CLIMECS and ^{14}C experiments completed

Milestone no. 7

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Summary report

A key objective of the PAPILLONS project is to understand the fate and effects of micro- and nanoplastics in soil ecosystems. Within the project, a series of experiments were designed to track this at different scales of environmental control and environmental realism. Mesocosm experiments bridge the gap between laboratory experiments and field studies, offering a degree of control over environmental parameters whilst also simulating ecosystems. Two such experiments were conducted to investigate the fate and effects of both micro- and nanoplastics, respectively.

CLIMECS experiments

CLIMECS stands for CLImatic Manipulation of ECosystem Samples. It is a facility established at the Vrije Universiteit Amsterdam that houses 40 soil columns which are individually controlled for different environmental conditions, such as temperature, humidity, and precipitation. The columns can be established to simulate soil ecosystems – with the addition of soil, vegetation, and fauna.

In the PAPILLONS project, three different CLIMECS experiments were performed (Table 1). Two experiments focused primarily on tracking effects of microplastics, whilst one was designed to investigate fate. In each experiment, Lufa 2.2 soil with a native microorganism community was used to reconstitute soil columns of different lengths. Springtails and earthworms were added and the columns were planted with lettuce (Figures 1 & 2). The experiments were maintained for a period of 12-13 weeks. Several endpoints were tested across the different experiments, including MP content in soil and earthworms, soil concentrations of plastic additives, growth and stress parameters of the lettuce, earthworm survival and stress indicators, microbial activity, and soil properties such as pH and penetrability (Figure 3).

Microplastic test materials produced from real agricultural mulching films by WP1 were used across all three experiments. The first experiment used one polymer type: starch-polybutylene adipate terephthalate (PBAT) blend, a certified biodegradable-in-soil material commonly used for mulching films in the European market. The second experiment used a 1:1 mixture of starch-PBAT blend and low density polyethylene (LDPE) microplastics. The final experiment replicated CLIMECS 1, but with LDPE particles – with one test concentration of PBAT included to compare CLIMECS 1 and CLIMECS 3.

Two experiments were initially planned in the PAPILLONS project, and these were completed prior to the deadline for this milestone (Month 27). However, the findings from CLIMECS 1 and 2 warranted further investigation and a third experiment was designed and executed.

Table 1. Summary of the three CLIMECS experiments included in the PAPILLONS project

Experiment	Primary focus	Timing (duration)	Test materials
CLIMECS 1	Effects	M10-M13 (3 months)	PBAT MPs
CLIMECS 2	Fate	M17-M20 (3 months)	PBAT and LDPE MPs
CLIMECS 3	Effects	M27-M30 (3 months)	LDPE MPs, with one test concentration using PBAT MPs

Figure 1. Photographs of the CLIMECS facility

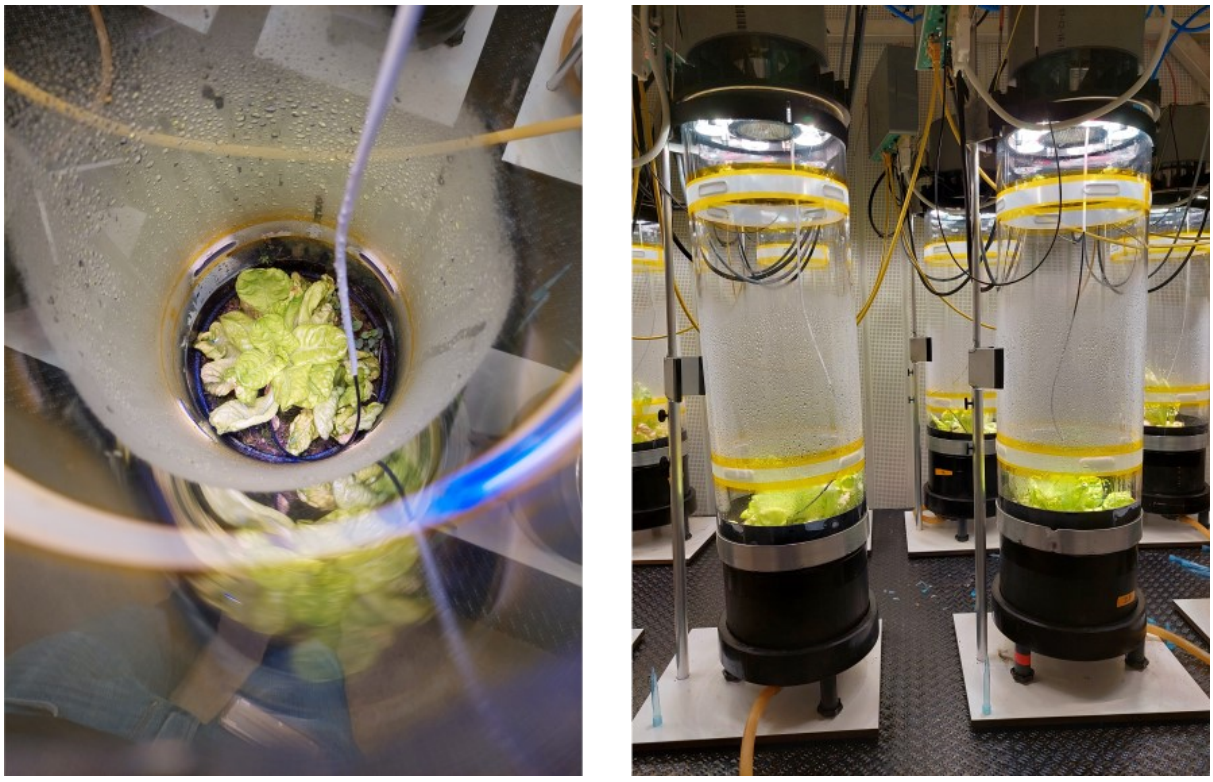

Figure 2. Photographs from the maintenance of a CLIMECS experiment (CLIMECS 1)

Figure 3. Photographs of the sampling for different endpoints at the end of a CLIMECS experiment (CLIMECS 2)

^{14}C experiment

Whilst the CLIMECS experiments utilised test materials derived from mulching films and this may have contained a proportion of particles in the nanoplastic size range, these could not be identified given challenges in analytical detection of small particles in complex environmental matrices. Yet, PAPILLONS also aimed to understand the fate and effects of nanoplastics in soil. To specifically address this, a second mesocosm experiment was designed. This was hosted by Forschningszentrum Jülich in Germany. To overcome the analytical difficulties in identifying and quantifying nanoplastic particles, a batch of nanoplastic particles were synthesised by Nanjing University using ^{14}C labelled polystyrene. This allows for an efficient and quantitative measurement of the particles in different environmental matrices, such as soil, plants and water (e.g., leachate of the soil of the mesocosms), by liquid scintillation measurement directly without isolating particles from the environmental matrices.

Intact soil monoliths were extracted from an experimental field with a known history (the same field that hosted the field plot experiment in Germany) and allowed to settle for a period of 3 months. A portion of soil was homogenised and spiked with ^{14}C nanoplastics and added as an upper 10 cm layer. Four columns were sown with spring barley (*Hordeum vulgare*) and five columns with lettuce (Lactuta sativa). In parallel, five control columns were sown for lettuce and six columns for winter barley, without any addition of nanoplastics. The experiment was maintained in outdoor conditions for a period of three months. During this time, samples of plants at different growth stages were collected to effects endpoints and to determine nanoplastic uptake. Leachate collected from the base of the columns was also analysed. Figure 4 shows photographs taken at different stages during the experiment.

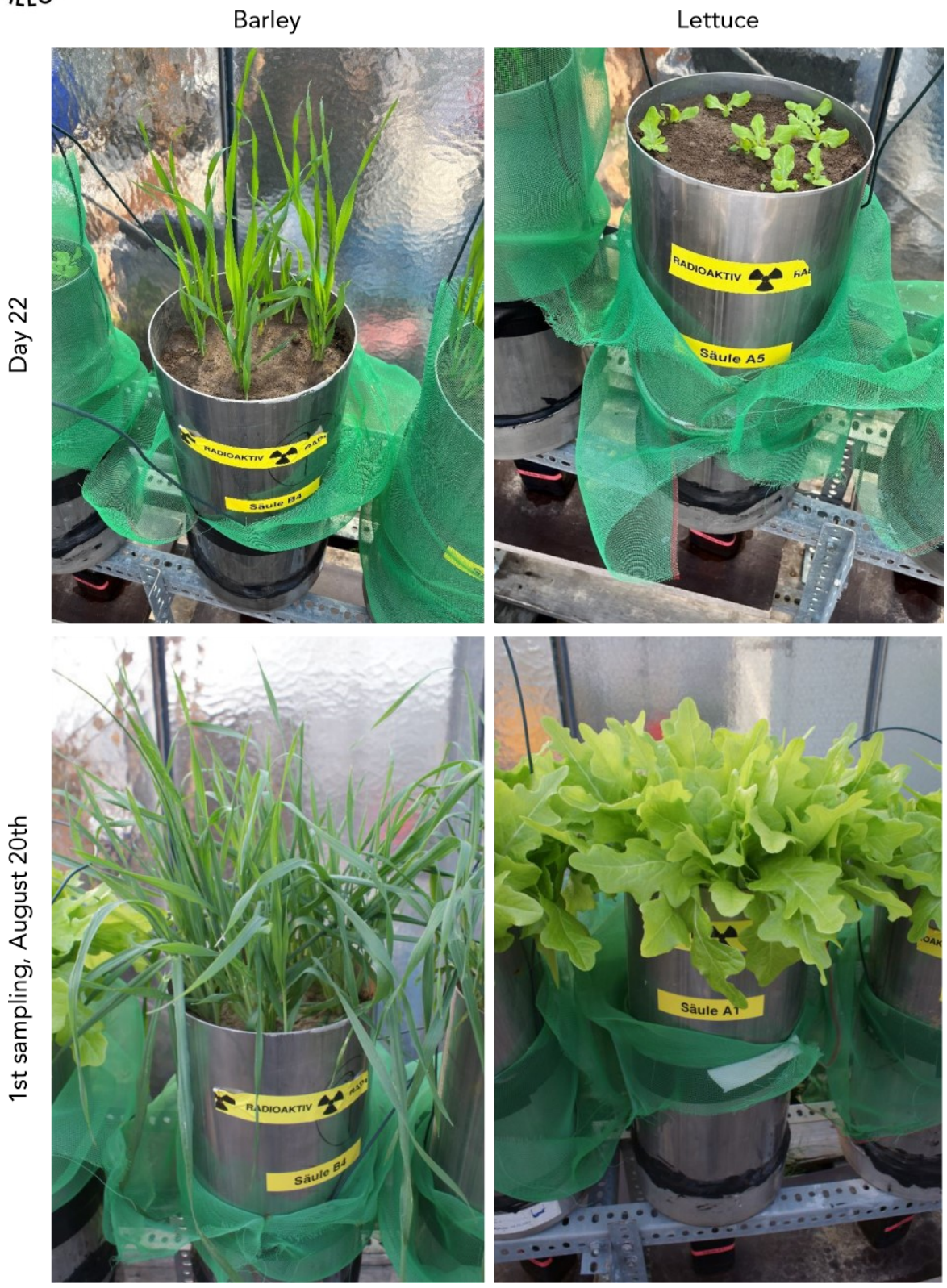

Figure 4. Photographs from the duration of the ^{14}C experiment

The ^{14}C experiment experienced a series of delays which postponed the onset by several months, and placed the completion of this milestone in delay. First, the production of the test materials was challenging and required some additional time to generate a sufficient amount of material in the target size of 100 nm, with a high standard of quality. This was successfully achieved with a small delay. Following the production of the test materials, a further delay was incurred due to logistical challenges associated with shipping ^{14}C radiolabelled materials from China (NJU) to the experimental site in Germany (FZJ). The material was successfully delivered during Autumn 2023, which represented a substantial delay, as this was aligned with the expected conclusion of the experiment (M27). As the ^{14}C mesocosm is run outdoors, the timing of the experiment depends on seasonality. As such, it was necessary to postpone the experiment until Spring 2024. A long period of inclement weather in Germany during Spring 2024 (heavy precipitation and flooding) delayed the commencement of the experiment until July 2024 as it prevented the soil monoliths from being extracted due to waterlogged conditions in the field. The experiment eventually commenced in July 2024 (M38) and ran until October (M41), which marked the completion of this milestone.