Completed laboratory studies on the influence of MNP's on soil properties

Milestone no. 11

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Summary report

Here, we report the activities carried out in the laboratory scale to elucidate the effects of MNPs on physicochemical soil properties (Objective 3.1).

The activities focused on properties crucial for soil fertility and biota. This included measuring soil pH, soil aggregation, and water retention characteristics. Effects of one and/or two types of MNPs on water retention characteristics i.e., pore-size distribution and water holding capacity (WHC), and aggregation were studied in controlled laboratory experiments using different types of agricultural soils, including the LUFA 2.2 soil and the soils from the Papillons field plot experiments. In addition, the impacts of four different types of MPs on soil pH and WHC were studied using the LUFA 2.2 soil spiked with MNPs for the single species invertebrate tests. These measurements were done in 3-4 laboratories (REC, SYKE, UL, VU) using the same methodology.

Table 1. Overview of the analyses reported in this Deliverable and of the used MNP test materials in reported studies. All materials were produced by cryomilling.

Soil Properties	Status	PAPILLONS Code for used MNP	Primary polymer type	Source material
Soil water retention characteristics (LUKE)	IP	M-rPE-black-A0; P3 M-rBIO-black-A0; P4	LLDPE Starch-PBAT blend	Recycled film pellets Recycled film pellets
Soil aggregation (FUB)	final	M-rPE-black-A0; P3 M-rBIO-black-A0; P4	LLDPE Starch-PBAT blend	Recycled film pellets Recycled film pellets
Soil aggregation (BONN)	final	M-rPE-black-A0; P3	LLDPE	Recycled film pellets
WHCs (REC, SYKE, UL, VU)	final	M-rPE-black-A0; P3 M-rBIO-black-A0; P4 M-BIOEL-15-black-A0; P5 M-PEDE-45-black-A0; P6	LLDPE Starch-PBAT blend Starch-PBAT blend LDPE	Recycled film pellets Recycled film pellets Mulching films Mulching films
pH (REC, SYKE, UL, VU)	final	M-rPE-black-A0; P3 M-rBIO-black-A0; P4 M-BIOEL-15-black-A0; P5 M-PEDE-45-black-A0; P6	LLDPE Starch-PBAT blend Starch-PBAT blend LDPE	Recycled film pellets Recycled film pellets Mulching films Mulching films

Soil pH and water holding capacity

Four types of MNPs were used in the experiments: two types of conventional polyethylene microplastics (PE-MP; P3, P6) and two types of biodegradable polybutylene adipate terephthalate microplastics (PBAT-BD-MP; P4, P5). Test materials P3 and P4 were made of ground pellets produced from recycled mulching films and test materials P5 and P6 were made of ground mulching films (Table i). The test materials were spiked in soils in 6-9 concentrations ranging from 0.0016% to 5% (w MNPs/w dry soil mass)

For exposures, MNPs were mixed in with the test soils, as dry powder, after which the soils were moistened to an optimal moisture content for the respective soil organisms, often 50-60% of the

WHC. For details on the single species toxicity tests performed within PAPILLONS, see Deliverable 3.1 (Progress report on single species invertebrate tests).

To assess soil pH-$CaCl_2$, soil samples were shaken for 2 hours with a 0.01 M $CaCl_2$ solution at a ratio 1:5 (w/v). After settling of the soil particles, typically overnight, pH of the suspension was recorded using a pH meter.

For measuring the WHC of the test soil with and without MNPs, the approach described in OECD guideline 232 was used. A portion of soil (typically 30-50 g) was placed on a filter paper in a container with a gauze of filter paper bottom. The container was placed in a tray with enough water to flood the soil. After 3 hours, the container with saturated soil was placed on a sand bed and allowed to drain for 2 hours. Finally, a soil sample was taken and dried at 105 °C to determine its moisture content, which then is defined as its WHC (in % of dry soil).

Soil water retention characteristics

Soils for the experiment were collected from the sites of the field plot experiments of the PAPILLONS project located in Germany (GE), Spain (SP) and Finland (FI). In addition, LUFA 2.2 soil used in mesocosm experiments and in ecotoxicological studies was included. Soils differed according to their particle size distribution, the LUFA 2.2 soil being coarse-textured and the soil from Spain having the highest clay content. The soils were homogenised, passed through a 5-mm mesh-size sieve and stored at 5 °C until the laboratory experiments.

The effect of MPs on soil water retention characteristics and pore-size distribution were studied for two MP types and at three different concentrations. MPs have been characterized in detail in PAPILLONS deliverable 1.1 “A catalogue and batches of MP Preference materials, reference spiked soils and report on validation of pyr-GC/MS method for MNP analysis”. Bulk densities (BDs) were 0.53 and 0.21 g cm^{-3} for M-rBIO-black-A0 (Starch-PBAT blend, referred here as MP1) and M-rPE-black-A0 (LLDPE, referred here as MP2), respectively (Fig 1). The tested MP concentrations were 0.05, 0.5 and 1.25 mass-% and the masses of soil and the two MPs weighed for each treatment are listed in Table 2. Due to the differences in BDs, the volume of the added MP differed between the two materials and therefore also the volumetric percentage of the MP differed within the same treatment between the two MPs. However, the mass-based treatment concentrations were selected in such way that the volumetric percentage of the lighter (lower BD) MP (LLDPE, referred here as MP2) at the highest concentration (1.25 %) falls fairly near the volumetric percentage of the heavier (higher BD) MP (Starch-PBAT blend, referred here as MP1) at the second highest concentration of 0.5 %.

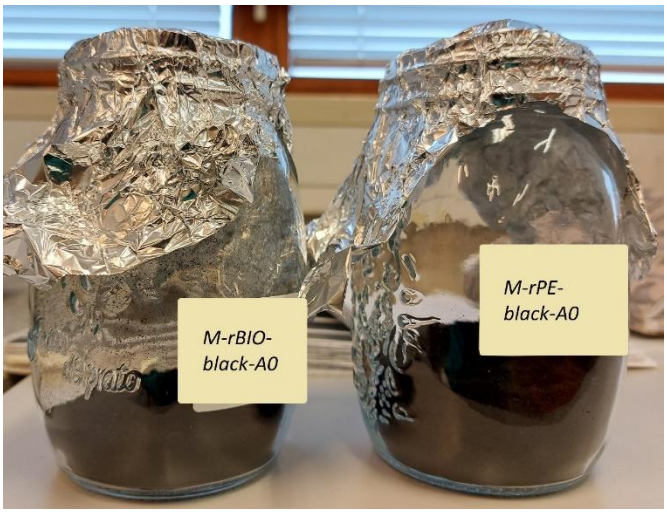

Figure 1. c. 30 grams of each MP (M-rBIO-black-A0, primary polymer type being Starch-PBAT blend and referred here as MP1, and M-rPE-black-A0, primary polymer type being LLDPE and referred here as MP2) used in the laboratory experiment /JOHANNA NIKAMA

Table 2. Masses of soil and MP weighed for 200 cm3 cylinders to reach the mass-based treatment concentrations, volumes of the two different MPS for each soil in each treatment and the resulting volumetric percentage of the MPs in each soil.

	Soil	**MP**		**MP1,** M-rBIO-black-A0 (Starch-PBAT)		**MP2,** M-rPE-black-A0 (LLDPE)	
	mass, g	**g 100 g^{-1}**	**g**	**cm^3**	**% (V/V)**	**cm^3**	**% (V/V)**
LUFA 2.2	240	0.05	0.12	0.23	0.11	0.57	0.29
		0.5	1.20	2.26	1.13	5.70	2.85
		1.25	3.00	5.65	2.83	14.25	7.13
GE	234	0.05	0.12	0.22	0.11	0.56	0.28
		0.5	1.17	2.21	1.10	5.57	2.79
		1.25	2.93	5.52	2.76	13.93	6.96
FI	213	0.05	0.11	0.20	0.10	0.51	0.25
		0.5	1.06	2.00	1.00	5.06	2.53
		1.25	2.66	5.01	2.50	12.64	6.32
SP	221	0.05	0.11	0.21	0.10	0.52	0.26
		0.5	1.10	2.08	1.04	5.25	2.62
		1.25	2.76	5.20	2.60	13.12	6.56

Preparation of the pF-cylinders

Metal cylinders with the volume of approximately 200 cm^3 (diameter 72 mm, height 48 mm) were used in water retention measurements. Packing was done as in Turunen et al (2021). First, each soil type was packed by pouring, with the aid of a collar, approximately 300 cm^3 of loose soil into a bottom-sealed 200 cm^3 cylinder and by compacting the soil with 35 g cm^{-2} weight under vibration generated by dropping a 2.2 kg weight for five times from a 50-mm height at a 100 mm distance

from the cylinder. After the compaction, the collar was removed, and soil surface was carefully levelled to the cylinder rim and the weight of the packed soil was measured (Figure 2). Bulk densities (BDs) of the soil packed into 200 cylinders varied between 1.103 and 1.199 g cm^{-3}.

Figure 2. Packing of cylinders was done with the help of vibration generated by dropping a 2.2 kg weight for five times from a 50-mm height/JOHANNA NIKAMA

Samples amended with the MPs were prepared by thoroughly mixing the desired mass of MPS with a mass of each soil required for filling the cylinder at the desired BD (Table 2.). The cylinders were packed as described above in four replicates. After levelling (Figure 3.), the remaining excess material of MP amended soils was collected and weighed and used in highest suction pressures measured in desiccators by vapour pressure since in the highest suction pressures, it would take a very long time for a whole cylinder to equilibrate to the desired water content. Furthermore, four in-house reference soil samples (clay-% 7, silt-% 19, sand-% 74) per each soil type were included in the determinations and they were prepared in the same manner as the non-amended samples.

Figure 3. Levelling of cylinders after packing.

Water retention measurements

Prior to the water retention measurements, the samples were incubated in a sandbox apparatus (Eijkelkamp, Giesbeek, The Netherlands) for 14 days by raising the (standing) tap water level to the midpoint of the cylinders and thereafter, the free water level was lowered to a level corresponding to a 0.1 kPa suction pressure. The soil moisture characteristic curves were determined by weighing the drying samples after equilibration in suction pressures of 0.1, 0.3, 6.3, 10, 20, 32, 100, 320, 1600 and 39,000 kPa. At the start, the samples were saturated with water from the bottom to the top in boxes with plastic foam at the bottom by holding the water level at the middle of the cylinder height for 5 d. After that the water level in the boxes was lowered to a level corresponding to a suction pressure of 0.3 kPa. Equilibration steps 0.1 and 0.3 kPa were conducted in the sand boxes (Fig. 4.) and steps from 1.0 to 320 kPa on ceramic plates in pressure extractors (Soil moisture Equipment Corp., Goleta, USA) (Fig. 5.). During the measurements, the equilibration times ranged from two to 21 days. After each drying step, changes in the sample height (vertical shrinkage) (Fig. 6.) and diameter (horizontal shrinkage) were measured at six points with a digital vernier calliper and at four points with a feeler gauge, respectively. The highest suction pressures, 1600 and 39,000 kPa, were measured in desiccators by vapour pressure equilibrium with saturated ammonium oxalate and sodium chloride solution, respectively, using unpacked samples (a 1.0 g air-dried, ground with a Culatti´s® Micro-impact mill to pass a 2-mm mesh size sieve).

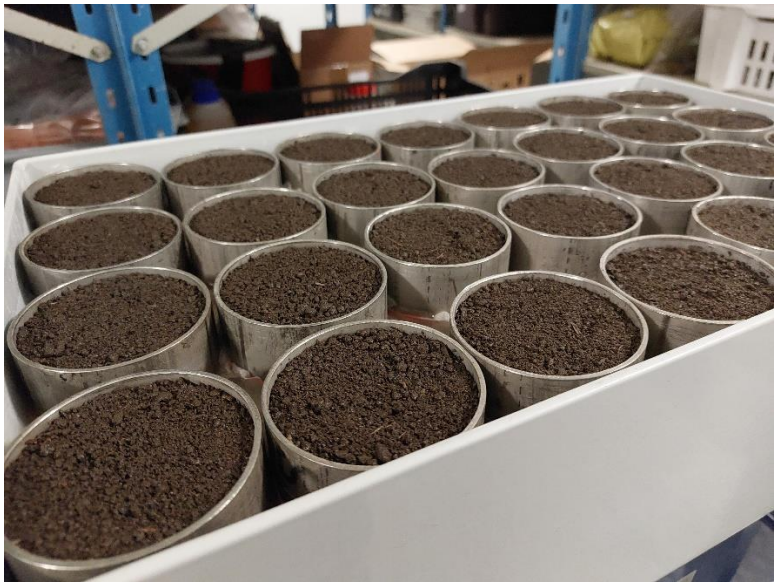

Figure 4. Cylinders in the sand box /JOHANNA NIKAMA

Figure 5. Cylinders on the on ceramic plates in pressure extractors on their way to pressure extractors /JOHANNA NIKAMA

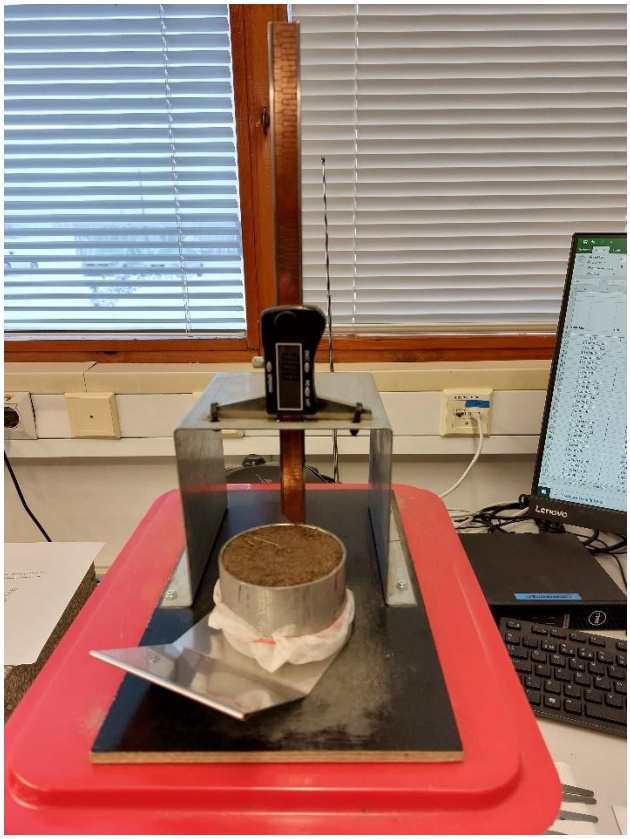

Figure 6. Measuring the shrinkage of the soil within the cylinder /JOHANNA NIKAMA

Effect of MNPs on aggregate stability and embedding of MNP within aggregates

Soils for the aggregate stability experiment were collected from the sites of the field plot experiment of the PAPILLONS project located in Germany (GE), Spain (SP) and in Finland (FI). In addition, LUFA 2.2 soil used in mesocosm experiments was included. The field plot experiments included five treatments: a control without any amendment, and two types of MPs (LLDPE M-rPE-black-A0 and biodegradable Starch-PBAT blend M-rBIO-black-A0) at two different concentrations (0.05% and 0.005%).

Soil samples were air-dried at room-temperature for 1 week and sieved through 4 mm-sieve. Each soil sample (20-21 g) was gently passed through a set of stacked sieves (4000, 2000, 1000, and 250 µm), and the weights of four separated fractions were recorded to determine the proportions (%) of each soil aggregate size class.

Soil samples were air-dried at room-temperature for 1 week and sieved through 4 mm-sieve. Each soil sample (4-4.5 g) was placed into a 0.25 mm-sieve for rewetting in deionized water for 5 min. We used a wet-sieving machine (Eijkelkamp, Netherlands) to apply vertical sieving conditions for 3 min (stroke = 1.3 cm, 34 times min^{-1}), and this process causes slaking of unstable macroaggregates. The samples were separated into water-unstable and water-stable (>0.25 mm) fractions (soil macroaggregates are defined as > 0.25 mm and are retained on the sieve when stable), and each fraction was dried at 60°C for 24 h. The water-stable fraction was corrected for any included debris and sand (i.e., coarse matter), and weighed respectively. The percentage of water stable aggregates (%) was determined as (water-stable fraction – coarse matter)/(4.0 g – coarse matter).

Samples for studying the embedding of MNP's within the aggregates were collected from the 3 sites of the field plots experiments s (Spain, Germany, Finland) – during the first sampling campaign carried out in summer 2022, shortly after harvesting. To ensure that MP contents were still above the background concentration even after aggregate fractionation, analyses were restricted to the LLDPE M-rPE-black-A0 variants (0.05%). For MP analysis, 50 g of air-dried soil were sieved and partially pushed through a 5 mm mesh to remove large debris, ensure consistency, and facilitate sample processing in the subsequent steps. After sieving, a first density separation was conducted to extract the fraction of free", i.e., not in aggregates embedded MPs (Fig. 7).

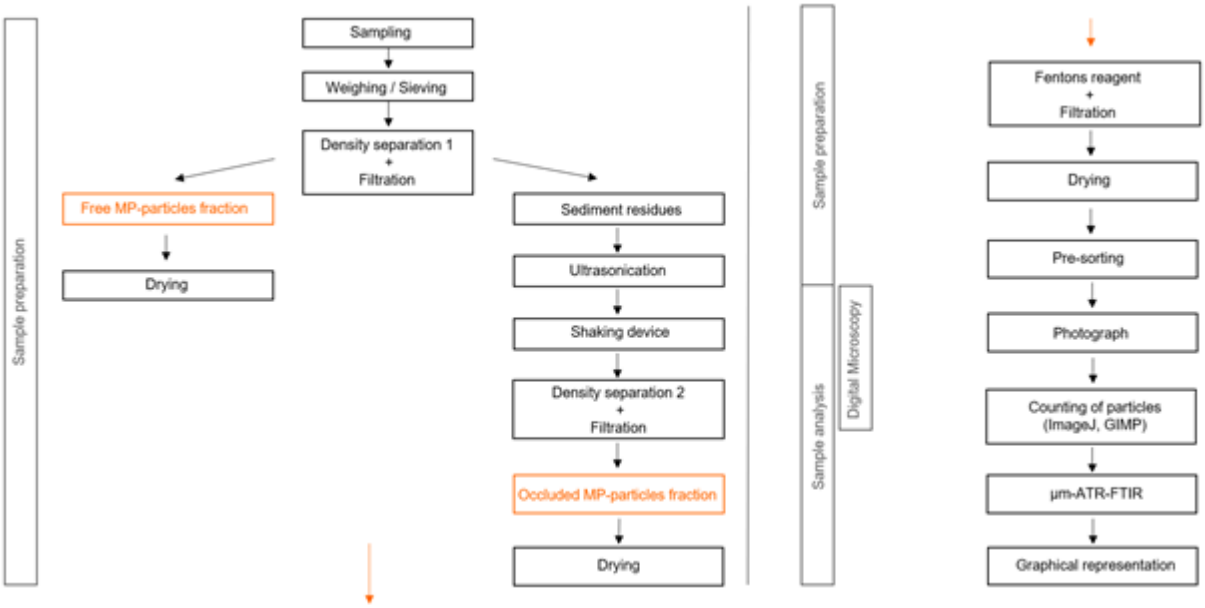

Figure 7. Scheme of the separation of aggregate occluded and not-occluded (free) MNPs, their extraction and analyses

Density separation was conducted according to Braun et al. (2021), using $ZnCl_2$ (technical zinc chloride powder; $ZnCl_2$, UN 2331 zinc chloride, anhydrous, VWR Life Science, Radnor, USA), mixed with distilled water. The density solution was adjusted to a density of 1.8 kg L^{-1}. The sieved soil was placed in a 1000 ml beaker which was then filled up with $ZnCl_2$. This mixture was gently stirred two to three times to avoid destroying the aggregates (Six et al., 2002) and left for at least twelve hours to settle (Möller et al., 2022). The supernatant, representing the free, not occluded fraction, was collected, and filtered through stainless steel mesh filters (Ø 47 mm) with a mesh size of 10 µm. After rinsing the filters with distilled water, they were dried at 40 °C and directly forwarded to soil organic mattertreatment. The remaining sample in the baker, consisting of the aggregate fraction, was subjected to ultrasonication (Branson Sonifier W-250 D) and a horizontal shaking to break down the aggregates. To ensure complete destruction of aggregates 100 J ml^{-1} were used as energy input instead of 60 J mL^{-1} (Amelung et al., 2023).

After the ultrasonic treatment, the samples were shaken in a shaking device (GFL analogues back and forth shaker, type 3018), filled back into a beaker and sedimented for another twelve hours for a second density separation. The supernatant, including MNPs of the occluded fraction was filtered, dried at 40°C and subjected to SOM treatment. For soil organic matter removal, Fentons reagent was used according to a modified protocol of Möller et al. (2022). Extracted MNPs of both fractions were identified via digital microscope (DVM, model: VHX 10000, Keyence) with afterwards manual as well as automated data evaluation. Additionally, to verify that particles identified via microscope were plastic and not other black materials, i.e., black carbon, a portion of particles was additionally analysed via FTIR (Bruker, Lumos II).