Completed experiments of the interaction of MNPs in plant-soil systems

Milestone no 14.

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Document Information

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Summary report

The aim of WP4 is to elucidate the effects of micro- and nanoplastics (MNPs) on plant seed germination and development, plant traits and environmental factors on crop growth and performance in representative European agroecological zones.

Table 1. Activity and progress of WP4 experiments on the interaction of MNPs in plant-soil systems

Activity	Timing and duration	Research question	Material / plant	Stage
Pot plant experiments in laboratory	Sep-Nov 2022	Effects of LLDPE and PBAT on plant growth of four crops	LLDPE and PBAT / Lettuce, carrot, wheat, barley (acute), lettuce and barley (chronic)	Zantis et al. 2024
Climecs 1 mesocosm setup	March-June 2022	Effects of PBAT-BD-MPs on lettuce under environmentally relevant conditions	PBAT / Lettuce	Submitted manuscript, Adamczyk et al.
Climecs 2 mesocosm setup	Oct 2022 - Jan 2023	Focus on fate of MPs, in addition effects of MPs on lettuce growth and biochemical parameters (see table 2).	Mixture LLDPE and PBAT / Lettuce	IP (in progress)
Climecs 3 mesocosm setup	Sep - Dec 2023	Effects of LLDPE and PBAT-BD-MPs on lettuce under environmentally relevant conditions	LLDPE and PBAT / Lettuce	IP
Field experiment, three geographical locations	2022 and 2023	Effects of LLDPE and PBAT-BD-MPs on barley growth and development under environmentally relevant conditions	LDPE and PBAT / Barley	IP
Leachate experiment	May 2024	Effects of MPs additives on plant growth and biochemical endpoints of four crops	Leachate of LLDPE and PBAT / Lettuce, carrot, wheat, barley (acute), lettuce and barley (chronic)	Planned activity
^{14}C	2024	Uptake	38um SYN-14C-PS-NP / Lettuce and barley	Planned activity

Table 2. Measured plant growth and stress indicators by April 2024

Activity	Measured parameters
Pot plant experiments in laboratory	Length, biomass, Specific leaf area, number of leaves, chlorophyll content
Climecs 1 mesocosm setup	Length, biomass, necrotic tissues, Specific leaf area, number of leaves, N and C content, chlorophyl content, lipid peroxidation, total phenolic content, salicylic acid, enzymes activity of SOD and CAT
Climecs 2 mesocosm setup	Length, biomass, necrotic tissues, Specific leaf area, number of leaves, chlorophyll content, lipid peroxidation, total phenolic content
Climecs 3 mesocosm setup	Length, biomass, necrotic tissues, Specific leaf area, number of leaves, N and C content, chlorophyll content, lipid peroxidation, total phenolic content, salicylic acid, enzymes activity of SOD and CAT, ammonia and nitrate, sugars, proteins
Field experiment	Biomass, Specific leaf area, Necrotic tissue, Chlorophyll content, N and C content, chlorophyl content, lipid peroxidation, total phenolic content, salicylic acid, sugars, proteins

Photographic documentation

1. **Pot-plan experiments**
   1.1. Seed germination and early development

We aim to elucidate how LLDPE and PBAT-BD-MPs affect the seed germination and early development (acute testing) of two monocotyledonous plants (barley, *Hordeum vulgare*, and wheat, *Triticum aestivum*) and two dicotyledonous plants (carrot, *Daucus carota*, and lettuce, *Lactuca sativa*).

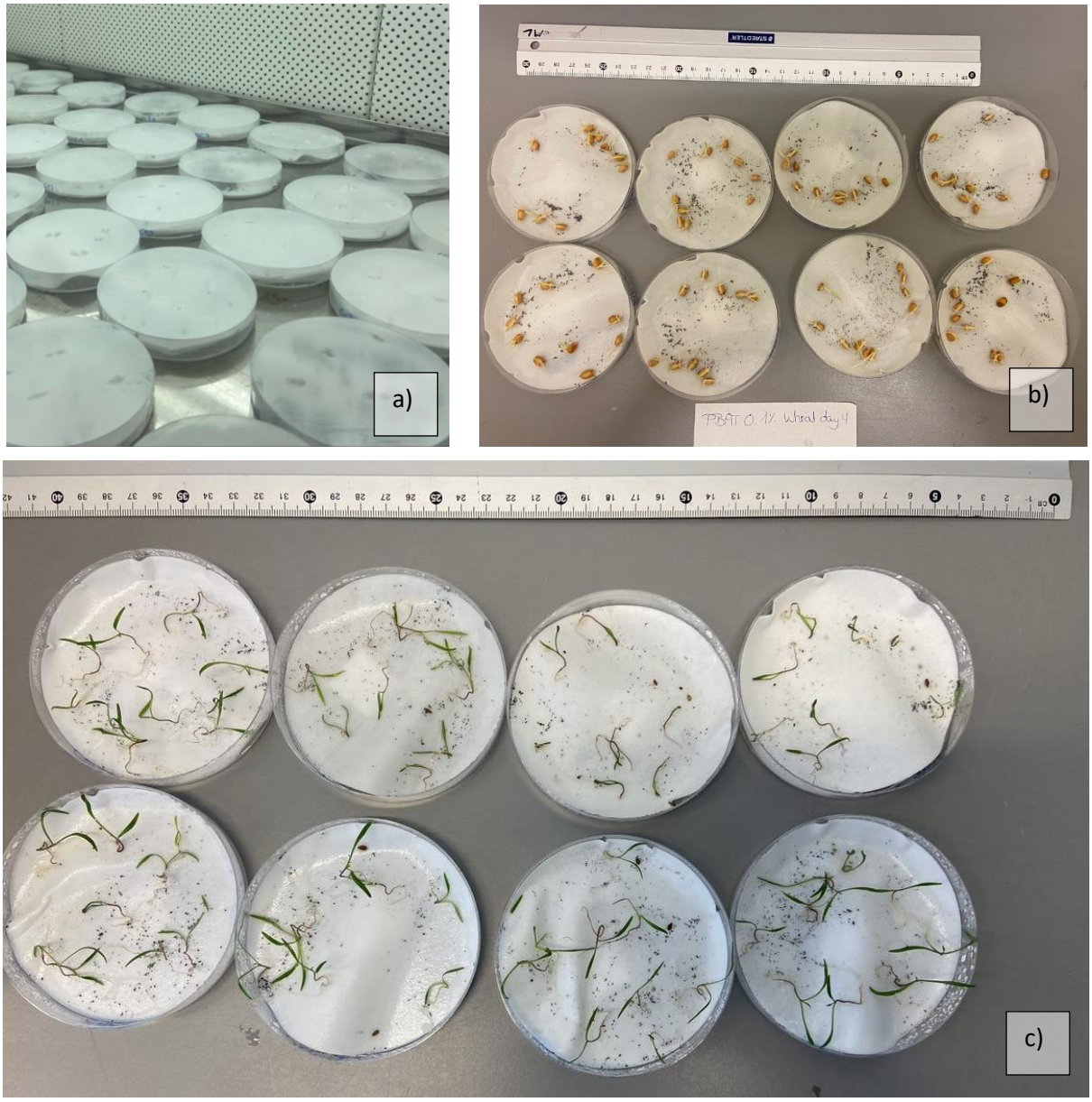

Figure 1.1. a) Experimental setup of seed germination of lettuce buds exposed to different concentrations of LLDPE and starch-PBAT blend MPs for 4 days. b) Seed germination of wheat buds exposed to 0.1% starch-PBAT blend MPs for 4 days. c) Seed germination of carrot buds exposed to 0.1% starch-PBAT blend MPs for 14 Photos: Laura Zantis.

1.2. Chronic pot-plant experiment

Using a pot-plant setup, we aim to elucidate how LLDPE and PBAT-BD-MPs affect the plant growth and development (chronic testing) of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa*).

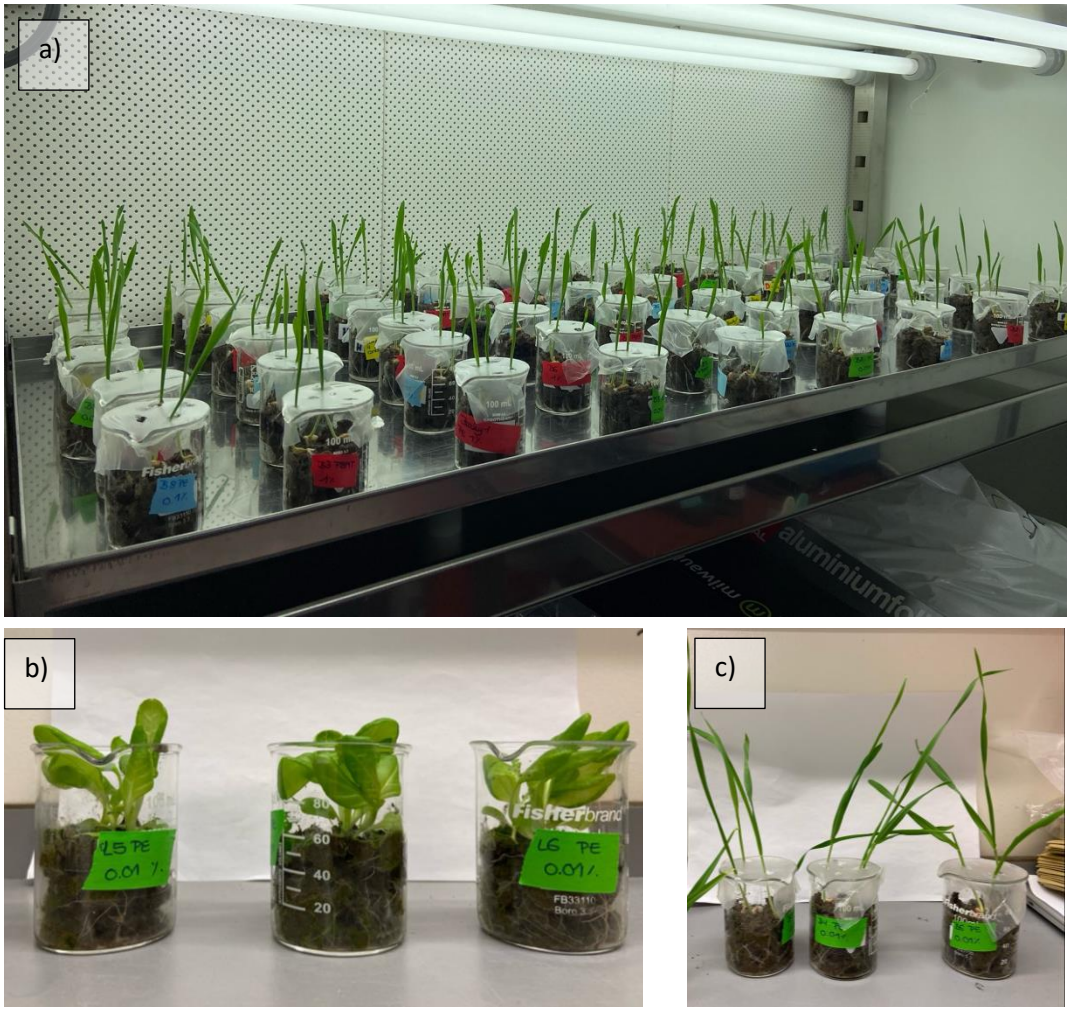

Figure 1.2. a) Experimental setup of barley exposed via soil to LLDPE and starch-PBAT blend MPs. This picture was taken after 10 days of exposure. b) Growth of lettuce seedlings after exposure to 0.01% w/w LLDPE MPs via the soil for 21 days. c) Growth of barley seedlings after exposure to 0.01% w/w LLDPE MPs via the soil for 14 days. Photos: Laura Zantis.

2. Mesocosm experiments

2.1. CLIMECS 1

Using a mesocosm setup as a model of terrestrial ecosystems containing a plant (lettuce, *Lactuca sativa*), natural soil, soil microorganisms, and soil invertebrates, we aim to elucidate how PBAT-BD-MPs affect plants under environmentally relevant conditions.

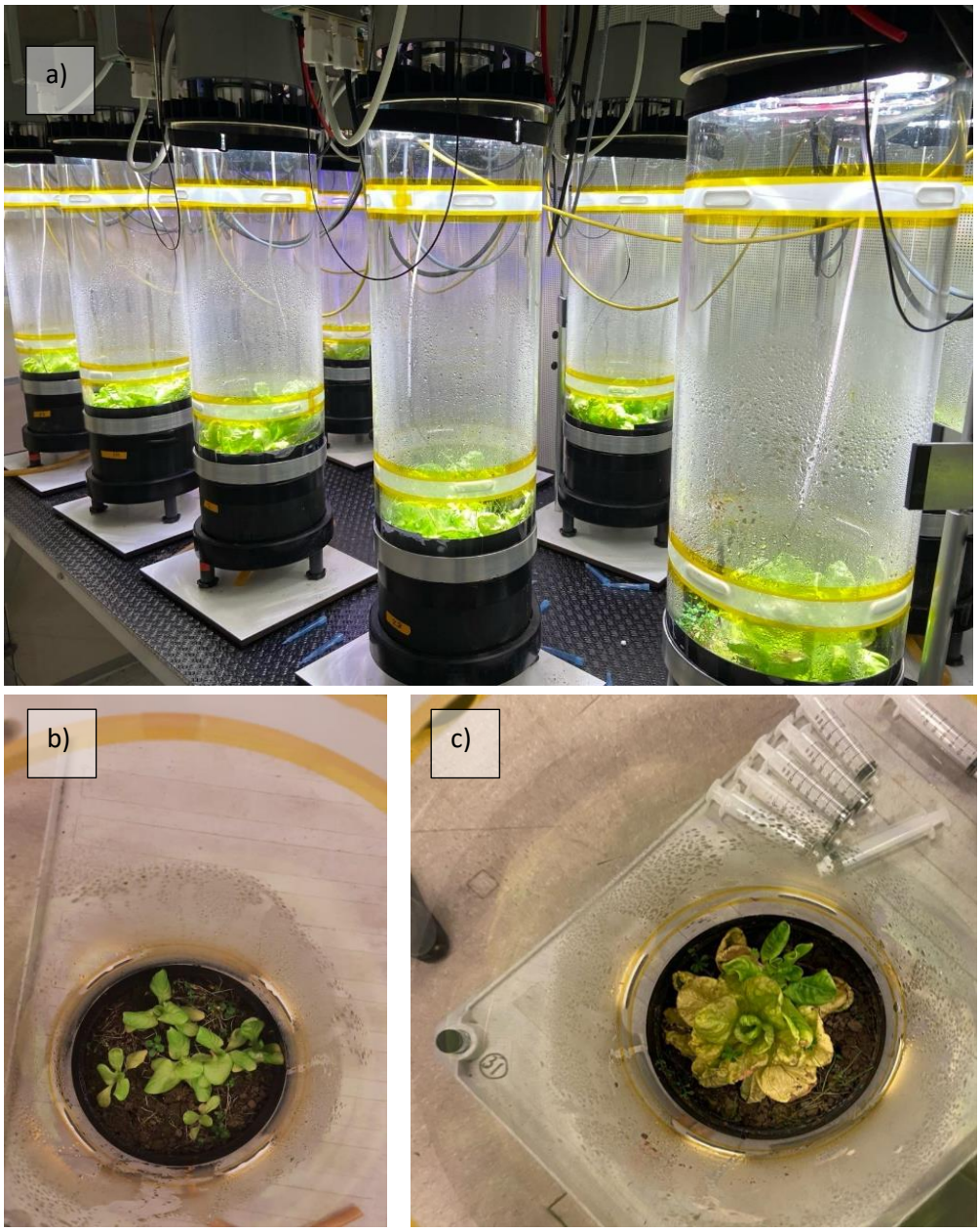

Figure 2.1. a) CLIMECS columns filled with soil and lettuce. This picture was taken after 8 weeks of growth and the soil was mixed with starch-PBAT blend. b) Top view of one CLIMECS column filled with soil and lettuce. This picture was taken after 4 weeks of growth and the soil was mixed with starch-PBAT blend. c) Top view of one CLIMECS column filled with soil and lettuce. This picture was taken after 8 weeks of growth and the soil was mixed with 0.05% w/w starch-PBAT blend. Photos: Laura Zantis.

2.2. CLIMECS 2

The second experiment run in the CLIMECS facility focused on fate of MPs in soil. More specifically, we investigated the influence of different watering regimes and the presence or absence of earthworms on the vertical transport of MPs through soil columns. We also assessed the impact of different mixtures of LLDPE and PBAT-BD-MPs on the growth of lettuce (*Lactuca sativa*).

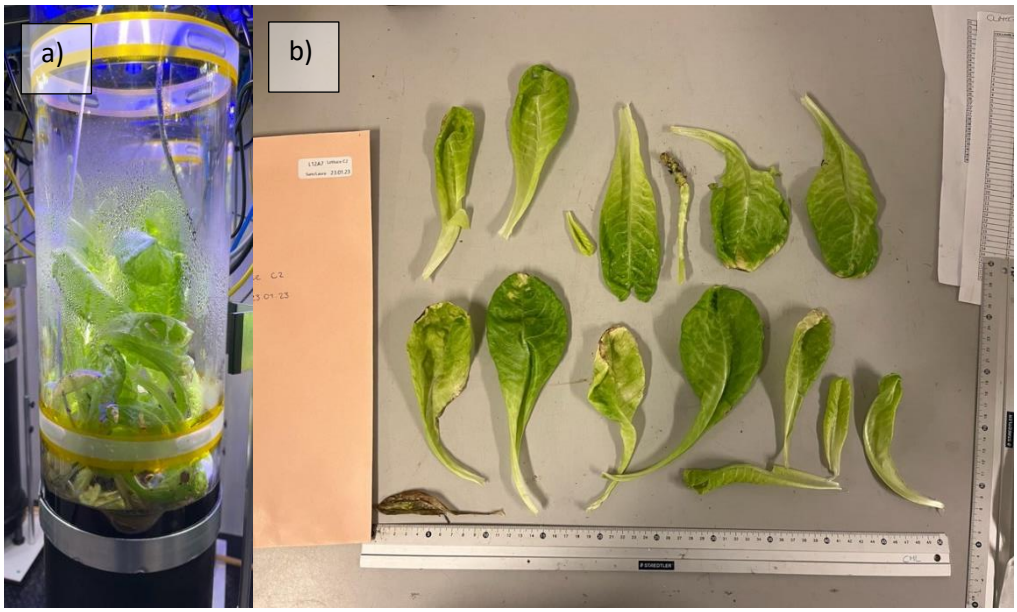

Figure 2.2. a) CLIMECS columns filled with soil and lettuce. This picture was taken after 12 weeks of growth. b) Measurement of total leaves area of lettuce seedling grown in a CLIMECS columns filled with soil. This picture was taken after 12 weeks of growth. Photos: Laura Zantis.

2.3. CLIMECS 3

Using a mesocosm setup as a model of terrestrial ecosystems containing a plant (lettuce, *Lactuca sativa*), natural soil, soil microorganisms, and soil invertebrates, we aim to elucidate how LLDPE and PBAT-BD-MPs affect plants under environmentally relevant conditions. To enable comparison of the results of this experiment with those of the first CLIMECS experiment, three concentrations of LLDPE MNPs will be tested and one concentration of PBAT MNPs, the latter being similar to the highest one tested in the first CLIMECS experiment.

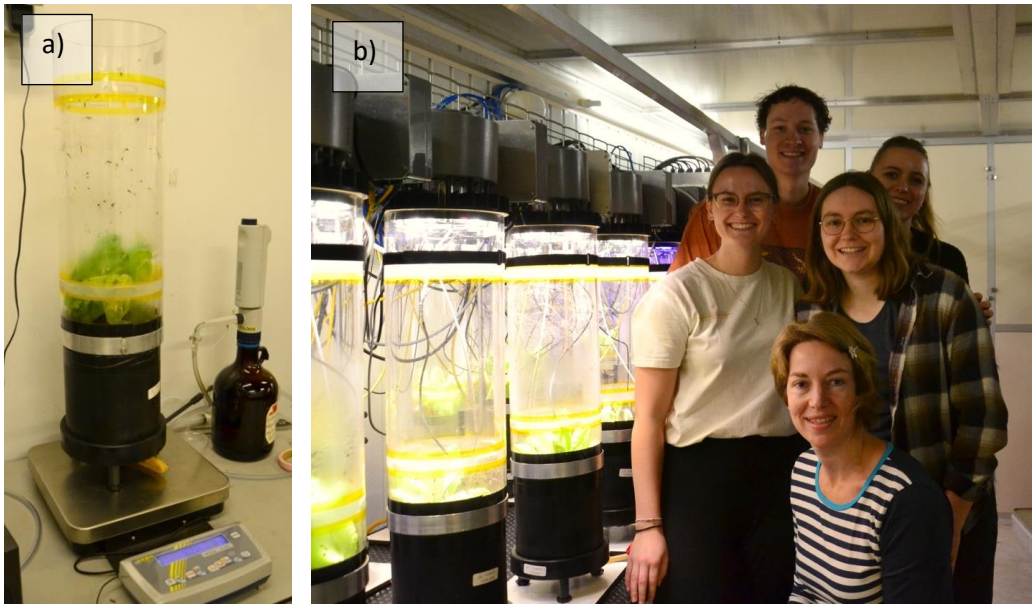

Figure 2.3. a) Weight measurement of a CLIMECS columns filled with soil and lettuce. This picture was taken after 12 weeks of growth. Photo: Laura Zantis. b) Group picture (from left to right, from top to down) with Sam van Loon (Vrije Universiteit Amsterdam - VU), Nienke Schut (VU), Laura J. Zantis (Leiden University - LEI), Lotte de Jeu (VU) and Klára Šmídová (RECETOX) during the final sampling of CLIMECS 3. Photo: Klára Šmídová.

2.4. ^{14}C experiment

To track the fate of the nanoparticles in the complex matrix soil, 14C labelling of the nanoparticles is used. Due to the difficult production of ^{14}C-labeled nanoparticles, this part of the project has been considerably delayed.

Figure 2.4. Soil column setup in a rack with several soil columns, column bases and brown glass bottles for leachate collection. Photo: Fabian Suttner.

3. Field experiment

We are studying, the effect of conventional (PE) and bio-plastics (PBAT) on plant health in the field scale in Finland, Spain and Germany.

Figure 3.1. Field plot experiment with barley (Hordeum vulgare) in Jokionen, Finland. Photos: Sylwia Adamczyk

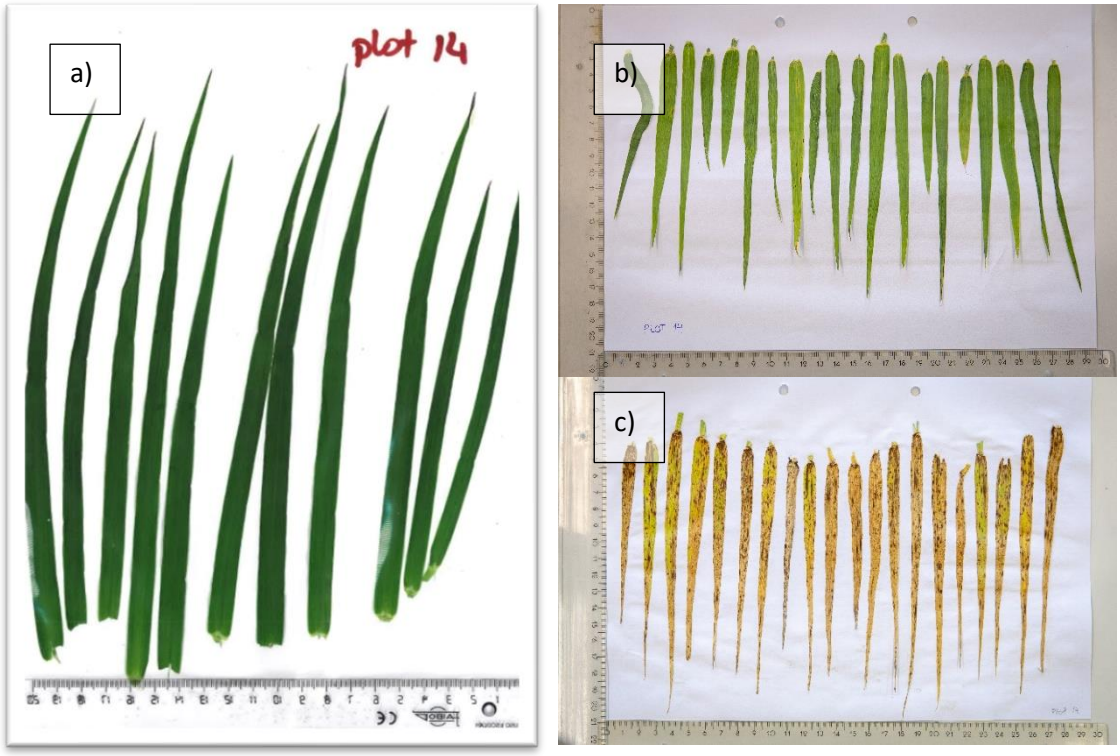

Figure 3.2. Leaves of barley prepared for specific leaf and necrotic tissue analyses in different growing stage (GS) (a- GS32, b- GS38, c- GS58). Photo: Sylwia Adamczyk

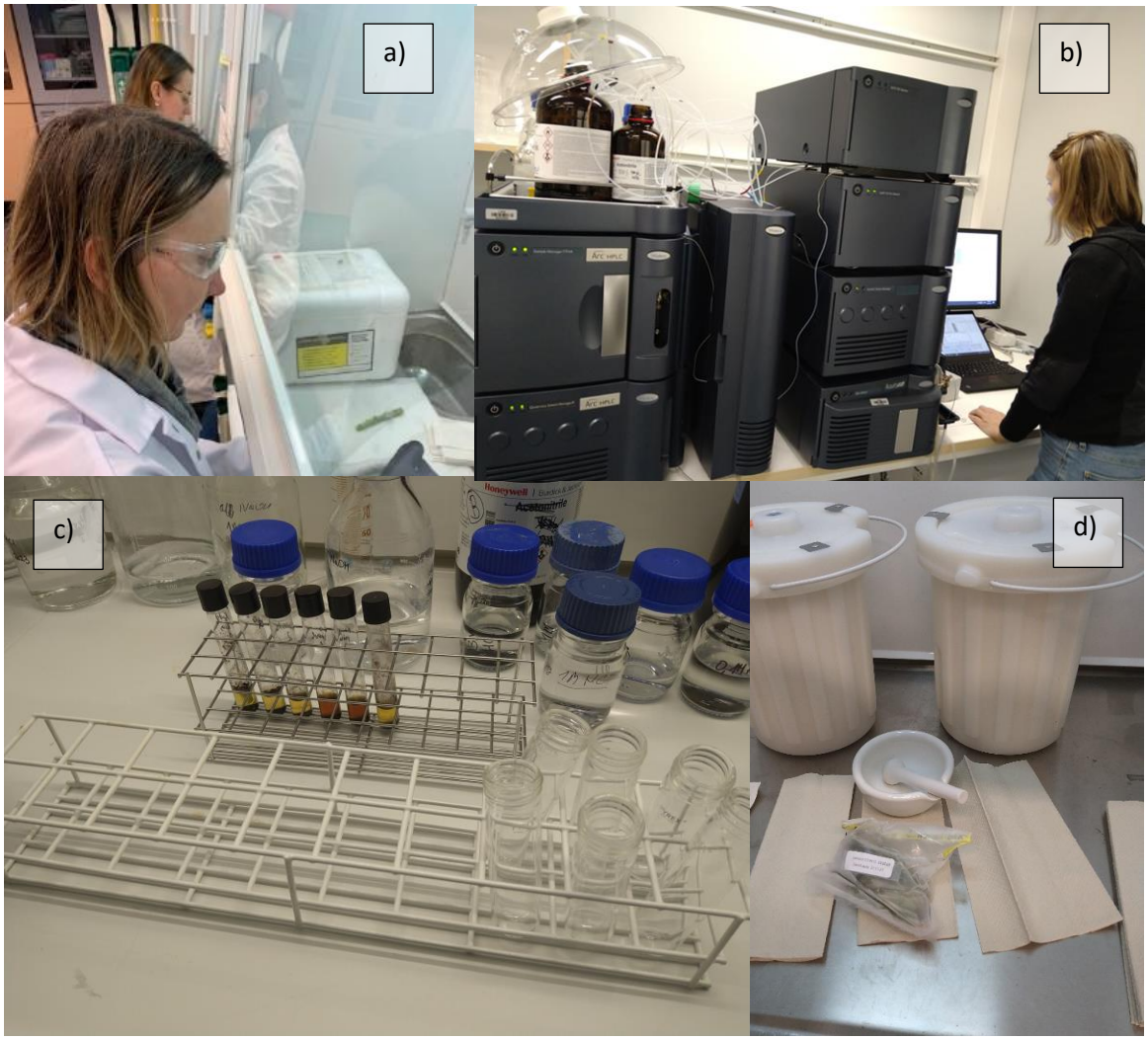

Figure 3.3. Analysing of samples from field experiment in Luke's laboratory, Helsinki. Preparing samples by grinding in liquid nitrogen, (a) in the front Sylwia Adamczyk. Photos: Sannakajsa Velmala and Sylwia Adamczyk

4. Leachate experiment

4.1. Seed germination and early development

In contrast to the previous experiment (section 1), in this experiment, we used the leachate of LLDPE and PBAT-BD-MPs. We aim to elucidate how the plastic additives and breakdown products of LLDPE and PBAT-BD-MPs affect the seed germination and early development (acute testing) of two monocotyledonous plants (barley, *Hordeum vulgare*, and wheat, *Triticum aestivum*) and two dicotyledonous plants (carrot, *Daucus carota*, and lettuce, *Lactuca sativa*).

4.2. Chronic pot-plant experiment

In contrast to the previous experiment (section 1), in this experiment, we used the leachate of LLDPE and PBAT-BD-MPs. We aim to elucidate how the plastic additives and breakdown products of LLDPE and PBAT-BD-MPs affect the plant growth and development (chronic testing) of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa*).

Publications

Adamczyk et al. Biodegradable microplastics induce profound changes in lettuce (*Lactuca sativa*) defense mechanisms and to some extent deteriorate growth traits in mesocosm experiment. Submitted to review.

Zantis et al. 2024. Comparing the impact of microplastics derived from a biodegradable and a conventional plastic mulch on plant performance. Science of the Total Environment 935, 173265, https://doi.org/10.1016/j.scitotenv.2024.173265