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# 1 Nanoplastic occurrence in a soil amended with plastic debris

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9

10 **ABSTRACT:** While several studies have investigated the potential impact of nanoplastics,  
11 proof of their occurrence in our global environment has not yet been demonstrated. In the  
12 present work, by developing an innovative analytical strategy, the presence of nanoplastics in  
13 soil was identified for the first time. Our results demonstrate the presence of nanoplastics with  
14 a size ranging from 20 to 150 nm and covering three of the most common plastic families:  
15 polyethylene, polystyrene and polyvinyl chloride. Given the amount of organic matter in the  
16 soil matrix, the discrimination and identification of large nanoplastic aggregates are  
17 challenging. However, we provided an innovative methodology to circumvent the organic  
18 matter impact on nanoplastic detection by coupling size fractionation to molecular analysis of  
19 plastics. While photodegradation has been considered the principal formation pathway of  
20 nanoplastics in the environment, this study provides evidence, for the first time, that plastic  
21 degradation and nanoplastic production can, however, occur in the soil matrix. Moreover, by  
22 providing an innovative and simple extraction/analysis method, this study paves the way to  
23 further studies, notably regarding nanoplastic environmental fate and impacts.

**Key Words:** Nanoplastics, soil, municipal wastes, Py-GCMS, AF4

## 1. Introduction

Despite a consensus that plastic debris will accumulate in the environment, its presence has drastically increased, reaching almost 360 million tons in 2018 (PlasticsEurope 2019). Plastic pollution is now considered one of the main environmental challenges and represents an emerging threat for all living species, especially due to the chemical species (additives and other adsorbed elements) that are associated with plastics (Velzeboer, Kwadijk, et Koelmans 2014; Alimi et al. 2018; Davranche et al. 2019; Shen et al. 2019). Leading governments have even classified plastic pollution as a critical problem comparable to climate change (Programme 2016).

In marine systems, despite knowledge gaps regarding the occurrence and fate of microplastics, the recent discovery of nanoplastics (Ter Halle et al. 2017) has opened the door to new considerations in terms of their properties and environmental fate and behavior. Nanoplastics are defined as plastic particles with sizes covering the colloidal range (1 nm to 1 µm) and with a Brownian motion in aqueous system (Gigault et al. 2018). By contrast, compared to marine systems, terrestrial systems are largely ignored, as noted by Rillig (2012).

However, plastic-based materials such as plastic mulches and polytunnels are widely used to increase agricultural production efficiency or life duration (Steinmetz et al. 2016; Liu et al. 2018; Gao et al. 2019). Sewage sludge products also contribute to the incorporation of microplastics into soils (Habib, Locke, et Cannone 1998; Corradini et al. 2019). Both inputs, together with many others (Hurley et Nizzetto 2018), such as landfills (He et al. 2019), lead to the accumulation of plastics in soils, which could represent an even larger pollution pool than that in the oceans (Horton et al. 2017). Once plastic debris is present in soil, it mixes and

reacts with soil organic matter (OM) and minerals and may persist for up to a few hundred years (Bläsing et Amelung 2018). The prolonged residence time of plastics in soil could lead to the formation of micro- and nanodebris. The formation of such debris represents a severe challenge for environmental protection, especially for long-term farming based on plastics utilization (Steinmetz et al. 2016). Some recent studies have started investigating nanoplastic impacts on soil biota (Zhu et al. 2018; Awet et al. 2018), their interactions with contaminants (Velzeboer, Kwadijk, et Koelmans 2014; Davranche et al. 2019) and their transport through model soils (Hu et al. 2020; Pradel et al. 2020). However, to the best of our knowledge, evidence of nanoplastics in a natural soil environment has never been demonstrated. This lack is clearly due to the need for appropriate analytical methodologies for the identification of nanoplastics in the complex and heterogeneous soil matrix (Pinto da Costa et al. 2019). The size, shape, concentration (at the trace level) and carbon composition of nanoplastics make their identification and quantification highly challenging, especially due to the amount of natural OM, which is also composed of carbon and covers the entire colloidal size range.

Due to their small size and high surface reactivity, nanoplastics could exert a direct and indirect effect on soil ecosystems, including groundwaters. They could indirectly influence the soil physicochemical properties, whereas direct effects were already reported on soil microorganisms and fauna communities (Zhu et al. 2018; Awet et al. 2018). Since soils and groundwaters are major human resources, evaluating the occurrence, fate and impact of nanoplastics in soils is crucial and is a main political and governmental priority, leading to legislation such as the European Commission directive on plastics on soil regulation.

Regarding the difficulties to extract and detect nanoplastics in a soil matrix, our objective is not to realize an exhaustive study of nanoplastic presence in various kind of soils, but to demonstrate that nanoplastics are present and can be produced directly in the soil matrix. The challenge was here to find a soil sufficiently contaminated by plastics and for

enough years to allow the potential production of nanoplastics. For this purpose, we chosen to work on a soil contaminated by plastic debris through several municipal waste amendments performed around fifteen to thirty years ago. Nanoplastics were extracted using a water extraction procedure and subsequently identified by an innovative high-resolution analytical method combining size fractionation and molecular analysis.

## **2. Methods**

### *2.1. Soil sampling and geochemical analysis.*

The soil samples are agricultural soils collected in central France in February 2018. They correspond to the upper most horizon (0 to 10 cm, organo-mineral (Ah) horizon) of a well-drained cambisol (VWR classification), enriched in pebbles and developed from alluvial deposits. The contaminated soil received two household wastes amendments mostly composed of plastics around thirty and fifteen years ago. The wastes were crushed and mixed with organic compost that was spread on the soil. The field was regularly tilled and used as a meadow for the last ten years. Plastic debris is thus clearly visible on and in the soil horizons from the surface to 40 cm depth. Plastic crushing has probably promoted the plastics degradation in the soil (Ng et al. 2018). Regarding the contamination origin, treatment and dates, this soil is therefore the ideal target for studying the potential nanoplastic production in a soil matrix.

A soil control was sampled from a non-amended plot close to the contaminated plot. The same extraction protocol and characterization methods were used for the amended and control soil samples. Soil samples were dried in ambient air, sieved at 2 mm and stored in ambient air in the dark. To ensure no plastic pollution, both soils were similarly stored and treated. The geochemical composition of both soil samples is summarized in the Supplementary Materials, [Table S1](#). Geochemical analyses were performed at the “Service d’Analyse des Roches et des Minéraux” (SARM). The major element concentrations were determined by inductively

coupled plasma-atomic emission spectrometry (ICP-AES, Thermo ICap 6500). Trace element concentrations were determined by ICP-mass spectrometry (Thermo Elemental X7). The samples were digested by alkaline fusion using lithium metaborate ( $\text{LiBO}_2$ ) as a fusion flux.

## *2.2. Nanoplastic water extraction experiments.*

To extract nanoparticles from the soil samples, soil-water extraction was performed. Ultrapure water was added to soil at a soil/water ratio of 1:4 (200 g/800 g) and stirred at 300 r  $\text{min}^{-1}$  for 72 h without any pH adjustment. Soil solutions were sampled over time. After pH and conductivity measurements, each sample was filtered to 0.8  $\mu\text{m}$  (Sartorius filters). Total organic carbon (TOC) was determined using a TOC analyzer (Shimadzu TOC-V CSH). The accuracy of the TOC measurements was estimated at  $\pm 5\%$  for all samples using a standard solution of potassium hydrogen phthalate. The extraction experiments were performed in duplicate.

## *2.3. Nanoplastics identification and characterization.*

Water-extract filtrates were fractionated by asymmetric flow-field flow fractionation coupled to UV spectroscopy and static light scattering (AF4-UV-SLS, Wyatt Technology, Germany, and Agilent Technologies, France). The AF4 channel thickness was fixed by a 250- $\mu\text{m}$  Mylar film. The channel had a length of 26.5 cm and a width that narrowed from 2.1 to 0.6 cm. The accumulation wall was defined by a 10-kDa PES membrane purchased from Wyatt Technology. The method used was described by [Gigault et al. \(2017\)](#). The detailed parameters are presented in the Supplementary Materials. Three hundred microliters of each sample were injected into the AF4 instrument. The sizes (gyration radius,  $R_g$ ) obtained from SLS were determined by the first-order Berry model using ASTRA-6 software. The AF4

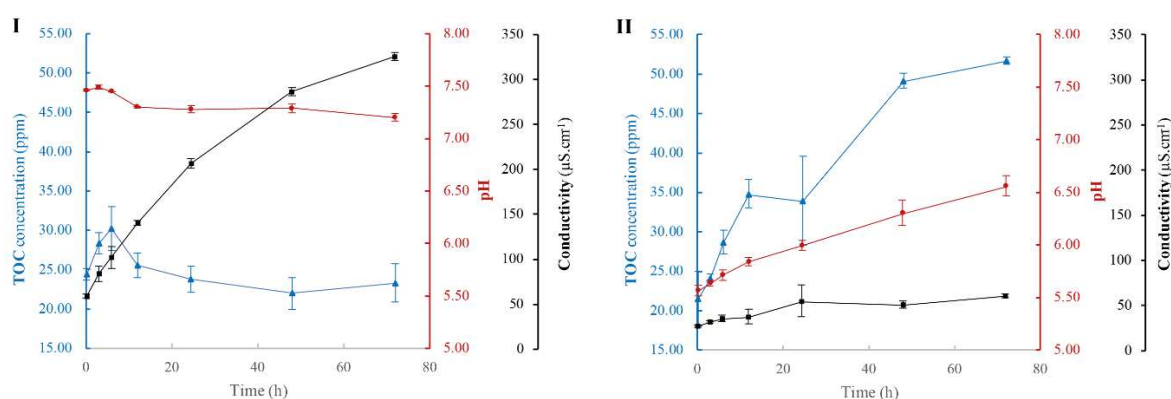
instrument was calibrated (Supplementary Materials, [Fig. S1](#)) using polystyrene spherical models (PSL, NIST traceable standard) to convert the elution time for a sphere with the equivalent hydrodynamic diameter ( $d_{zH}$ ). Both the AF4 extracts and  $< 0.8 \mu\text{m}$  water extract fractions were analyzed by pyrolysis (PY-3030D - Frontier Lab, Japan) coupled to gas chromatography and mass spectrometry (Py-GCMS). For this experiment,  $40 \mu\text{L}$  of sample was introduced into an analysis cup and placed on a heating table at  $40^\circ\text{C}$  until complete evaporation of the solvent. This step was repeated one time to concentrate the samples before injection. The method followed for Py-GC/MS was described by [Dehaut et al. \(2016\)](#), and the corresponding settings are detailed in the Supplementary Material. A blank control was performed to ensure that none of the instruments or materials contaminated the samples.

### 3. Results and Discussion

#### *3.1. Extraction and aggregation of the nanoparticle fraction.*

During soil-water extraction, pH, conductivity and TOC were monitored in the  $<0.8 \mu\text{m}$  fraction of the soil water-extracts ([Fig. 1](#)). For the contaminated soil ([Fig. 1.I](#)), in the first 6 h, TOC increased from  $24$  to  $30 \text{ mg L}^{-1}$  and then decreased to reach a pseudo equilibrium from 24 h with an average concentration of  $23.7 \pm 1.8 \text{ mg L}^{-1}$ . The pH remained stable ( $7.35 \pm 0.2$ ), while the conductivity continuously increased along the experiment, from  $59.0$  to  $325.7 \mu\text{S cm}^{-1}$ . The TOC variations could be explained by an aggregation. Aggregates were formed with the running time experiments and were removed by the filtration at  $0.8 \mu\text{m}$ , leading to the TOC decrease. This hypothesis is also supported by the conductivity increase, which could be responsible of the colloids material aggregation. By contrast, the TOC, pH and conductivity of the control soil all increased along the experiment ([Figure 1.II](#)) but with values much lower than for the contaminated soil. The control soil did not reach equilibrium during the experiment. Moreover, the control soil has lower concentrations of CaO and  $\text{CO}_2$  total than the contaminated soil ([Table S1](#), Supplementary Materials). The contaminated soil

is thus enriched in carbonates as compared to the control sample. Such carbonates are dissolved during the water-extraction which buffer the pH and involve the conductivity increase. This carbonates enrichment could be the result of agricultural practices such as liming but also of the presence of weathered plastics debris. [Hahladakis et al. \(2018\)](#) showed that plastics can have carbonate-based fillers representing until 50% of the total plastic weight.

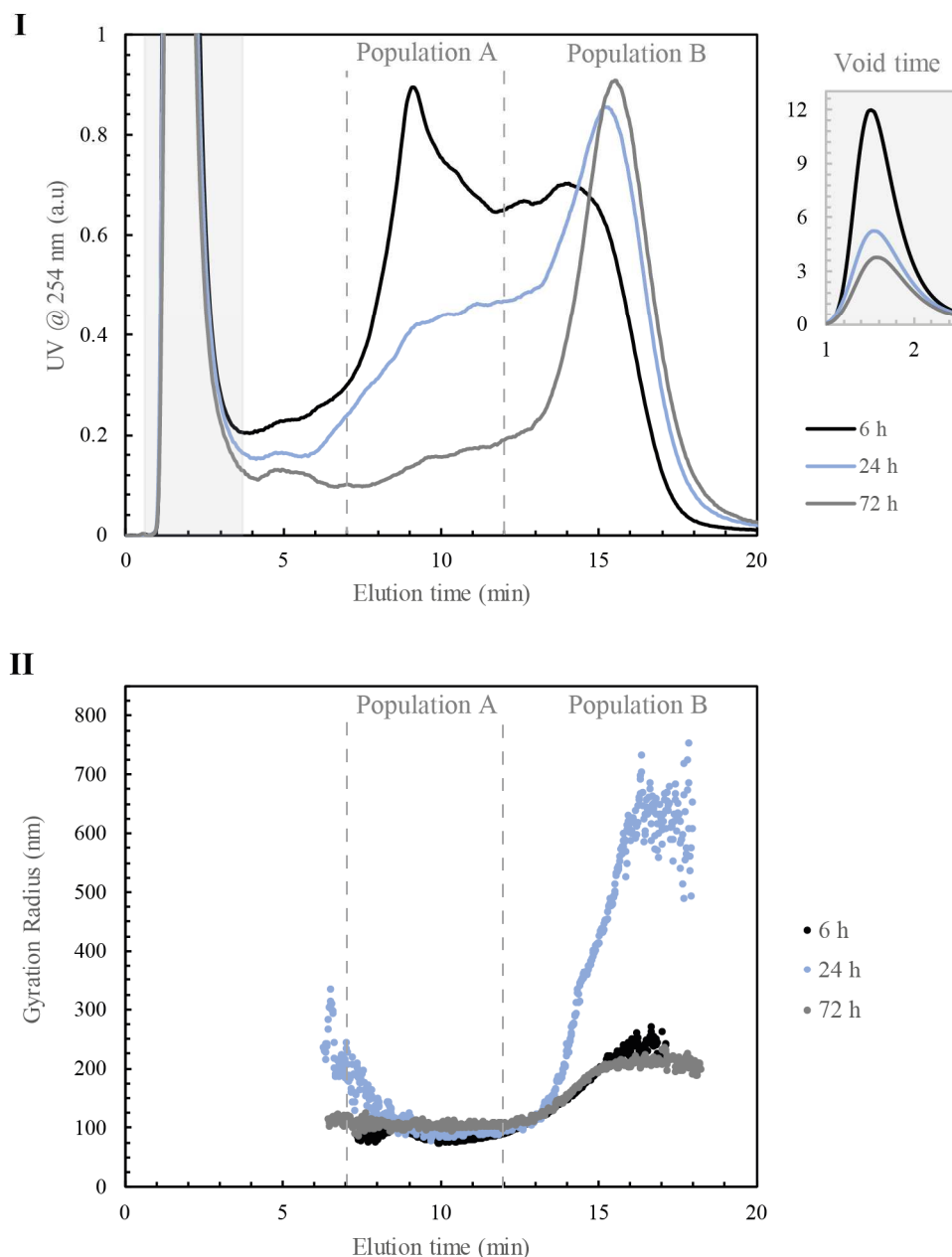


**Fig. 1:** Evolution of TOC, pH, and conductivity with extraction time. **I:** contaminated soil, **II:** soil control. The error bars correspond to the standard deviation calculated from duplicates.

To verify such hypothesis and better understand the behaviors in presence, the soil water extracts were characterized by AF4-UV-SLS. Regarding the TOC evolution, only 3 samples were collected and analyzed at 3 extraction times (6 h, 24 h and 72 h). [Fig. 2](#) presents the AF4-UV-SLS analysis results at different sampling times for the  $<0.8 \mu\text{m}$  solutions. According to the UV results, 3 populations (void time, A and B) were identified for all extraction times ([Fig. 2.I](#)). The void time population from 0 to 2.5 min corresponds to small particles (0 to 5 nm) that are generally not colloidal ([Chevalier et al. 2018](#)) and correspond to low-molecular-weight OM. They were not considered in the present study. Two colloidal populations were identified: **A** from 7 to 12 min and **B** from 12 to 19 min, which correspond to hydrodynamic sizes ( $d_{\text{ZH}}$ ) of 20 to 150 nm and 150 nm to 500 nm, respectively (*eq. (1)*, [Supplementary Materials](#)). The variation in  $R_g$  (gyration radius), obtained by SLS, provides



167 information on the particle size. From 6 h to 24 h, while  $R_g$  did not vary for **A**,  $R_g$  increased  
168 from 200 to 600 nm for **B** and finally decreased at 72 h to reach 200 nm, the initial value (Fig.  
169 2.II). Such  $R_g$  variations indicate particle aggregation (Frimmel, Kammer, et Flemming 2007).  
170 To characterize the nature of the particle aggregation,  $R_g$  was combined with the  
171 hydrodynamic radius ( $r_H$  – obtained from AF4 elution time, *eq. (1)*) to determine the shape  
172 factor  $S$  (i.e.,  $R_g/r_H$ ). While  $S = 0.778$  corresponds to hard spherical particles, a deviation to 1  
173 indicates the presence of inhomogeneities on the sphere structure (Brewer et Striegel 2011).  
174 Here,  $S \gg 1$  indicates the formation of polymorphic aggregates. Our results therefore show  
175 the formation of large and polymorphic aggregates. This aggregation process can be  
176 confirmed by the UV signal evolution with time. From 6 to 72 h, the UV signal of **A**  
177 decreased, while it increased for **B**, indicating the aggregation and transfer of the **A**  
178 population to **B**. Moreover, from 6 to 72 h, the maximum of the peaks in the **B** population is  
179 shifted to the right, which means that the particles size increased. In addition, from 6 to 72h,  
180 the total UV signal decreased for the same elution time interval (7 to 20 min). The total area  
181 of the peaks was 367 at 6 h, 314 at 24 h and 213 at 72 h which correspond to the TOC  
182 decrease and particles loss. Thus, after 24 h, large aggregates with sizes  $> 0.8 \mu m$  were  
183 formed, and the remaining particles in the  $<0.8 \mu m$  fraction were smaller, as confirmed by the  
184  $R_g$  decrease.



**Fig. 2: I:** Fractograms (UV trace at 254 nm) of the soil water extracts at 6 h, 24 h and 72 h from the contaminated soil with respect to elution time. **II:** Evolution of the gyration radius (Rg) with the elution time for the 3 soil water extracts.

### 3.2. Are extracted nanoparticles nanoplastics?

To discriminate the presence of anthropogenic nanoparticles, populations **A** and **B** identified by AF4 were collected and further analyzed by Py-GCMS. Analysis by Py-GCMS

of different plastic materials provides specific molecular markers, which are reported in commercial databases (Tsuge, Ohtani, et Watanabe 2011; Supplementary Materials, table S2). Fig. 3 presents the pyrograms obtained at different extraction times for A only. The most abundant signal was obtained at 24 h (no signal at 72 h). At 6 h and 24 h, the global pyrograms present a series of multiple peaks at constant time intervals that are characteristic of polyethylene (PE) (Tsuge, Ohtani, et Watanabe 2011). By extracting the appropriate ion masses ( $m/z$ , Supplementary Materials, table S2), specific plastic molecular markers were identified at particular retention times ( $t_R$ ), as follows:

- Naphthalene ( $m/z$  128) at  $t_R=11.3$  min and naphthalene-1-methyl ( $m/z$  142) at  $t_R=12.3$  min are characteristic of polyvinyl chloride (PVC; Fig. 3.II)
- Styrene monomer ( $m/z$  104) at  $t_R=8.5$  min and toluene ( $t_R=7$  min) are markers of polystyrene (PS; Fig. 3.III).
- Finally, as previously described, the pyrogram (Fig. 3.IV) shows the presence of PE with a series of alkenes: 1-decene (C10), 1-undecene (C11), 1-dodecene (C12), 1-tridecene (C13), 1-tetradecene (C14) and 1-pentadecene (C15).

Moreover, as demonstrated by Ter Halle et al. (2017), the presence of the triplet n-alkadiene, n-alkene and n-alkane with a bimodal distribution indicates a typical molecular formation pathway of PE during pyrolysis, rather than OM (Fig. 3.IV). Considering the Py-GCMS results and by comparison with the control soil, nanoplastics are effectively present in the amended soil. Indeed, for the control soil, 24 h of extraction time also allowed the most important signals to be obtained, but these signals had typical markers that are characteristic of natural organic matter (Supplementary Materials, Fig. S2). No specific markers of plastics were determined in the control soil water extracts at any time.

For the contaminated soil, a first approximation gives a size ranging from 20 to 150 nm (A population) with heterogeneous shapes. Surprisingly, no signal was obtained for B

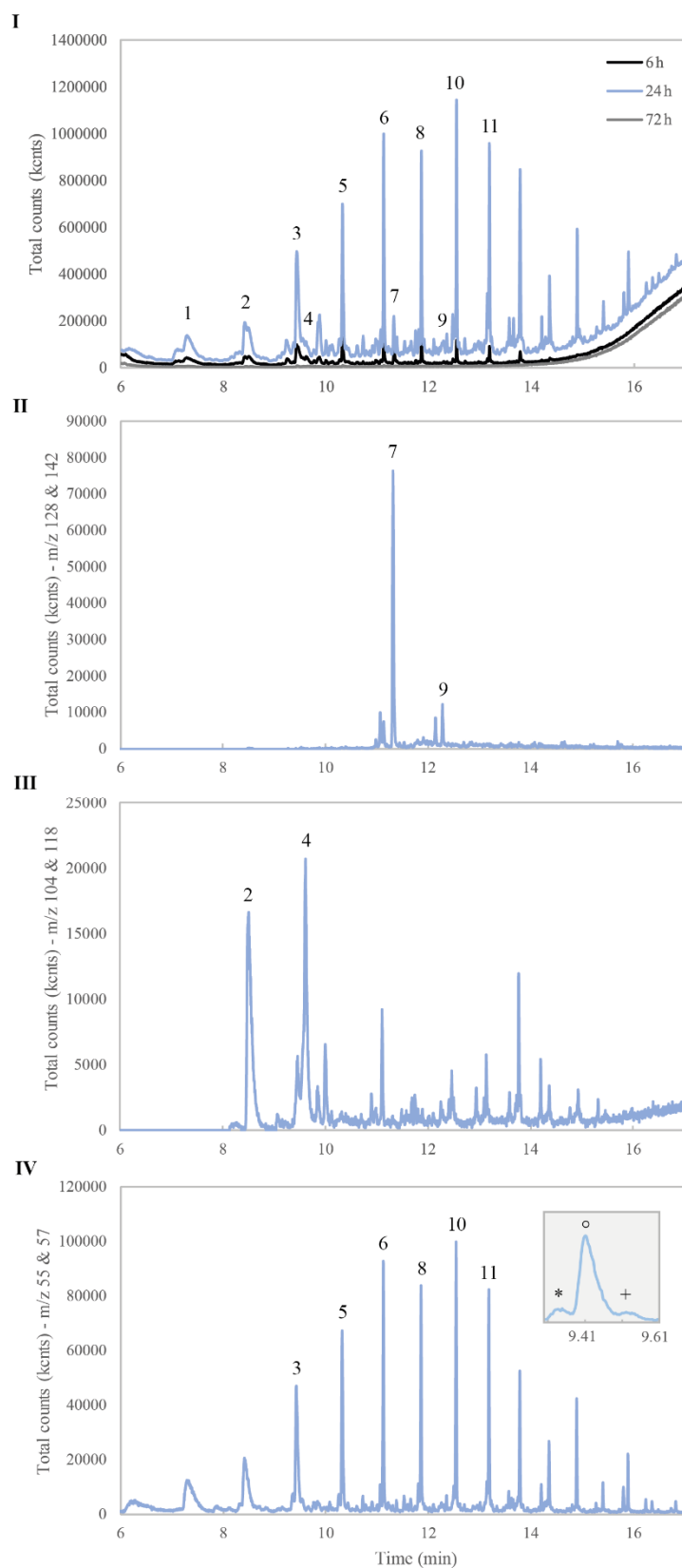
despite the aggregation suggested by the AF4 results and TOC evolution. Different hypotheses could be made: (i) the nanoparticles identified in **A** aggregated to form micrometric particles that were transferred to **B** while the nanoplastics remained stable; or (ii) OM became predominant in **B** and interfered with the pyrolysis signal. Nanoplastics could be stabilized by their bindings with organic macromolecules or present a different aggregation behavior. However, the comparison of the water extracts with and without AF4 analysis and fraction collection (Fig. 4) showed that the pyrolysis signals were enhanced after size fractionation despite sample dilution. Therefore, AF4 can be used as a purification technique to remove organic and dissolved species that may interfere with detection. The amplification of the Py-GCMS signal of plastics after AF4 purification demonstrates that OM mitigates the detection of plastics. A higher proportion of OM than plastics could hide the plastic signature in the Py-GCMS results for the **B** population. This assumption explains the higher pyrolysis signal at 24 h than at 6 h and the plastic disappearance at 72 h. To summarize, in the first 6 h, OM was quickly released and aggregated after 24 h. In both cases, OM was present in a higher proportion than plastics and interfered with the plastic pyrolysis signal until plastic detection became impossible at 72 h. By contrast, at 24 h, OM had just begun to aggregate, and its proportion was less than that of nanoplastics, enhancing the plastic PY-GCMS signal.

Finally, despite the increase in detection sensitivity achieved using A4F or other colloidal purification techniques (ultracentrifugation and ultrafiltration), it is still challenging to attribute specific markers to nanoplastics or OM. An interesting way to discriminate the OM contribution from that of plastics is the toluene-to-styrene ratio (Tol/Sty). Styrene and toluene are also produced during the pyrolysis of OM (Fabbri, Trombini, et Vassura 1998). Tol/Sty ranges from 4 to 5 for substrates of OM origin, in contrast to values of 0.001 to 1 for polystyrene plastics (Fabbri, Trombini, et Vassura 1998; Watteau et al. 2018). This difference in ratios was confirmed by analyzing polystyrene nanoplastics and humic acid standards

(Dignac et al. 2005). Tol/Sty did not exceed 0.03 for the polystyrene standards, while 6.3 was obtained for the humic acids. In the soil-water extracts, for A, Tol/Sty was 2.8. Recently, Watteau et al. (2018) compared the Tol/Sty values of leachates from a soil amended with municipal solid wastes. For the 0-2  $\mu\text{m}$  fraction, potentially containing nanoplastics, Tol/Sty was approximately 3 to 5 for their nonamended soil sample (Watteau et al. 2018). Despite this difference in range from typical values, the authors claimed no plastic occurrence since Tol/Sty was over 1. However, the confidence of the nanoplastics detection in our samples is based on (i) the coherent polystyrene detection associated with PE and PVC nanoplastics identification and (ii) the contribution of OM to plastics markers at the nanoscale (i.e., high specific surface area). Note that in the literature, Tol/Sty ratios were determined from microplastic debris with sizes generally  $>500 \mu\text{m}$ . Nevertheless, plastic debris (micro- and millimetric) is generally coated by biofilms and OM with a thickness less than  $0.4 \mu\text{m}$  (Besseling et al. 2017). Therefore, the mass proportion of OM on large debris is insignificant. Nanoplastics (like other nanoparticles) are also associated with OM (surface sorption, heteroaggregate formation, surfactant-type associations, etc.) (Hotze, Phenrat, et Lowry 2010). However, regarding the high specific surface area and similar size of OM and nanoplastics, the OM mass contribution to core materials (i.e., plastics) is substantially larger (Delay et al. 2011). Indeed, by decreasing the size from  $200 \mu\text{m}$  to  $200 \text{ nm}$ , the OM volume on plastic increases by more than 4 000 times (Supplementary Materials, Fig. S3). This OM distribution on nanoplastics leads to different Tol/Sty values from those of large microplastics.

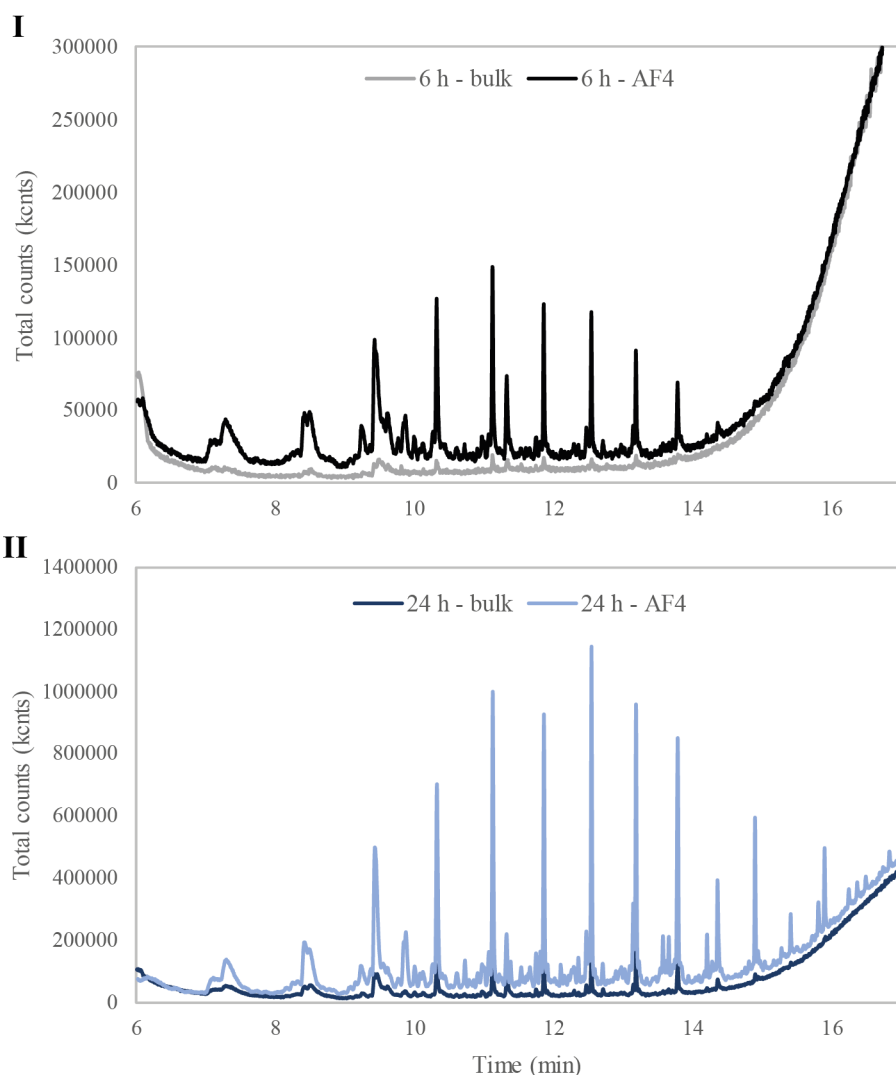
To summarize, in our experiment, nanoplastics are released into the soil solution concomitantly with OM, which controls the stability and aggregation pathways of nanoplastics (Hotze, Phenrat, et Lowry 2010). OM-nanoplastics associations are thus a key factor in their detection in complex environmental matrices. This study demonstrated the

268 mandatory use of AF4 or other size-separation techniques for proper detection. The  
269 development of an analytical strategy based on Py-GCMS is therefore needed (pyrolysis  
270 temperature optimization, ramp program, etc.). Preliminary sample treatment (UV or H<sub>2</sub>O<sub>2</sub>) to  
271 separate nanoplastics from OM is also of major concern in such analytical strategies. Finally,  
272 this study also rises the question of plastics impacts on soil chemical properties. The  
273 occurrence of plastic debris seems indeed to involve different geochemical behaviors of both  
274 studied soils.



**Fig. 3: I.** Pyrograms of the 3 soil water extracts at 6, 24 and 72 h. Numbers correspond to specific markers of plastic: 1: toluene, 2: styrene, 3: 1-decene, 4:  $\alpha$ -methyl styrene, 5: 1-undecene, 6: 1-dodecene, 7: naphthalene, 8: 1-tridecene, 9: naphthalene 1-methyl, 10: 1-tetradecene, 11: 1-pentadecene. **II, III** and **IV**: Ion chromatograms at

279 24 h for  $m/z=128$  and  $142$ ,  $m/z=104$  and  $118$ , and  $m/z=55$  and  $57$ , respectively. Finally, the box in the top right  
 280 corner in **IV** details the triplet n-alkadiene \*, n-alkene ° and n-alkane +.



**Fig. 4:** Comparison between bulk solutions and solutions fractionated by AF4. **I:** 6 h, **II:** 24 h.

### 3.3. Environmental implications.

In addition to the first proof of nanoplastic presence in a soil contaminated by plastic debris, this study demonstrates that such debris is formed from larger pieces of plastic directly in the soil matrix. While the main degradation pathways of plastics in ocean and surface waters are mechanical abrasion and photo-thermo-oxidative degradation (Andrady 2015), these mechanisms only occur in the first centimeters of the soil. Unfortunately, experimental data on plastic degradation in soils are limited and rather scattered, as is knowledge of the



parameters that control these degradation processes (Scalenghe 2018). To elucidate this uncertainty concerning nanoplastic formation pathways, the degradation of carrier plastic bags in soil, open-air and marine environments was recently compared over a 3-year period (Napper et Thompson 2019). After 3 years, plastic bags were still functional after exposure to soil and marine environments but had degraded into microplastics under open-air conditions. Therefore, degradation is slower in soil and marine environments than under open-air conditions. Our results suggest that in a 30-year time frame, plastic degradation in soil occurs to produce nanoplastics, which is similar to the degradation time observed in oceanic systems. This degradation pathway in soil thus suggests the implication of mechanisms other than photo-thermo-oxidative processes. Scalenghe (2018) reported that plastics degradation in soil could be enhanced by microorganisms.

Nanoplastics production in soil also raises the question of their environmental fate and their final impact on living organisms. The nanoscale of nanoplastics allows them to pass through the cell membranes (Bouwmeester, Hollman, et Peters 2015). As soils support our food sources, there is an urgent need to investigate the fate of nanoplastics: Are they taken up by plants? Do they accumulate in the food chain? Are they transferred to surface and groundwater? Their ability to cotransport contaminants (Velzeboer, Kwadijk, et Koelmans 2014; Davranche et al. 2019) as well as to release additives in the environment (Shen et al. 2019) could also impact water quality. These issues represent considerable societal and economic impacts that need to be urgently characterized to better anticipate them.

To conclude, it is important to note that the aim of the present study was not to provide an exhaustive study of the nanoplastic occurrence in various soil families and pedo-climatic conditions. Regarding that no study has already provided evidence of the potential nanoplastic production and presence in natural soil under environmental conditions, we chose to study a soil sample contaminated in plastics during a sufficient timeframe to be able to prove that

nanoplastics can be produced directly in a soil matrix. For this, we developed an innovative and single extraction/analytical procedure allowing to detect nanoplastics even with a high amount of OM. Moreover, the present results suggest that nanoplastic production in soil seems to be rather slow, 30 years maximum, since photo-degradation is only limited to the first cm of the uppermost soil horizon.

#### 4. Supplementary Materials

Table of the elementary composition of the top soil of the contaminated and the uncontaminated soil (Table S1), detailed methods, A4F calibration (Fig. S1), plastic markers for the Py-GCMS detection (Table S2), pyrograms of the soil water extracts from the uncontaminated soil (Fig. S2), comparison between OM volume and plastic debris surface (Fig. S3).

#### 5. Acknowledgments

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