study could be 1 field from which 5 patches are selected. From each patch 5 samples are collected and analysed. This leads to 5*5 = 25 samples. At a price of 30.000 kr per sample this amounts to 750.000 kr for the analysis. On top of this it would be reasonable to add 250.000 kr for other activities, leading to an overall minimum cost of roughly 1.000.000 kr. The duration of such action could be 24 months. The study is scalable and would optimally be performed with 3 fields, costing roughly 3.000.000 kr and taking 36 months.</td>
</tr>
<tr>
<td><strong>1.2 Background microplastics concentration levels in soils that have not received sludge or other organic waste products as fertilizer</strong></td>
<td>The general concentration level of microplastics in soils of different types is unknown. A baseline needs to be established to allow to quantify the general background concentration of microplastics in Danish soils, to which soils that have received sludge or other organic waste products as fertilizer can be held up against.</td>
<td>To establish such baseline knowledge, a number of fields must be sampled thoroughly. To ensure that spatial variability is kept at a minimum, a large number of sub-samples are taken and thoroughly mixed before extracting a subsample for analysis. A minimum dimensioning of such study could be 10 fields from which for example 20-30 subsamples are taken all over the field, mixed, and analysed. At a price of 30.000 kr per sample this amounts to 300.000 kr for the analysis. On top of this it would be reasonable to add 200.000 kr for other activities, leading to an overall minimum cost of roughly 500.000 kr. The duration of such action could be around 18 months. The study is scalable and would optimally be performed with 30 fields, costing roughly 1.500.000 kr and taking 24 months</td>
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<tr>
<td><strong>1.3 Impact of sludge on the microplastics concentration in agricultural fields</strong></td>
<td>Wastewater treatment plant sludge contains microplastics. But we have no experimental proof that the sludge causes a significant increase in microplastics in the fields that have received the sludge. This is a key knowledge gap as it governs whether or not wastewater treatment plant sludge can be viewed as a significant polluter when used as fertilizer.</td>
<td>This action depends on action 1.2 being conducted, as it compares microplastics in fields that have not received sludge to microplastics in fields that have received sludge. It is furthermore crucial that the two studies are conducted with precisely the same sample preparation and analytical methods, as comparison otherwise is problematic. Action 1.2 and 1.3 could hence also be conducted as one action.</td>
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A number of fields that have received sludge must be sampled thoroughly. To ensure that spatial variability is kept at a minimum, a large number of sub-samples are taken and thoroughly mixed before extracting a subsample for analysis.

A minimum dimensioning of such study could be 10 fields from which for example 20-30 subsamples are taken all over the field, mixed, and analysed. At a price of 30,000 kr per sample this amounts to 300,000 kr for the analysis. On top of this it would be reasonable to add 200,000 kr for other activities, leading to an overall minimum cost of roughly 500,000 kr. The duration of such action could be around 18 months.

The study is scalable and would optimally be performed with 30 fields, costing roughly 1,500,000 kr and taking 24 months.

| 1.4 | Assessment of the relative importance of sludge to the microplastic concentration in fields |
| Wastewater treatment plant sludge contains microplastics. But we have no overall national estimate on how much the sludge can contribute to an increase in microplastics in the fields that have received the sludge. This is a key knowledge gap as it governs whether or not wastewater treatment plant sludge can be viewed as a significant polluter when used as fertilizer. |
| An overall assessment of the load to the agricultural fields can be made by knowing the background contamination in the fields that have not received sludge, and the concentration in the sludge which is applied to the fields. This is a desktop study which takes data from action 1.2 and 2.2 and estimates what the maximum increase in plastics would be assuming no degradation after the plastics have been applied. The duration of such action could be 6 months. It can be done within a limited budget, for example 200,000 kr. This action depends on action 1.2 and 2.2 being conducted. |

| 1.5 | Transport of plastics to agricultural fields |
| Very little is known on where the microplastics we find in the fields come from. Is it mainly agricultural activities, is it related to long-distance atmospheric deposition, or is it mainly wind-borne transport of macroplastics which then degrades to microplastics? |
| This is a quite complex research question and demands a major scientific investigation. It is, though, rather specific and its size can be dimensioned accordingly. Dimensioning should be no less than 1 PhD students/Postdocs working on this topic for 3 years, corresponding to no less than 3,500,000 kr |

| 2 | Microplastics in sludge from wastewater treatment plants |
| Variability of microplastics in sludge |
| A proper sampling of microplastics in sludge requires that the variability of microplastics in the sludge is known. Variability must be known within larger batches of sludge (truck loads), and over time, for example over a year. Typical size distributions must also be known to ensure that sample sizes are adequate to cover the particle size range which |
| One or several treatment plants are selected. Each plant is thoroughly sampled and the samples analysed. For example by collecting and analysing a number sludge batches by thorough sub-sampling, and sample a number of batches over the year. A minimum dimensioning of such study would be 1 treatment plant from which 5 batches are selected. From each batch 5
is envisioned. Here is the rule that large particles are comparatively less abundant and hence requires larger sample volumes than smaller particles.

samples are collected and analysed. This leads to $5 \times 5 = 25$ samples. At a price of 25.000 kr per sample this amounts to 625.000 kr for the analysis. On top of this it would be reasonable to add roughly 200.000 kr for other activities, leading to an overall minimum cost of roughly 850.000 kr. The duration of such action could be 24 months.

The study is scalable and would optimally be performed with 3 treatment plants, costing roughly 2.550.000 kr and taking 36 months.

| 2.2 Concentration levels in sludge – mapping Danish treatment WWTP sludge | The general concentration level of microplastics in sludge from different treatment plants is unknown. A baseline needs to be established | To establish such baseline knowledge, a number of treatment plants must be sampled thoroughly. To ensure that temporal and batch variability is kept at a minimum, a large number of sub-samples are taken over a year and thoroughly mixed before extracting a subsample for analysis.

A minimum dimensioning of such study would be 10 treatment plants from which for example 20-30 subsamples are taken over the year, mixed, and analysed. At a sample price of 25.000 this amounts to 250.000 for the analysis. On top of this it would be reasonable to add 200.000 for other activities, leading to an overall cost of roughly 450.000 kr. The duration of such action could be around 18 months.

The study is scalable and would optimally be performed with 30 fields, costing roughly 1.350.000 kr and taking 24 months.

| 2.3 Microplastic balances on wastewater treatment plant internal streams | Most studies addressing wastewater treatment plants have looked at inlet and outlet. Few have addressed what goes on internally in the plant. The lack of such information means that the treatment plant designers and operators cannot optimize the design and operation of treatment plants to minimize the amount of microplastics that ends up in the digesters, and finally in the sludge on agricultural fields. | This is a quite complex research question and demands a major scientific investigation. It is, though, rather specific and its size can be dimensioned accordingly. Dimensioning should be no less than 1 PhD students/Postdocs working on this topic for 3 years, corresponding to no less than 3.500.000 kr.

| 2.4 Fate of microplastics during anaerobic digestion – influence of treatment operation | Another significant knowledge gap is how different treatment processes and their operating conditions affect microplastics. Anaerobic digestion (AD) is a common treatment process to stabilize wastewater sludge, which is the ultimate receptor of microplastics from industry, households and urban run-off. Residues from AD (i.e. digestate) is a valuable resource and applied on agricultural soil as fertilizer. Without removal | This is a quite complex research question and demands a major scientific investigation. Its size must be dimensioned accordingly, and a proper investigation would require a consortium of research groups having various specialties. Dimensioning should be no less than 3 PhD students/Postdocs working on this topic for 4 years, corresponding to no less than 10.000.000 kr. This
(degradation) of microplastics during AD they will be transferred to the terrestrial environment through the application of digested sludge on agricultural fields. Only few plastics are reported as being degradable under anaerobic conditions and published reports are up to now, non-conclusive and contradictory. Furthermore is it unknown how pre-treatment of sludge before it enters a digester will affect the plastics. Such pre-treatment could for example be high-temperature/pressure treatment or mechanical disintegration. Studies are needed to understand the fate of microplastics during AD and if/how the treatment process can be optimized for microplastics degradation. It would also be beneficial to identify and investigate if bioaugmentation can optimize degradation. Work would logically include Research Action 6.6 on development of traceable model particles as a central activity. The work could also be sub-divided into smaller tasks, however, coordination of the work is important to cover the whole problem-area.

3 Fate of microplastics in the terrestrial environment

| 3.1 Breakdown and degradation of microplastics in agricultural soils | The knowledge on breakdown and degradation of microplastics in agricultural fields is very limited. Numerous detailed questions remain open, for example which plastic types are more readily broken down, which conditions enhance this breakdown, which mechanisms are involved, whether the breakdown is primarily a fragmentation to smaller particles or a true mineralization, where the fragments end up, and so on.

A more fundamental issue relates to the fact that existing test guidelines to determine biodegradability of substances are not tailored to microplastics. This is acknowledged also in the recent ECHA restriction proposal for intentionally added microplastics, stating that (ECHA, 2019): “Variations to existing standardised (bio)degradation testing methods, or potentially entirely new standardised testing methods, are likely to be necessary to appropriately assess the (bio)degradability potential of some microplastics in the environment”.

It should be noted that microplastics are in some cases also directly and deliberately applied to soils, e.g. plastic used as capsulation agent in controlled release of fertilizing products. Development of new and suitable approaches to determine the (bio)degradation of microplastics will therefore also support future European regulation. This is a quite complex research question and demands a major scientific investigation. Its size must be dimensioned accordingly, and a proper investigation would require a consortium of research groups having various specialties. Dimensioning should be no less than 3 PhD students/Postdocs working on this topic for 4 years, corresponding to no less than 10.000.000 kr. This work would logically include Research Action 6.6 on development of traceable model particles as a central activity. The work could also be sub-divided into smaller tasks, however, coordination of the work is important to cover the whole problem-area. |
| 3.2 | Vertical distribution of microplastics in soil | While only few studies have addressed occurrence in soils, none have addressed how concentrations develop with depth. In other words, whether microplastics migrate from the top soils and further into the sub-soils. It seems reasonable to assume that microplastic concentration within the plowing layer are somewhat homogeneous, but this is not known per se. Furthermore, concentrations below the plowing layer is unknown. In other words, we do not know if microplastics is transported further into the soil below the plowing layer. | This can be addressed by an action analysing a number of depth profiles. It can be designed in various ways, for example by choosing one field (or several) and profiling it (them) thoroughly at a number of locations. For example 5 locations per field, and analysing for microplastics at 5 depths. This leads to 5*5 = 25 samples. At a price of 30.000 kr per sample this amounts to 750.000 kr for the analysis. On top of this it would be reasonable to add 250.000 kr for other activities, leading to an overall minimum cost of roughly 1.000.000 kr. The duration of such action could be 24 months. The study is scalable and would optimally be performed with 3 fields, costing roughly 3.000.000 kr and taking 36 months. |
| 3.3 | Transport of microplastics through soil | Information on the transportation of microplastics through soils is limited. Existing assumptions are based mainly on theoretical considerations and experimental data from related fields (colloidal transport). There is a clear need for studies that examine the actual transport of microplastics, with a view to both particle and soil characteristics. | This work would include dedicated soil column experiments to estimate rates of movement of microplastic particles, evaluate the roles of the various factors (soil and microplastics) potentially influencing this transport. The study can be scaled to cover few or more soil types and geological conditions. To ensure a meaningful dataset, representative of Danish conditions, the study should have a duration of no less than 12 months and a cost of no less than 700.000 kr |

### 4 Biological impacts on the terrestrial environment

| 4.1 | Impacts on invertebrate fauna | Very little is known on the impact of microplastics on invertebrates of agricultural soils. A huge knowledge gap is to understand if, and if then how, invertebrates are affected at environmentally realistic conditions, using environmentally relevant microplastics. | This is a quite complex research question and demands a major scientific investigation. It is, though, rather specific and its size can be dimensioned accordingly. Dimensioning should be no less than 1 PhD students/Postdocs working on this topic for 3 years, corresponding to no less than 3.500.000 kr |
| 4.2 | Impacts on soil ecosystems | Very little is known on the impact of microplastics on agricultural soil ecosystems. A huge knowledge gap is to understand if, and if then how, ecosystems are affected at environmentally realistic conditions. | This is a quite complex research question and demands a major scientific investigation. Its size must be dimensioned accordingly, and a proper investigation would require a consortium of research groups having various specialties. Dimensioning should be no less than 3 PhD students/Postdocs working on this topic for 4 years, corresponding to no less than 10.000.000 kr. This |
The work could also be sub-divided into smaller tasks, however, coordination of the work is important to cover the whole problem-area.

### 5 Chemical impacts and interactions on the terrestrial environment

#### 5.1 Microplastics as vector for micropollutants

Microplastics can contain harmful chemicals. We do not know whether or not the contribution of microplastics is significant compared to other sources.

This topic can be addressed in two different ways: A desktop study and an experimental study.

a) Based on data of microplastics occurrence in soil and sludge (actions 1.2 and 2.2), and literature values on how much of various harmful chemicals can be present in microplastics, an upper estimate of concentrations can be made and used for risk assessment. This is a desktop study which can be done within a limited budget, for example 300,000 kr and a duration of for example 9 months.

b) Specific microplastics additives can be measured in sludge and agricultural soils. Because the additives also can come from other sources, this only gives an upper boundary on how much additives could be present in the microplastics. However, when combining measurements of additives and microplastics, an estimate can be made on whether there could be a relationship between such additives and microplastic concentrations.

This is a quite complex task and demands a major scientific investigation and probably the collaboration between specialists in analysing organic micropollutants and specialists in analysing microplastics. It is, though, rather specific and its size can be dimensioned accordingly. Dimensioning should be no less than 1 PhD students/Postdocs working on this topic for 3 years, corresponding to no less than 3,500,000 kr.

This action depends on availability of information on how much microplastics is on the fields, and how much is delivered by the sludge. Hence it depends on action 1.3 and the actions enabling it.
| 5.2 | Microplastics as sorption/desorption agent | Microplastics can sorb and desorb harmful chemicals. We do not know whether or not the contribution of microplastics is significant compared to other sorption materials in soil systems. | This is an experimental study which compares sorption of selected substances to selected microplastic types, sizes, and aging. It also compares sorption of these substances to naturally occurring soils without plastics and to specific compounds of these soils (for example organic matter, clay).
This is a quite complex task and demands a major scientific investigation. It is, though, rather specific and its size can be dimensioned accordingly. Dimensioning should be no less than 1 PhD students/Postdocs working on this topic for 3 years, corresponding to no less than 3,500,000 kr |

| 6 | Methods for analysing microplastics in the terrestrial environment |  |

| 6.1 | Standard reference particles | No certified reference particles exist which are suitable for quality control of an analysis. This is especially a problem for recovery tests, where recovery will depend on size, shape, aging and polymer type. Commercially available materials are restricted to beads of a limited number of materials, and are only of limited value when ensuring the internal quality of analysis. | Establishing a library of reference materials which have certified concentrations (number and mass), size distributions, mass distribution, polymer types, shapes and aging. The library shall contain around 10 of the most common polymers and cover a set of size intervals ranging from 10 µm to 1000 µm. For example as 10-20, 80-120, 300-500, and 800-1000 µm fractions.
Creating such standard particles has caused substantial problems for a number of labs that have tried to do so. However, it is crucial that such materials exist in order to ensure the quality of microplastic analytics. Even though this might sound as a trivial task, it must be stressed that it is not. There are quite a large number of practical issues which must be sorted out, and the task is hence significant. An estimated cost for this would be 2,000,000 kr with a duration of 2 years.
This work is a prerequisite for proficiency testing (action 6.3) and also linked well to the development of traceable model particles (action 6.2). |

| 6.2 | Traceable microplastics for laboratory experiments | In general, our understanding of the transport, fate degradation of microplastics is hampered by methodological and analytical challenges, especially for smaller (<10µm) particles, as mentioned previously. This is especially true when present in a complex matrix such as soil or sewage sludge. Being able to trace microplastics is of imperative importance in controlled laboratory experiments to develop and optimise of treatment technologies, as well as when studying their transport or degradability in soil. | This is a highly interdisciplinary study, linking polymer chemistry and environmental sciences. Different options for polymer labelling can be considered including trace metals (e.g. gold) or isotope labelling of the polymer backbone. The task will involve both polymer synthesis and testing the applicability of the material in a suitable system – likely as an iterative process. This is a substantial tasks and dimensioning should be no less than |
Incorporating a tracer into the polymer enables us to track the fate of microplastics by e.g. standard trace metals analysis (in the case of metal doped microplastics), thereby bypassing several analytical pitfalls.

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<th>Action</th>
<th>Description</th>
<th>Details</th>
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<tr>
<td>6.3</td>
<td>Proficiency materials for intercalibration</td>
<td>No proficiency material exist to intercalibration of laboratories for microplastics analysis. This is a major obstacle when it comes to ensuring cross-laboratory analysis quality. Proficiency materials depend on the availability of standard particles which can be used to spike samples with little or no plastics. This action hence depends on action 6.1. It furthermore need soils and sludge with low or no microplastics. Such soils can probably be obtained from remote areas, while sludge has to be generated under controlled laboratory conditions (pilot reactors simulating wastewater treatment plants which generate digested sludge). Known amounts of microplastics has then to be added to batches of clean soil / sludge. Creating proficiency material has caused substantial problems for a number of labs that have tried to do so. However, it is crucial that proficiency material exist in order to ensure the quality of microplastic analytics and ensure quality between laboratories. Even though this might sound as a trivial task, it must be stressed that it is not. There are quite a large number of practical issues which must be sorted out, and the task is hence significant. An estimated cost for this would be 2.000.000 kr with a duration of 2 years. Proficiency testing is a prerequisite for a reference laboratory, as a reference laboratory (action 6.5) would organize and ensure intercalibration rounds between laboratories.</td>
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<td>6.4</td>
<td>Guidelines for sampling and analysis of microplastics in soil and sludge</td>
<td>Guidelines for sampling and analysis must be available for monitoring of microplastics in soil and sludge. They must be so specific that results can be compared between different laboratories conducting the analysis. The guidelines must specify how fields/soils as well as sludge should be sampled, what type of data must come out of an analysis, and how the proficiency of the laboratory to analyse these systems/matrixes must be documented. The part regarding the sampling depends on the completion of action 1.1 and 2.1. The guidelines must further specify how limits of quantification and detection can be established for a laboratory. This is a desktop study which can be done within a limited budget, for example 300.000 kr. However, while it as such is doable to describe what must be done and how, the actual implementation of the guidelines can be hampered by practical problems such as a lack of standard reference particles and proficiency materials. Hence the practical implementation of the</td>
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<td>6.5</td>
<td>Establishing a reference laboratory for microplastics analysis</td>
<td>There is no laboratory that can act as reference laboratory to ensure that other laboratories, e.g. commercial laboratories, conduct analysis of soil and sludge to a certain standard.</td>
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<td>6.6</td>
<td>Quantification of small microplastics particles</td>
<td>Methods for microplastics analysis down to around 10 µm are in place, however, what is lacking is validation of the accuracy of the methods for the small particle sizes (&lt;300 µm and especially &lt;100 µm). To analyse for microplastics, one first must extract the microplastics from the matrix. For a complex matrix like soil and sludge, this requires many steps, and many different chemical treatments. During these steps, microplastics can be lost, partly degraded, or fragmented. Quality control, improvement of sample preparation methods, and recovery of especially the smallest particles hence need to be addressed.</td>
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<td>6.7</td>
<td>Strategies for monitoring and regulation of microplastics in soil and sludge</td>
<td>When accepted methods for analysis and especially for their documentation and quality assurance are in place, monitoring and regulation can be put into place.</td>
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Prioritization and progression of actions

Certain actions depend on previous actions being conducted. These interdependencies are discussed in Table 3. Some actions are less pressing than others, leading to a prioritization of actions. The prioritization and progression of actions is shown in Figure 7. The prioritization focuses on what is needed for the Danish Environmental Protection Agency to implement a monitoring strategy and possible regulation. Especially the prioritization will always be a point of debate. However, it does seem reasonable that, for example, quantification of occurrence of microplastics in soil and sludge comes before their biological impacts on the soil biota. Knowing how much is out there is a prerequisite to judge whether or not microplastics constitute a risk for soil organisms. In this way, the authors have prioritized the actions according to their best knowledge and judgement.
Figure 7. Prioritization and progression of actions. The colour coding reflects the need for conducting certain actions before others in order to gain sufficient knowledge to monitor and regulate microplastics in agricultural soils. Prioritization will always be a point of debate, and the prioritization is not a judgment of what is 'important science' and what is not. The time-line is envisioned as a timeline of progression, indicating how much time is needed to conduct the individual tasks assuming they are all launched at the same time. See also the discussion on Page 32 and in Table 3.
References


