Completed experiments of the effects of MNPs and additives on single invertebrates species

Milestone no. 12

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Document Information

Work Package	WP3
Lead beneficiary	SYKE
Due date	M40
Delivered	M41

Summary report

We designed single-species testing approach using an array of organisms from different taxonomic groups focusing in particular on multi-generation testing. We employed different methodological approaches and also faced several challenges in testing which were successfully optimised (**Figure 1**).

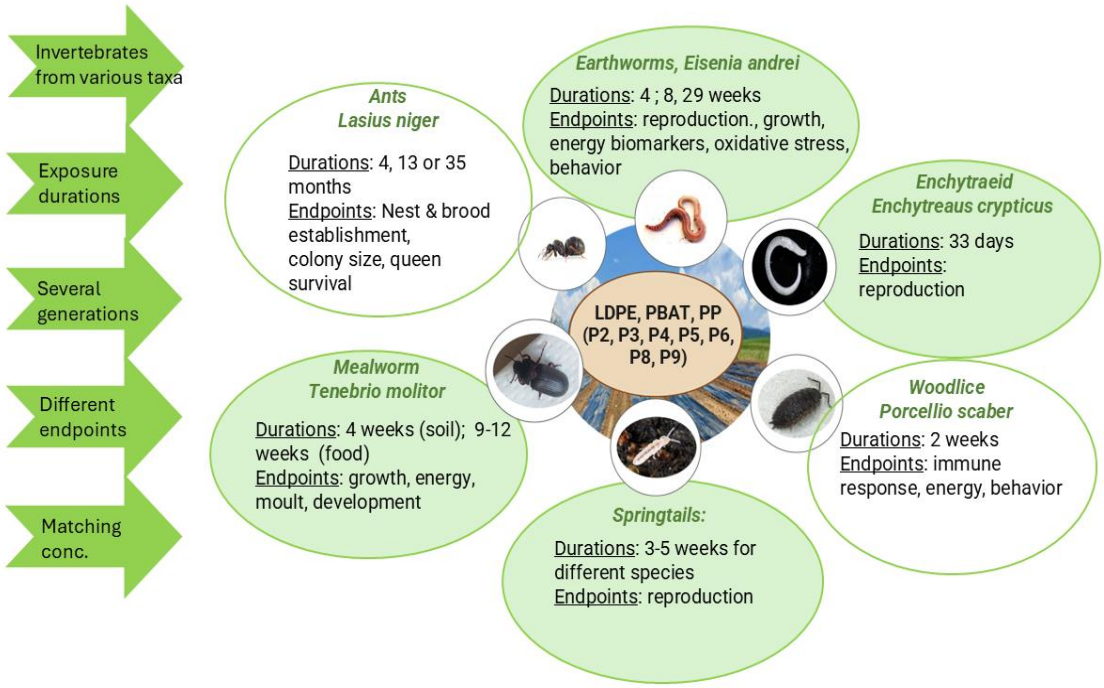

Figure 1. Summary of the integrated approach implemented in PAPILLONS. Green ballons resemble multigeneration testing.

We have performed several experiments employing different test organisms, endpoints and durations of exposure as listed in **Table 1**.

Table 1. List and details of performed experiments.

Lead beneficiary	Test organism	Number of generations / Duration of exposure	Endpoints	Number of tests and test materials

SYKE	Earthworm *Eisenia andrei*	2 generations / P generation 4 weeks, F1 generation 6-7 months	survival, reproduction, growth, energy-related traits	Two tests, one with P3 and one with P4
SYKE	Earthworm *Eisenia andrei*	1 generation / 8 weeks	survival, reproduction, growth, biomarkers for oxidative stress	Two tests, one with P5 and one with P6
SYKE	Earthworm *Eisenia andrei*	48 hours	Avoidance behaviour	One tests with P8 and P9
MU	enchytraeid *Enchytreaus crypticus*	2 generations / 4 weeks for each generation	survival, reproduction	Six tests, one with each test material (P3, P4, P5, P6, P8, P9)
VU	springtails *Sinella curviseta; Ceratophysella denticulata, Folsomia candida, Heteromurus nitidus*	1 generation / 3-4 weeks	survival, reproduction	Four tests, one with each test material (P3, P4, P5, P6)
VU	Springtails *Folsomia candida*	1 generation / 4 weeks	Survival, reproduction	Two tests, one with P8, one with P9
VU	Springtails *Folsomia candida*	5 generations / 4-5 weeks for each generation	survival, reproduction	Two tests, one with P3 and one with P4
MU	nematodes *Caenorhabditis elegans*	1 generation	survival, reproduction	Four tests, one with each test material (P3, P4, P5, P6)
UL	Woodlice *Porcellio scaber*	1 generation / 2 weeks,	survival, feeding, immune response, energy biomarkers	Four tests, one with each test material (P3, P4, P5, P6)
UL	Mealworms *Tenebrio molitor*	2 generations / 4 months	survival, development, moult, growth	Two tests, one with each test material (P3, P4)
UL	Woodlice *Porcellio scaber*	1 generation / 2 days	Behaviour tests	Four tests with group woodlice exposure, two with each material (P8, P9) Six tests with individual woodlice exposure, three with each material (P8, P9)
UBT	ants *Lasius niger*	1 generation / 4 months (MNPs in soil)	colony founding, growth, colony size, survival	P5 and P6
UBT	ants *Lasius niger*	1 generation / >1 year (MNPs in food)	Colony development, survival, colony size	P5 and P6

UBT	ants *Lasius niger*	1 generation / >2 years (MNPs in soil)	Colony development, survival, colony size	P5 and P6

P3: M-rPE-black-A0; P4: M-rBIO-black-A0; P5: M-BIOEL-15-black-A0; P6: M-PEDE-45-black-A0, P8: C3-PPDE-200-black-A0; P9: C4-PPDE-50-white-A0

Experimental set-up with earthworms

Figure 2: A) Two fully grown earthworms being washed before weighing, B) Pre-experiment: Juveniles after four weeks incubation in abundances of 10, 20 and 40 juveniles per jar, C) Removing of the juveniles from the jars using warm water bath, D) Test jars

Experimental set-up with enchytraeids

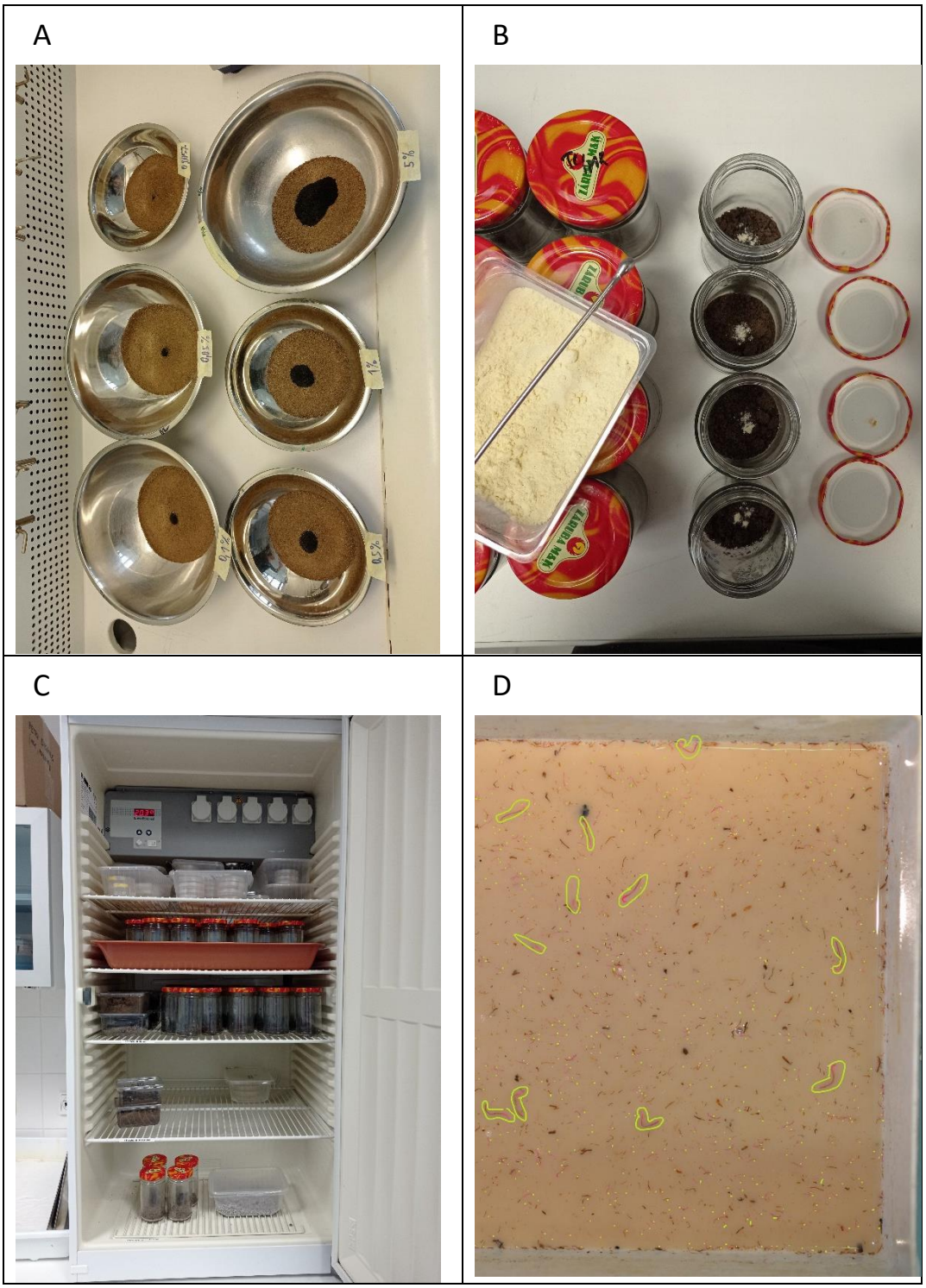

Figure 3: Experimental set-up with enchytraeids: A)Lufa soil with MPs before contamination, B) A look inside the test vessels with the soil, test animals (hidden in the soil) and food (corn flour), C) all test jars for one generation and two tested material in the incubator, and D) counting the juveniles and adults using a floating method..

Experimental set-up with nematodes

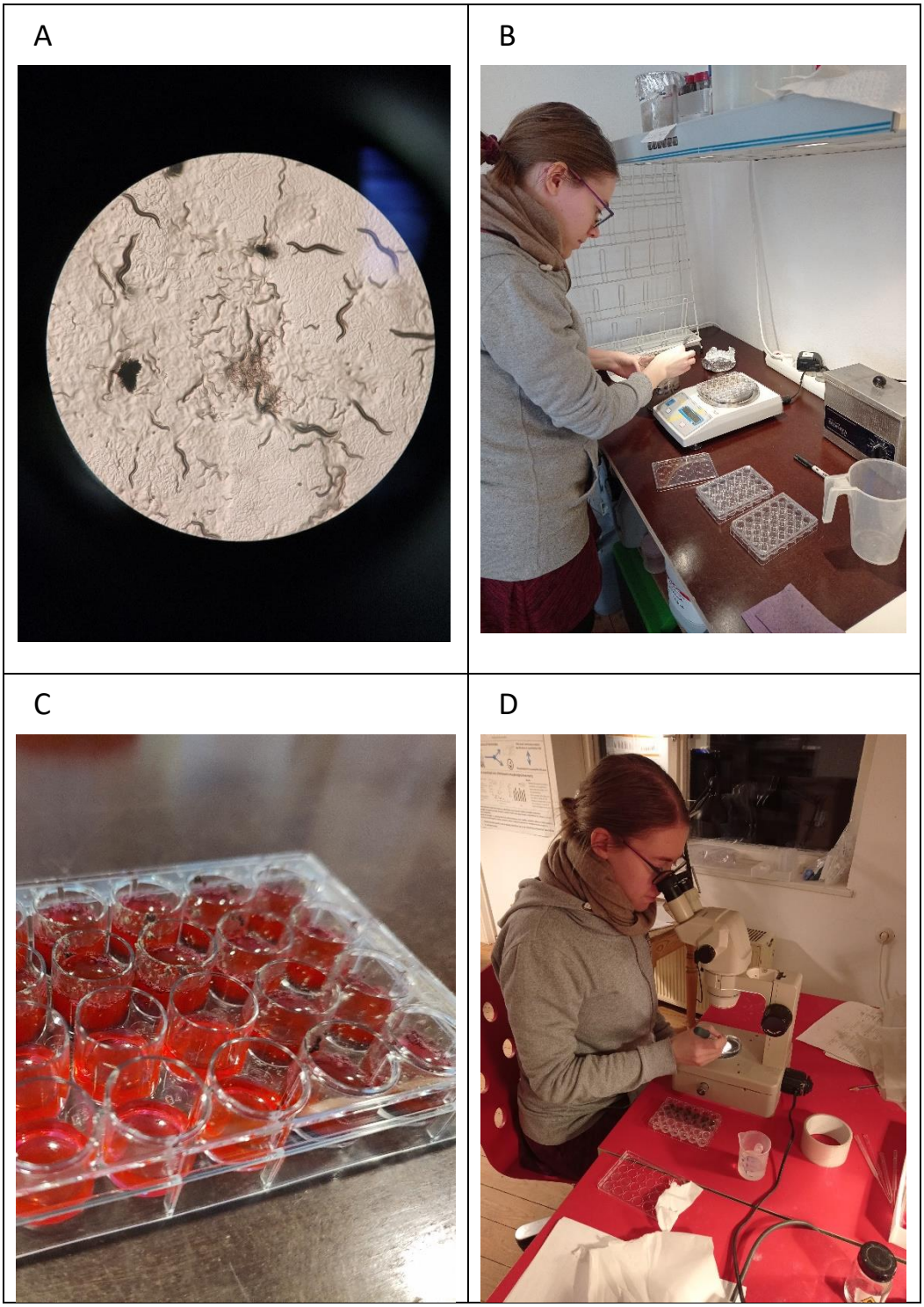

Figure 4: Experimental set-up with nematodes: A) A nematode culture under the microscope, B) Weighting the contaminated soil into the 24-well plate plates, C) Finished laboratory test in the testing plate, and D) counting juveniles and adults under the microscope.

Experimental set-up with springtails

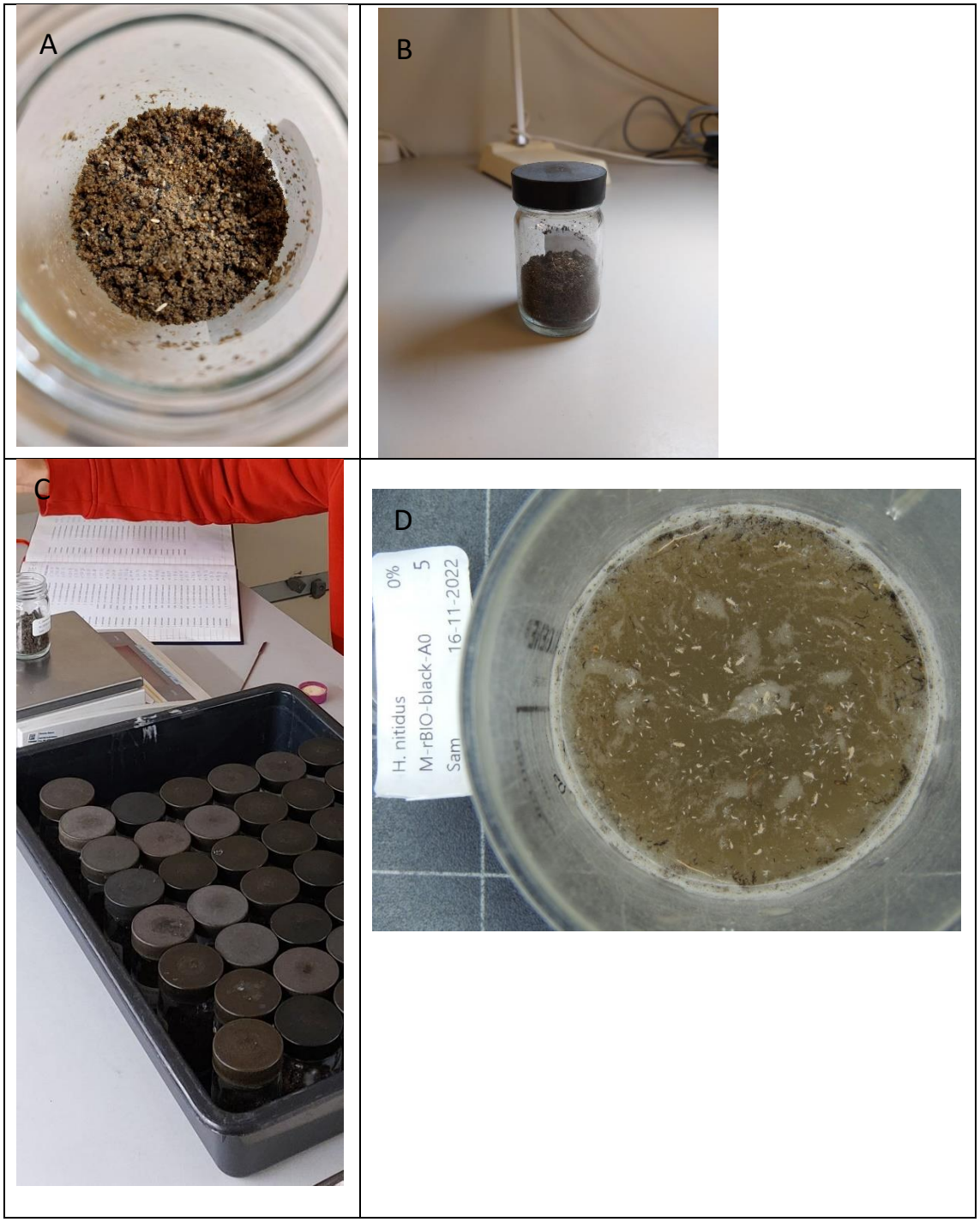

Figure 6: Experimental set-up with springtails: A) Inside test jars, with soil, animals, black reference material and yeast, B) test jar, C) all test jars are weighed weekly to weigh how much water evaporated and should be replenished, as well as food is added, D) picture made at the end of an experiment, used for counting adults and juveniles

Experimental set-up with woodlice

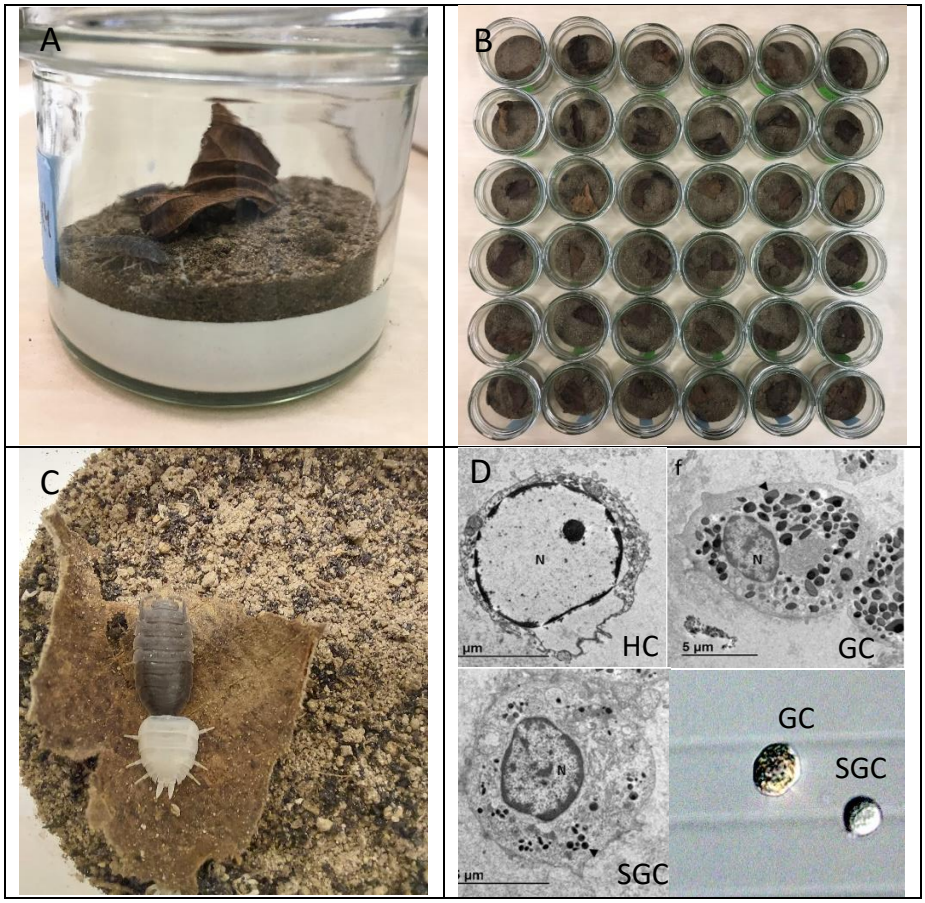

Figure 7. Experimental set-up with woodlice: A) test jar with plaster, soil and leaf; B) all test jars, C) woodlice on soil with microplastics, animal just moulted; D) different types of immune cells in woodlice haemolymph (after Dolar et al., 2020. Developmental & Comparative Immunology, 113, 103789.) SGC-semigranulocytes, GC-granulocytes, HC-hyalinocytes.

Experimental set-up with mealworms

Figure 8: Experimental set-up with mealworms: A) test jar with plaster and soil; B) larvae with marked black stripe, C) different life-stages of mealworms D) freshly moulted larvae and exuviae, E) set-up in food exposure

Experimental set-up with ants

Figure 9. Soil exposure experiments. Long term test B: A) gentle collection of mated queens with a custom-made vacuum suction; B) collected queens stored in boxes for transport to the laboratory; C), D) ant colony growth over 1 year; E), F) preparation of soil mixtures with MNP for long-term soil exposure experiments; G) Replacing colonies in the soil. After a few days, the ants rearranged their nests in the soil and the tubes were removed; H) overview of the test in the climate chamber.

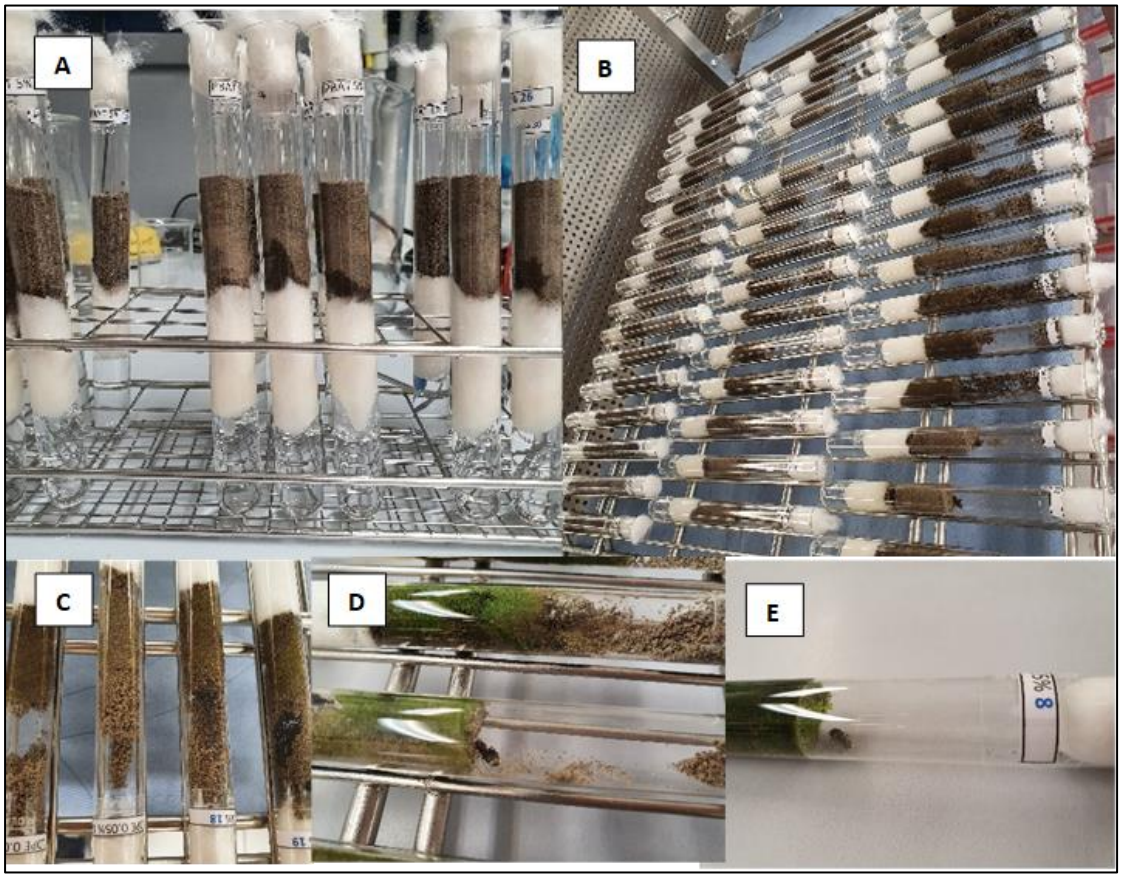

Figure 9. Soil exposure experiments with ants. Colony founding test A: A) preparation of ant housing for a colony founding test; B) overview of an ongoing test; C), D) ants digging nests; D), E) ants without excavated nests.