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Unearthing nanoplastics in soil: optimising extraction and purification while preserving particle integrity

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Abstract
Nanoplastics (NPs) are considered to be widespread environmental pollutants but little is known about their occurrence and properties in soils. Here, we evaluate and optimise an extraction and purification method for NPs in soil, aiming to preserve particle integrity and assess the potential for a single extraction workflow to support characterisation by both advanced microscopy techniques and mass-based techniques such as Py-GC-MS. This targets comprehensive characterisation of NPs, including size, shape, polymer chemistry, and mass concentration data. Individual extraction and purification steps were optimised, including density separation by centrifugation using a sucrose solution, filtration to < 1 µm by vacuum filtration and concentration combined with purification using direct flow ultrafiltration. Recovery tests using Pd-doped NPs aided baseline performance quantification. Recoveries were 38% for extraction (7% SD), 74% for density separation (18% SD), 92% for filtration (15% SD), and 74% for ultrafiltration (7% SD). The final combined method comprising these steps in sequence had a low recovery of 1.4% (0.4% SD), demonstrating the challenge of particle-preserving NPs extraction from soil. Next, we assessed the suitability of our final combined NP extraction method for analysis of NPs by Py-GC-MS and SEM. As part of this feasibility assessment, we tested a step to transfer the extracted NPs for Py-GC-MS analysis by dissolving them in a mixture of 1,2,4-trichlorobenzene and *p*-xylene and drying aliquots in pyrolysis cups (87% recovery, 41% SD). However, high sample dilution during the extraction resulted in a high method detection limit, which was unsuitable for quantitative Py-GC-MS analysis of NPs extracted from soil. In contrast, the method was well suited to qualitative analysis of NPs using advanced microscopy techniques, with SEM images revealing highly purified samples and minimal contamination from soil. As the first study to both evaluate recovery for a particle-preserving NP extraction method in soil and demonstrate its application to analytical techniques, this work provides a foundation for future improvements in NP extraction and analysis methods.

Keywords Nanoplastic, Soil pollution, Py-GC-MS, Microplastics, Environmental pollution, SEM, Filtration, Ultrafiltration, Microscopy

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Introduction

Nanoplastics (NPs, < 1000 nm) can be harmful for living organisms under certain circumstances [1–3]. They are expected to occur with high number concentration in the environment as the fragmentation of larger plastic debris forms an ever-increasing number of small particles [4, 5]. However, little is known about their presence in terrestrial environments due to analytical challenges in detection and quantification, especially in complex matrices such as soil. NP number concentrations may exceed those of microplastics (MPs, 1–5000 µm), as number concentrations of MPs increase with smaller particle size [6]. The small size of NPs causes higher availability than MPs to uptake by organisms, including into cells [7], and their colloidal behaviour influences their environmental transport and fate [8]. Although NPs have been identified in soil, characterisation of their size, shape, and concentration remains challenging [9–11]. Accurate NP characterisation would help to assess NP exposure, allowing design of environmentally relevant studies on the impact of NPs on soil health, transfer of NPs to different environmental compartments, and impacts on plants. However, the detection and quantification of NPs in soil remains a challenge due to their size, low mass concentration, and the complexity of the soil matrix.

Several analytical techniques can be used to characterise plastics in environmental samples, including spectroscopic, mass-based, and microscopic imaging techniques [12, 13]. Spectroscopic methods coupled with optical microscopy such as micro-Fourier-transform infrared (µFTIR) and µRaman spectroscopy are commonly used for MP analysis, providing size, shape, and polymer chemistry [14–16]. However, µFTIR detects particles only down to ~ 10 µm, and µRaman is limited to ~ 300 nm [13], missing smaller NPs which are environmentally relevant: NPs < 300 nm can be taken up by organisms and cause effects [17, 18]. Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) can quantify polymer mass and identify polymer chemistry, and has been used to provide information on the presence of NPs in soil [10, 11], water [19, 20], plants [21], and animal tissues [7]. However, this is at the expense of size and shape information as samples are destroyed during analysis. Furthermore, as NPs are expected to have a low mass concentration, Py-GC-MS requires sufficiently large initial soil sample volumes to ensure that the polymer mass in the final concentrated sample is above the method limit of detection (LOD) [22], which was between 0.02 and 18.1 µg polymer/g soil in previous studies [10, 23]. High-resolution, advanced microscopy techniques such as scanning electron microscopy (SEM), atomic force microscopy–infrared spectroscopy (AFM-IR), and scanning transmission X-ray spectro-microscopy (STXM) with near-edge X-ray absorption fine structure spectroscopy (NEXAFS) can detect particles in the nanoscale range [9, 24, 25]. However, while NPs can be imaged by SEM, it is difficult to distinguish them from mineral and organic particles and the polymer chemistry cannot be identified using SEM alone [9, 26]. This can be achieved by coupling of high-resolution microscopy techniques with spectroscopic techniques. For example, SEM coupled with energy-dispersive X-ray spectroscopy (EDX) can provide information on the elemental composition of the particles, allowing some differentiation from mineral particles but not between polymer types [27]. Furthermore, AFM-IR has been used to identify NPs in a drinking water treatment plant from 20 to 1000 nm through topographic imaging of samples along with collection of IR spectra to identify polymer chemistries [28]. Similarly, STXM-NEXAFS has been used to identify individual NPs in soil, allowing size, shape, and chemical identity of NPs to be determined [9]. However, due to the long time and analytical effort of obtaining images, it is not possible to quantify NPs using these methods. Regardless of final analysis method, NP extraction techniques from soil must provide effective separation from other soil particles and effective sample concentration, as impurities can interfere with analysis and the concentration of NPs in the sample must be detectable [20].

Previous work on the extraction and analysis of NPs in soil has focused either on particle-based analysis (imaging or identification of individual particles) or mass-based analysis (identification and mass quantification of NPs in bulk samples). To date, no recovery-tested method for the extraction of NPs from soil has been proposed which preserves the integrity of NPs while demonstrating compatibility with subsequent analysis using microscopy techniques or mass-based techniques. This is a significant research gap as this enables size and shape determination while being transparent about particle losses and providing a basis for future improvements. Various methods exist to extract MPs from soil, often including steps to remove mineral and organic soil particles through density separation and oxidation while preserving particle integrity for microscopic analysis [6, 29–31]. However, these approaches target a larger size fraction, so that any filtrations aim to retain rather than remove large particles, making them unsuitable for NPs [32].

NPs extraction from soil requires an initial extraction step, sample purification, and concentration for analysis. First, to separate NPs from soil, an extraction of particles or chemical digestion of soil can be used. Foetisch et al. [9] used tetrasodium pyrophosphate (TSPP), whereas Wahl et al. [11] used ultrapure water to disperse the soil. Others chemically digested samples using tetramethylammonium hydroxide (TMAH) [10, 23]. Monikh et al. [33] combined these approaches with a multistep extraction using MilliQ water to remove unbound NPs, followed by sodium dodecyl sulphate (SDS) solution to extract loosely soil-attached NPs and TMAH to isolate any remaining NPs. After extraction, further purification from interfering organic and mineral particles is needed. For example, asymmetric flow-field flow fractionation (AF4) can isolate particles based on size, aiding the removal of non-target particles [11]. Mineral particles and organic matter can be removed by density separation with sucrose solution followed by organic matter oxidation with H_2O_2 [9]. Alternatively, ammonium sulphate solution can be used in a density separation [29]. After TMAH digestion, centrifugation in ethanol can be used to separate natural organic matter (NOM) from NPs [23]. Another method involves filtration after TMAH digestion followed by centrifugation to remove mineral particles, so that the NP-containing supernatant can be flocculated with soil organic matter (SOM) [10]. However, in the studies involving TMAH, the final NP-containing sample is leached with dichloromethane (DCM) before Py-GC-MS analysis, dissolving the plastics and ruling out any imaging. Next, Py-GC-MS requires filtered < 1 µm samples to ensure only NPs are analysed, while microscopic techniques benefit from filtration to remove larger particles and avoid masking smaller ones. Membrane filters with pore sizes ranging from 0.8 to 1.2 µm are commonly used [5, 10, 11], although Albignac et al. [34] reported higher NP recovery using single-layer 5 µm stainless steel mesh compared with multilayer membrane filters. However, a 1 µm pore size is preferable to avoid analysing small MPs instead of NPs. Subsequently, samples must be concentrated prior to analysis regardless of analysis technique. Ultrafiltration has been applied for concentration of NP-containing soil and water samples [9, 19, 34–36], but can suffer from high particle losses through attachment of particles to the filtration membrane [35]: Albignac et al. [34] reported only 60% recovery. For Py-GC-MS, the samples must be further concentrated and/or dried, then resuspended or dissolved before transfer and drying in an analysis cup [20, 37]. For imaging methods such as SEM and STXM-NEXAFS, the sample is deposited on a substrate such as Si or Si_3N_4 through centrifugation or drop deposition [9]. However, current methods for NP extraction have various limitations: they have either not been tested for extraction recovery [9, 11], focus on limited polymer types [10], do not allow for analysis of single particles [10, 23], do not assess the applicability of the method for downstream chemical or imaging analysis [33], or lack size fractionation, leading to inclusion of MPs [23, 33].

An extraction procedure for NPs was recently used in our research group to identify individual NPs in water and soil by STXM-NEXAFS (Foetisch et al. [9]). The recovery of this approach was not evaluated, and application to further techniques was not explored, but it was chosen as a basis for our work as the method allowed analysis of NPs by microscopy techniques. Here, we build on this initial approach to systematically optimise and evaluate a particle-preserving extraction method for NPs in soil. We (1) optimise and evaluate individual steps such as extraction, density separation, organic removal, filtration, and concentration, aiming to make the method accessible to a wide range of laboratories, (2) evaluate the optimised particle-integrity-preserving extraction and purification method for NPs in soils by performing the first recovery testing for such a method, and (3) explore the applicability of the method for Py-GC-MS and SEM analysis to provide a foundation for combined mass- and particle-based analysis of NPs and support wider implementation of the method.

Materials and methods

Materials and chemicals

Fluorescent 100 nm (micromer®-redF, plain, 30–00–102, Zeta potential −56.3 mV) and 250 nm (micromer®-greenF, plain, 29–00–252, Zeta potential −56.9 mV) polystyrene (PS) NPs were purchased as aqueous suspensions from Micromod, Germany and characterised using SEM (Gemini 450, Zeiss, Oberkochen, Germany) and dynamic light scattering (DLS, Zetasizer Pro Blue (ZSU3200), Malvern Panalytical Ltd, Worcestershire, UK) (SI 1). These fluorescent PS NPs were used in method development requiring SEM imaging because their narrow size distribution and spherical shape allow for easy identification. Pd-doped NPs consisting of a polyacrylonitrile (PAN) core containing a Pd label and a PS shell were utilised as a tool for method development, where the metal was used as proxy for plastic to ease quantification challenges. Details regarding the synthesis of the Pd-doped NPs are provided in Mitrano et al. [38]. These NPs were characterised by inductively coupled plasma mass spectrometry (ICP-MS, Plasma Quant MS Elite®, Analytik Jena, Jena, Germany), SEM (JMS 7600 F, Jeol, Tokyo, Japan), and DLS (SI 1). In our tests, two different aqueous suspensions of Pd-doped NPs were used in a similar size range to the purchased fluorescent NPs, originating from different synthesis batches: ~210 nm (Zeta potential −49.7 mV) and ~ 150 nm (Zeta potential −51.1 mV), where the size variation is caused by unavoidable small variations during the synthesis. Due to limited availability of the Pd-doped NPs from each batch, some tests were carried out with one size and others with the second once the first batch was exhausted. As the two batches of Pd-doped NPs are in a similar size range with similar Zeta potential, we consider them comparable and interchangeable for method development purposes. In tests with Pd-doped NPs, samples were placed inside microwave vessels (PTFE, EasyPrep Plus, CEM Corporation, North

Carolina, USA). Sample drying was carried out in an oven at 95 °C, and on a hot plate at 130 °C or a sand bath at 95 °C in a fume hood in the case of samples containing ethanol. Samples were digested in a microwave (Mars 6, CEM Corporation, North Carolina, USA) using 8 mL HNO_3 (69%, supra, Carl Roth®, Karlsruhe, Germany), 1 mL H_2SO_4 (98%, Carl Roth®, Karlsruhe, Germany), and 600 µL H_2O_2 (30%, Carl Roth®, Karlsruhe, Germany), at 200 °C for 60 min. Digested samples were diluted to 50 mL with MilliQ water. ICP-MS was employed to measure ^{105}Pd concentrations, with ^{115}In as internal standard. Details of the instrumental parameters used in ICP-MS measurements can be found in SI 2. Calibration curves for Py-GC-MS were prepared with PE (polyethylene, CAS 9002-88-4, Thermo Scientific, Massachusetts, USA), PP (polypropylene, PP Borealen, Semadeni Group, Switzerland), and PS (polystyrene, CRT300, Carat GmbH, Bocholt, Germany). For recovery testing and evaluation of the application of the extraction method to SEM, an agricultural soil from Wageningen, Netherlands was used. Details of the soil characterisation can be found in SI 3. The soil was dried and sieved to 2 mm before use.

Tetrasodium pyrophosphate (TSPP, $Na_4P_2O_7$, > 95%, Sigma-Aldrich, USA), sucrose (≥ 99.5%, Sigma-Aldrich, USA), H_2O_2 (30%, Carl Roth®, Karlsruhe, Germany), 1,2,4-trichlorobenzene (99%, Thermo Scientific, Massachusetts, USA), *p*-xylene (≥ 99%, Carl Roth®, Karlsruhe, Germany), butylated hydroxytoluene (≥ 99.0%, Merck, Darmstadt, Germany), and ethanol (> 99.5%, Carl Roth®, Karlsruhe, Germany) were used as received. Two filtration setups were tested to isolate the < 1 µm size fraction. Syringe filtration used hydrophilic polytetrafluoroethylene (PTFE) membrane filters (1 µm pore size, 25 mm diameter, Omnipore, Merck, Darmstadt, Germany) in stainless steel filter holders (25 mm, Sartorius, 16214, Göttingen, Germany) connected to a glass syringe (20 mL, Luer-Lock, Fortuna Optima, Poulten Graf, Wertheim, Germany). Vacuum filtration was used with stainless steel mesh filters (1 µm pore size, 47 mm diameter, plain dutch weave, SS316, Négofiltres, France) mounted on a PTFE-coated screen mesh filter holder (47 mm, Sartorius, 16309, Göttingen, Germany). Ultrafiltration was performed using an Amicon® Stirred Cell (200 mL, Merck Millipore, Sigma-Aldrich, USA) fitted with 10 kDa polyethersulfone (PES) membranes (Merck Millipore, Sigma-Aldrich, USA). Centrifuge tubes (polypropylene, 50 mL, Corning® CentriStar™, USA) and high-strength glass centrifuge tubes (30 mL) with PTFE-lined caps from Kimble® Kimax® (USA) were used in method development. SEM images during method development were obtained using either Gemini 450, Zeiss at 10 kV acceleration voltage with a secondary electron detector or JEOL JMS 7600 F at 5 kV acceleration voltage with a secondary electron detector.

Extraction method optimisation and recovery testing

In the optimisation and evaluation of different particle-preserving method steps for NPs extraction from soil, we followed the framework of Foetisch et al. [9]. The method includes (1) extraction using TSPP through sample homogenisation and sedimentation, (2) density separation with sucrose solution to remove mineral particles, (3) organic matter oxidation using H_2O_2, and (4) concentration and washing by ultrafiltration. In the present study, we also include testing of a filtration to < 1 µm to remove large particles prior to ultrafiltration and evaluate the recovery of the full method in sequence.

The extraction step assessed here was initially developed for silver nanoparticles by Schwertfeger et al. [39] and adapted by Foetisch et al. [9] for NP analysis. 50 g of soil was spiked with ~ 150 nm Pd-doped NPs (188 mg/kg soil, 602 µg Pd/kg soil) and mixed with 1 L ultrafiltered TSPP 2.5 mM solution in a 2 L glass beaker. The foil-covered beaker was shaken for 30 min at 100 rpm (Universalschüttler SM 30 C, Edmund Bühler®, Bodelshausen, Germany), then ultrasonicated for 2 min at 35 kHz in an ultrasonic bath (Transsonic TS 540, Elma®, Singen am Hohentwiel, Germany) to release particles from the soil aggregates [39]. The samples were left to settle for 18 h to allow large particles to sediment. After settling, 100 mL of suspension was collected from the top 1 cm using a glass pipette. This approach assumes that NPs behave as colloids and remain evenly distributed in the supernatant. Recovery was assessed by ICP-MS analysis after drying and digesting 24 mL aliquots of the collected samples, corresponding to the volume used for the density separation in the next step.

Foetisch et al. [9] carried out a density separation to remove mineral particles from the suspension via ultracentrifugation, using a layer of concentrated sucrose solution below the sample. To increase accessibility, we adapted this to a benchtop centrifuge. In our tests, samples were gently layered on top of a sucrose solution (1.22 g cm^{-3}), then centrifuged at 5000 rpm (4696 *g*) for 8 h using a benchtop centrifuge (Megafuge ST1 Plus, equipped with a TX-400 rotor, Thermo Scientific, Massachusetts, USA). It should be noted that the density of the sucrose solution allows for analysis of PE, PP, PS, and polyamide (PA) among other low-density polymers, but excludes the higher-density PVC (polyvinyl chloride) and polyethylene terephthalate (PET), which are also commonly found in soil [40, 41]. The supernatant was collected to a depth of 5 mm inside the sucrose layer. To test if agglomeration of particles occurs during centrifugation, a 30 mL mixture of 100 nm and 250 nm fluorescent PS NPs (5 ppm, and 12.5 ppm, respectively) was layered over 5 mL sucrose solution and centrifuged. The supernatant was diluted 1000× using MilliQ water, drop-deposited on a Si wafer, and analysed by SEM (Gemini 450, Zeiss) to check for aggregation or agglomeration. As the drying of the suspension can cause artificial aggregation through capillary forces, aggregation or agglomeration of particles was carefully assessed through the assumption that any aggregates formed during the centrifugation would be three-dimensional rather than lying flat on the surface. To evaluate recovery during the density separation and causes of NP losses, we utilised 30 mL high-strength glass centrifuge tubes with PTFE-lined caps to avoid potential plastic contamination when the method is applied to environmental samples. Triplicate samples containing 24 mL of a 1:1 mixture of soil extract and 210 nm Pd-doped NPs suspension in 2.5 mM TSPP were layered on top of 4 mL of sucrose solution. After centrifugation, the pipette used for collection of the supernatant was washed with 10 mL ethanol, which was collected separately. The pellet obtained by centrifugation was resuspended in MilliQ water and collected. All fractions were analysed by ICP-MS after drying and digestion.

To oxidise organic matter in the samples, Foetisch et al. [9] applied 5% H_2O_2 at 2-hour intervals over 24 h. To improve the efficiency of this step, we investigated the effect of a higher H_2O_2 concentration on organic carbon oxidation. We simulated the sample matrix after density separation by performing the extraction and density separation steps. These were mixed in a 1:1 ratio with 30% H_2O_2 to reach 15% H_2O_2. Non-purgeable organic carbon (NPOC) concentration was measured before and after the addition of H_2O_2 at 60, 120, and 180 min (Vario-TOC cube, Elementar®, Langenselbold, Germany). Potential effects of 5–20% H_2O_2 on NPs were investigated by exposing 100 nm fluorescent PS NPs in a 2.5 mM TSPP solution for 2 h followed by SEM analysis (Gemini 450, Zeiss). The diameters of 100 particles per treatment were measured using ImageJ and this was compared to the diameters of unexposed particles. The reaction between H_2O_2 and sucrose was monitored by recording the temperature after 2, 5, 10, 20, 30, 60, and 120 min.

We evaluated the recovery of two different filtration protocols to isolate the < 1 µm size fraction. As a preliminary test, we used syringe filtration with PTFE membrane filters. However, clogging of these filters during testing limited its application, so follow-up tests included filtration through a stainless steel mesh filter via vacuum filtration. Description of the syringe filtration tests can be found in SI 4. In our vacuum filtration test, we used a 47 mm stainless steel mesh filter (1 µm pore size) supported on a PTFE-coated screen mesh. A concentrated suspension of Pd-doped NPs in 2.5 mM TSPP (~ 210 nm, 23.86 mg/L) was pre-filtered to remove any aggregated or agglomerated particles larger than 1 µm. To avoid potential clogging observed during the syringe filtration, particle concentration was reduced by diluting 25 mL of the filtered suspension to 200 mL with MilliQ water. This suspension was filtered into a 1 L glass collection flask. Additionally, the effect of washing the apparatus afterwards was tested. The sample was filtered and collected as before, then the filter and apparatus were washed using 10 mL ethanol. Filtrate and ethanol wash were combined. All samples were dried in microwave vessels, digested, and analysed by ICP-MS. SEM images of the filter surface before and after use were recorded (Gemini 450, Zeiss and JEOL JMS 7600 F).

The filtrate containing the size fraction < 1 µm must be washed and concentrated to remove dissolved impurities and enrich the sample prior to analysis. Previous work has often used ultrafiltration to achieve this [9, 19, 34–36]. The influence of the number of washing steps and stirring on the NP recovery after the ultrafiltration was investigated under three conditions: (1) two washes, no stirring, (2) one wash, 200 rpm stirring and (3) two washes, 200 rpm stirring. Suspensions of Pd-doped NPs in 200 mL MilliQ water were added to the ultrafiltration device. The volume was reduced to 10 mL, refilled to 200 mL with MilliQ water, and reduced again to 10 mL. For conditions 1) and 3), this washing step was repeated twice. The final 10 mL samples were transferred to microwave vessels. The ultrafiltration membranes were washed with 10 mL ethanol, and the washings were pooled with the samples. The samples were then dried, digested, and analysed by ICP-MS. Between samples, the ultrafiltration apparatus was cleaned with soap and water, ethanol, and MilliQ water. Next, the efficiency of sucrose and dissolved organic matter removal by the washing step was tested under condition 1 (two washes, no stirring) with samples prepared using the extraction and density separation steps described above. The NPOC concentration was recorded before washing, after 1 wash, and after 2 washes.

The optimised steps described above were carried out in sequence to test overall recovery (Fig. 1a). For each replicate ($n = 3$), 50 g of soil was added to a 2 L beaker and spiked with ~ 150 nm Pd-doped NPs (188 mg/kg soil, 602 µg Pd/kg soil). The soil was mixed with 1 L 2.5 mM TSPP using a glass rod, covered, shaken for 30 min, and ultrasonicated for 2 min. 2.5 mM TSPP (10 mL) was used to wash the sides of the beaker. The suspension was left to settle for 18 h to allow large particles to sediment. Using a glass pipette, 100 mL of suspension was collected in a glass bottle from the top 1 cm of suspension. Next, sucrose solution (4 mL, 1.22 g cm^{-3}) was added to the bottom of a 30 mL high-strength glass centrifuge tube, and 24 mL of sample was pipetted gently on top, taking care not to mix the layers. Tubes were closed with PTFE-lined caps and centrifuged at 5000 rpm (4696 *g*) for 8 h. The supernatant was collected to a depth of 5 mm into the sucrose cushion and transferred to a 400 mL glass beaker, then diluted to 200 mL with MilliQ water. The

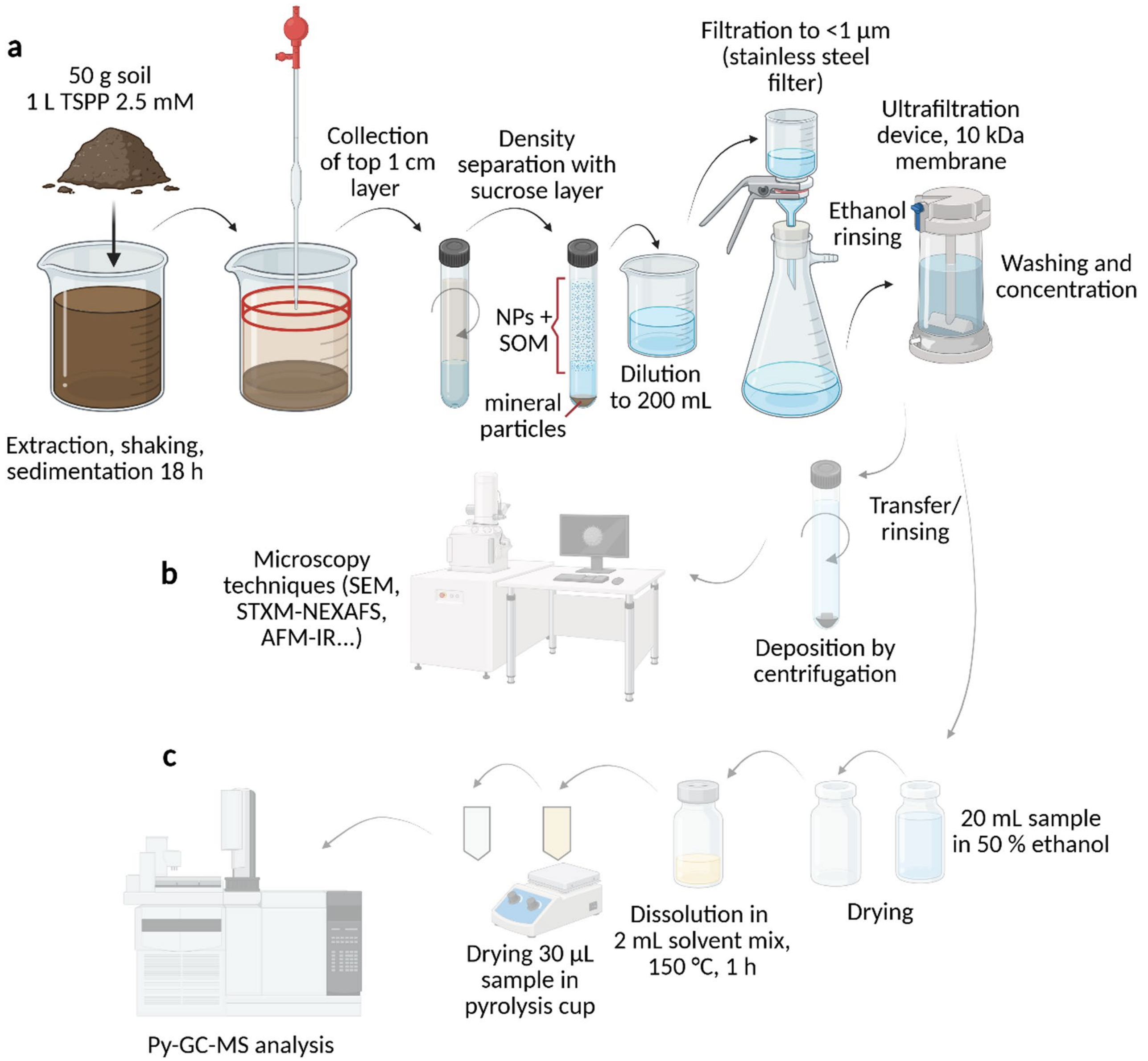

Fig. 1 Optimised extraction procedure applied to extract NPs from soil samples. **a** Optimised method for extracting NPs from soil. **b** Transfer of samples to a suitable substrate (Si or Si_3N_4 wafer) for analysis by microscopy techniques (SEM, STXM-NEXAFS, AFM-IR). **c** Transfer of samples to Py-GC-MS

diluted suspension was vacuum filtered using a 47 mm stainless steel mesh filter (1 µm pore size). The filtrate was transferred to an ultrafiltration device fitted with a 10 kDa PES membrane, and the stainless steel filter was washed with 10 mL ethanol, which was also added to the ultrafiltration device. The volume in the ultrafiltration device was reduced to 10 mL. Washing was carried out as follows: the 400 mL beaker previously containing the sample was filled to 200 mL with MilliQ water, this was filtered through the same filter used for the sample, then transferred to the ultrafiltration device. The sample volume was then reduced to 10 mL. This wash was repeated once more. The final 10 mL of sample was collected inside a 30 mL crimp-top glass vial. The membrane of the ultrafiltration device was washed with 10 mL ethanol via pipette directly into the sample vessel. Between samples, the filtration and ultrafiltration apparatus were cleaned with soap and water, ethanol, and MilliQ water. Samples were dried inside microwave vessels on a hot plate set to 130 °C inside a fume hood with HEPA-filtered air, then digested. Recovery was determined by ICP-MS.

SEM and Py-GC-MS analysis

To evaluate the applicability of the full extraction method to characterisation of NPs by microscopy techniques, 50 g of soil was spiked with a mixture of 100 nm and 250 nm fluorescent PS NPs. The full method described for the recovery tests using Pd-doped PS NPs was applied, comprising the steps in Fig. 1a and b. After the samples were washed and concentrated, 3 mL of sample was added to a 15 mL glass centrifuge tube containing a holder for a 0.5 × 0.5 mm Si wafer and the sample was centrifuged at 5000 rpm (4696 *g*) for 4 h to deposit NPs on the wafer. The samples were sputtered with Pt/Pd (80:20 ratio, 5 nm) and characterised by SEM (JEOL JMS 7600 F).

To provide a method to transfer samples for Py-GC-MS analysis, we adapted the protocol of Steinmetz and Schröder [42]. Recovery was tested in triplicate using a suspension of 100 nm fluorescent PS NPs in 50% ethanol (20 mL) in 30 mL glass crimp-top vials. Samples were dried on a hot plate in a fume hood with HEPA-filtered air at 120 °C. 2 mL of a 1:1 v/v mixture of 1,2,4-trichlorobenzene and *p*-xylene containing 100 mg/L butylated hydroxytoluene was added using a glass pipette (Acura® manual 835 macropipette with Pasteur pipette fitting, Socorex, Switzerland), and the vials were sealed with PTFE-lined crimp caps. The samples were placed in an oven at 150 °C for 1 h and vortexed twice to facilitate dissolution and collect any particles adhered to the side of the vials. 30 µL aliquots of each sample were dried in pyrolysis cups for 10 min at 150 °C. The process is shown schematically in Fig. 1c. Recovery was evaluated by Py-GC-MS analysis of the dried and dissolved samples compared with the original suspension added to the vials.

To evaluate the LOD of the method for Py-GC-MS analysis, calibration curves were prepared for PE, PP, and PS. The solid polymer powder was dissolved and transferred as described above (Fig. 1c). The target polymers (PE, PP, and PS) were analysed using a pyrolyzer (EGA/PY-3030D, Frontier Lab®, Fukushima, Japan) equipped with an autosampler (AS-1020ET, Frontier Lab®, Fukushima, Japan) coupled to a gas chromatograph (Trace™ 1310 GC, Thermo Fisher Scientific, Dreieich, Germany) and a mass spectrometer (ISQ 7000™, Thermo Fisher Scientific, Dreieich, Germany). Samples were pyrolyzed under helium in double-shot mode to evaporate residual solvent and volatiles before polymer pyrolysis in order to protect the source. In the first shot, the temperature was held at 240 °C for 5 min, ramped to 250 °C at 10 °C/min, and held for 1 min. The resulting gas was transferred to the GC (split ratio 100, flow rate 0.8 mL/min) operated at 150 °C for 1 min, then ramped to 310 °C at 50 °C/min and held for 3 min. The second shot was performed at 250 °C for 2 min, and the gas produced was also transferred to the GC (split ratio 100, flow rate 1.2 mL/min) operated as follows: 310 °C for 2 min, ramped to 310 °C at 10 °C/min, held for 4 min.

After the first and second shots, the remaining sample in the same pyrolysis cup was then pyrolyzed at 610 °C for 18 s and transferred to the GC (split ratio 40). The pyrolysis interface and GC split/splitless injector were maintained at 300 °C. Pyrolysates were separated on a TraceGOLD TG-5MS capillary column (30 m × 0.25 mm, 2.5 µm film thickness; Thermo Fisher Scientific, Dreieich, Germany). The GC oven program was started at 60 °C (3 min hold), ramped to 320 °C at 20 °C/min, and held for 14 min. The transfer line between the GC and MS as well as the ion source of the MS (electron ionization, 70 eV) were held at 250 °C. For pyrolysate identification, the mass spectrometer collected data in scan mode with a mass range of 40 to 600 m/z. For quantitative analysis, SIM mode was used to determine the peak intensity of the selected mass for each pyrolysate. The pyrolysates selected and their indicator ions are given in SI 5, Table SI 5. Full details of the calibration curves and calculation of the instrumental LOD (limit of detection) and LOQ (limit of quantification) are provided in SI 5.

In the above tests, blank samples containing no Pd-doped NPs were processed in parallel. Reported values for Pd-based NP concentrations and NPOC analysis are blank subtracted. Measures for contamination prevention are reported in SI 6. During Py-GC-MS analysis, blank sample cups were subjected to the same heating steps as the samples. During extraction of samples for SEM analysis, a water sample spiked with 100 nm and 250 nm fluorescent PS NPs as well as a blank sample were included.

Results

Optimisation and evaluation of individual steps of NP extraction and purification

Testing the initial extraction step by homogenisation and sedimentation with TSPP as described by Foetisch et al. [9] gave a recovery of 38 ± 7% (n = 3, mean ± SD) using Pd-doped NPs. The recovery of the density separation step was 74 ± 18% (n = 3, mean ± SD). The relatively high standard deviation can likely be attributed to sensitivity of the step to sample handling, such as differences when collecting 5 mm into the sucrose layer. The recovery could potentially be increased by collecting deeper into the sucrose layer. However, sucrose could interfere with microscopy by forming a layer on the substrate that obscures particles and could also hinder Py-GC-MS analysis by crystallising during drying, thereby shielding particles from dissolving in the solvent. Therefore, we deemed this recovery sufficient due to the need of keeping the sucrose concentration in the sample low. When evaluating sources of particle loss, 7 ± 1% (n = 3, mean ± SD) of the Pd-doped NPs were found in the pellet, and only 0.1 ± 0.1% (n = 3, mean ± SD) in the pipette used to collect the supernatant, indicating the remainder (~ 19%) were retained on the tube walls or left in the remaining sucrose solution (Fig. 2a). For density separation in the presence of sucrose, we found only very minimal aggregation (or agglomeration) of the 100 nm and 250 nm PS

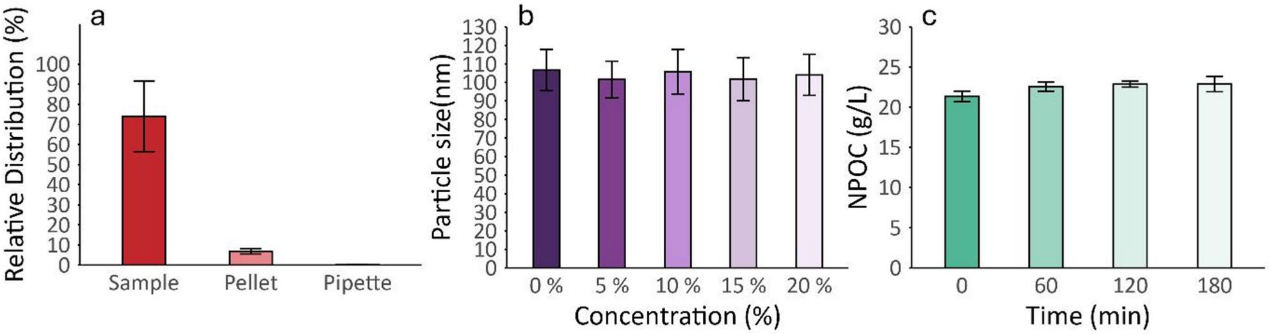

Fig. 2 Evaluation of the density separation and the oxidation of organic matter in the samples. **a** Relative distribution of NPs in the sample collected from the supernatant, pellet, and pipette after density separation. Values indicate proportion of NPs found in each. Error bars represent the standard deviation from triplicate tests. **b** Average size of 100 nm fluorescent PS NPs after treatment with different concentrations of H_2O_2 for 2 h. Error bars represent standard deviation from size determination of 100 particles per sample using SEM. **c** Changes in NPOC concentrations over time after adding H_2O_2 to the test solutions containing sucrose and soil extract in 2.5 mM TSPP. Error bars indicate the standard deviation from triplicate tests

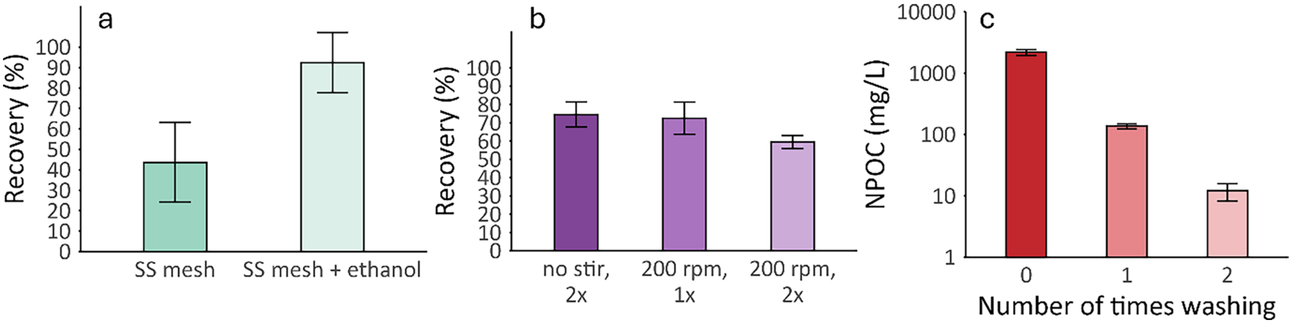

Fig. 3 Optimisation of NP extraction procedures. Error bars represent standard deviation from triplicate tests. **a** Recovery of stainless steel filtration protocols (SS = stainless steel). **b** Recovery of different washing and concentration protocols using an ultrafiltration device. **c** Concentration of NPOC in the sample after different numbers of washing and concentration steps using an ultrafiltration device (note the logarithmic scale on the Y axis)

NPs in SEM images after centrifugation, caused by drying after the drop deposition on the Si wafer. However, no 3D structures were detected, indicating that aggregates or agglomerates did not form due to centrifugation (SI 7, Fig. SI 2). To remove natural organic matter and sucrose, we tested 15% H_2O_2 for oxidation, rather than the 5% H_2O_2 used by Foetisch et al. [9]. SEM image analysis indicated no significant effects of H_2O_2 concentrations up to 20% on the particle size of fluorescent 100 nm PS NPs after treatment with H_2O_2 (Fig. 2b, SI 8, Fig. SI 3). However, 15% H_2O_2 did not effectively remove sucrose, as seen in Fig. 2c, with no significant differences between NPOC concentrations of sucrose-containing test solutions exposed to 15% H_2O_2 after 0, 60, 120, or 180 min. Furthermore, the temperature of the solutions did not increase significantly over time, indicating that no strong chemical reaction was occurring, as the reaction of H_2O_2 with NOM is exothermic (SI 8, Fig. SI 4). Because it failed to remove sucrose, this step was excluded from the final method.

Two filtration approaches were tested to isolate the < 1 µm NP fraction. In our preliminary test using syringe filtration with a PTFE membrane filter (1 µm pore size), the recovery was only 25 ± 1% ($n = 3$, mean ± SD) and we observed significant clogging of the filters. Furthermore, qualitative analysis of SEM images of the filters revealed retention of many particles < 1 µm due to the filter's multilayer structure and undefined pores (SI 9, Fig. SI 5). Vacuum filtration with a stainless steel mesh filter improved the recovery. Without washing, the recovery was 44 ± 19% ($n = 3$, mean ± SD), but increased to 92 ± 15% ($n = 3$, mean ± SD) after rinsing the apparatus with ethanol after filtration (Fig. 3a). SEM images showed that the filters had well-defined pores and a single-layer structure, while few particles were detected on the surface post-filtration (SI 9, Fig. SI 5).

Due to the removal of the organic matter oxidation from the protocol, the washing and concentration step using ultrafiltration became more critical for sucrose removal. We observed that stirring created a vortex, which may have caused particles to adhere to the ultrafiltration membrane. To test this, we examined the impact of stirring and the number of washes on recovery (Fig. 3b). Fewer washes increased recovery, while stirring decreased it. We also investigated the removal of sucrose during the washing by NPOC analysis (Fig. 3c).

After 1 wash, the concentration of NPOC was still high (136 mg/L), so the option of washing the sample twice without stirring was chosen (12 mg/L NPOC). This had a recovery of 74 ± 7% (*n* = 3, mean ± SD).

Evaluation of the optimised extraction method

The recovery of the complete extraction method sequence (Fig. 1) was evaluated up to the end of the washing and concentration step using Pd-doped NPs. The measured overall recovery was 1.4 ± 0.4% (*n* = 3, mean ± SD). The recoveries of each of the finally chosen steps in the full extraction procedure are shown in Table 1.

Analysis with SEM and Py-GC-MS

To demonstrate the applicability of the method developed here for analysis of NPs with advanced microscopy techniques, we subjected soil samples spiked with a mix of smooth, spherical 100 nm and 250 nm fluorescent PS NPs to the full extraction procedure. A spiked water sample and an unspiked water blank were included to assess the influence of soil and cross-contamination. SEM images (Fig. 4, SI 10) of the spiked soil and water extracts showed that the spherical 250 and 100 nm particles were well separated and largely free of contamination. The particles retained their shape and size, confirming that NPs can be successfully extracted and imaged using our method while preserving particle integrity. Some minimal contamination could be observed in the form of thin, elongated structures < 1 µm in width and variable in length in the spiked soil extracts, which were absent in the spiked water extract. EDX spectra showed these structures to be organic materials. However, as the NPs were spheres of 100 nm and 250 nm, they could be easily distinguished based on their shape and size. Additionally, in all samples, the substrate and particles appeared to be covered by a thin residue in some places (shown in SI 10). EDX spectra suggest this residue is organic and is likely sucrose due to high levels of C and O. In the blank sample, very few NPs were detected, indicating negligible cross-contamination. SEM images and EDX spectra of this contamination and residue are provided in SI 10. Overall, NPs were retained as individual, intact particles, and the samples exhibited high purity with minimal residual material, showing the success of our method in enabling analysis of soil NPs using advanced microscopy techniques.

In the transfer step to Py-GC-MS through drying the sample and dissolving in an organic solvent mixture, the recovery was 87 ± 41% (*n* = 3, mean ± SD). The peak areas found are shown in SI 11. No peaks were detected in the calibration blanks, so the LOD was determined from the lowest concentration standard. Values for LOD and LOQ are given in Table 2. Details on the calibrations can be found in SI 12.

Table 1 Overview and description of each individual optimised method step as well as the final combined optimised method including mean recovery and standard deviation of recovery, assessed by using Pd-doped NPs

Method step	Description	Mean recovery	Standard deviation of recovery
1. Extraction of NPs from soil	Extraction of NPs from soil with TSPP 2.5 mM, homogenisation, sedimentation for 18 h	38%	7%
2. Density separation	Separation of NPs from mineral soil components using saturated sucrose solution layer in a benchtop centrifuge	74%	18%
3. Filtration	Dilution of sample to 200 mL, filtration through stainless steel mesh filter using glass vacuum filtration apparatus, washing with 10 mL ethanol	92%	15%
4. Washing and concentration	Washing twice with 200 mL MilliQ water in an ultrafiltration device, concentration to 10 mL, no stirring, washing membrane with 10 mL ethanol	74%	7%
All (calculated from recoveries of individual steps)	Calculation of theoretical recovery from all method steps above	19%	7%
All (measured experimentally)	All method steps above carried out in sequence	1.4%	0.4%

Discussion

Optimisation and evaluation of individual steps of NP extraction and purification

The initial extraction with 2.5 mM TSPP resulted in a low recovery of 38%, indicating that the extraction of the NPs from soil is the limiting step of the overall extraction protocol. The extraction approach assumes colloidal behaviour of NPs [9, 39], such that they remain evenly distributed in the suspended fraction after settling. In soil systems, NPs could associate with soil particles during extraction, potentially affecting their distribution between fractions [43]. The extraction protocol was adapted from Schwertfeger et al. [39], who reported an 84% recovery for extracting silver nanoparticles from soil. However, as silver nanoparticles and NPs have different surface charge, density, hydrophobicity, and chemical reactivity, their interactions with soil particles are likely to differ. They could be associated with different types of soil particles or have different binding strengths through electrostatic vs. hydrophobic interactions [44,

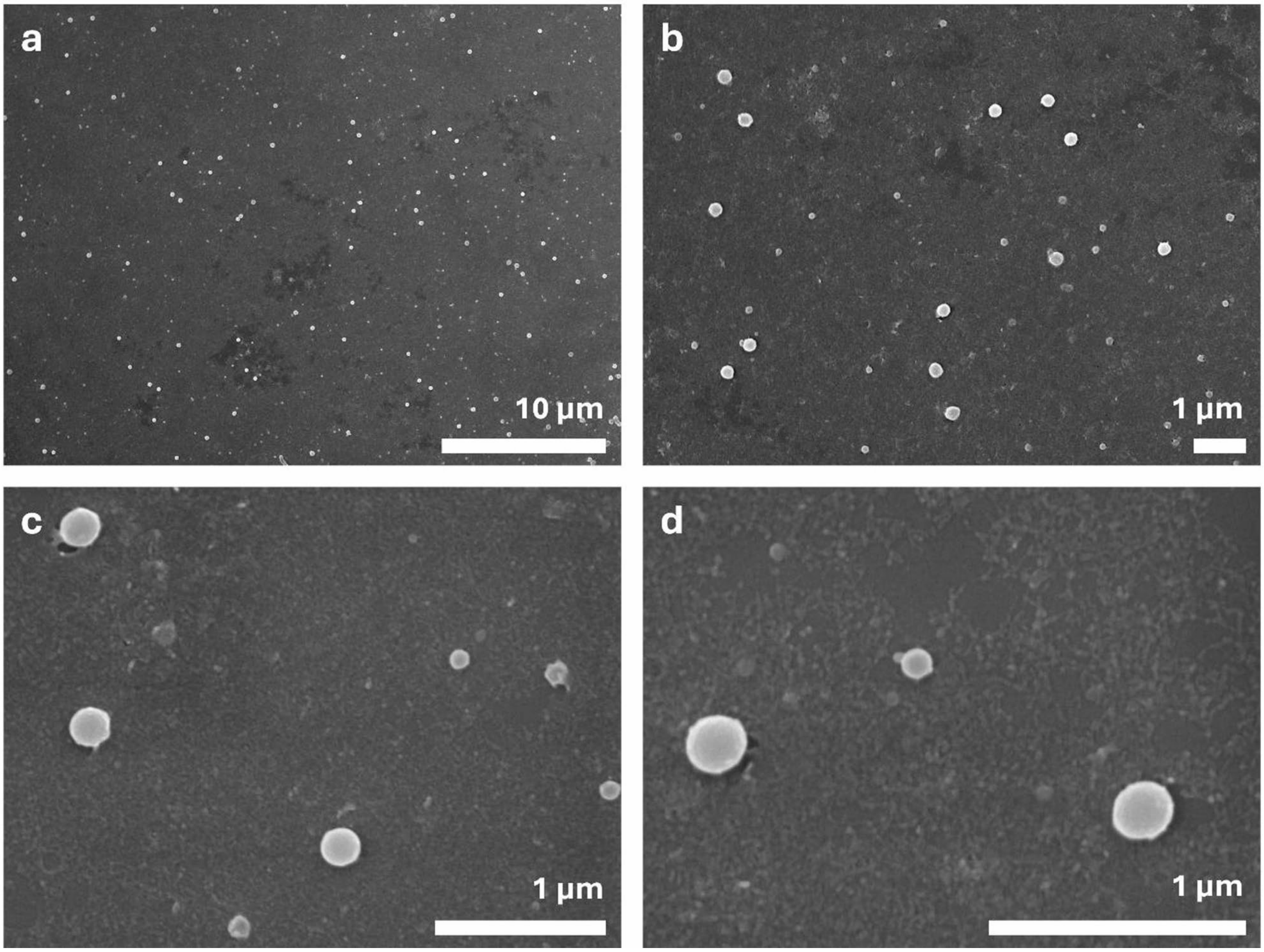

Fig. 4 Representative SEM images of extracts of Wageningen soil spiked with 250 nm and 100 nm fluorescent PS NPs. **a** 3,000 × magnification, **b** 10,000 × magnification, **c** 30,000 × magnification, **d** 50,000 × magnification

Table 2 LOD and LOQ for each polymer (PE, PP, or PS). The instrumental LOD and LOQ represent the absolute masses measured by the instrument. The method LOD and LOQ values in μg/g are calculated based on the instrumental LOD and LOQ, extrapolating from the 30 μL sample measured to the original 50 g soil in 1 L extractant, and accounting for the recovery of the extraction procedure (1.4%)

Polymer	Instrumental LOD (μg)	Method LOD (μg/g soil)	Instrumental LOQ (μg)	Method LOQ (μg/g soil)
PE	0.064	257	0.19	779
PP	0.053	211	0.16	641
PS	0.057	227	0.17	688

45]. Schwertfeger et al. [39] used an ultrasonic wand for the extraction, which increased recovery approximately 3-fold compared with an ultrasonic bath, likely due to the higher power of ultrasonication wands. They also observed that a soil-to-extractant ratio of 1:100 caused a 20-fold increase in the number of particles extracted compared with a ratio of 1:10. In contrast, we used a soil-to-extractant ratio of 1:25 to extract a large enough mass of soil in a manageable volume of solution, aiming for a high concentration of NPs for later detection. We opted for an ultrasonic bath for the extraction to avoid cross-contamination from an ultrasonic wand. Switching to an ultrasonic wand and lower soil-to-extractant ratio could nonetheless improve recoveries of NPs. However, any future attempts to increase the soil-to-extractant ratio for NPs extraction should aim to maintain a large volume of soil to avoid a further dilution of the initial samples which would increase the method LOD (Table 2). Monikh et al. [33] applied a three-step extraction protocol to soils spiked with Eu-doped PS NPs, including extraction with MilliQ water, SDS, and TMAH to isolate NPs bound to different soil fractions, following each step with a density separation using a 1.11 g cm^{-3} ammonium sulphate solution. This yielded a recovery of > 77% in the tested soils while preserving the integrity of the PS NPs. However, this method focused only on the extraction of particles and did not involve concentration or filtration of samples which would be required for SEM or Py-GC-MS. The extraction protocol developed by Monikh et al. [33] could be adopted in future work due to its higher recovery than TSPP, but testing would be required to ensure that the samples are sufficiently purified for downstream analysis. In addition, previous work has used TMAH ranging between 5 and 10% w/w with 90% recovery under optimised conditions to extract PS NPs from soils and reported approximately 25% higher recovery compared to TSPP [10, 23]. However, those studies involved subsequent leaching with DCM, making them not suited for imaging of any polymers soluble in DCM. While Li. Z et al. [10] show that TMAH does not affect the surface of PS NPs, applying TMAH to an extraction method which aims to preserve particle integrity would require testing to ensure compatibility with the further downstream steps, as it digests a certain proportion of the organic matter in the samples, significantly altering the sample matrix. This may necessitate further clean-up. Our tests of the density separation with sucrose showed the utility of sucrose as a density cushion, allowing preservation of NPs in their natural agglomeration state or as isolated particles, in contrast to density separation using certain concentrated salt solutions, which can cause NPs to agglomerate and thus rules out further size separation through filtration [46]. Despite the density of the sucrose solution (1.22 g cm^{-3}) leading to exclusion of denser polymer types such as PVC or PET (both 1.38–1.41 g cm^{-3}), it is the best option known to us as it still includes many common polymers such as PE, PP, PS, PA, and polycarbonate (PC), which have a lower density [41]. It should be noted that this step is sensitive to sample handling, and care must be taken to collect the top 5 mm of the sucrose layer to maintain the reproducibility. 1.8 M ammonium sulphate solution (pH 7, 1.11 g cm^{-3}) was also utilised in a density separation for NP extraction, with NPs remaining isolated when exposed to the solution for < 1 h, but the lower density excludes further polymer types [33]. We recommend that future work using sucrose solution in a density separation tests for the agglomeration of a wider range of polymer types. Next, we found no significant difference in NPOC concentration after treatment with 15% H_2O_2. We therefore omitted this step from the procedure altogether. Particulate organic matter was assumed not to interfere with Py-GC-MS analysis, as the solvent used is optimised for our target plastics. This was demonstrated by Steinmetz et al. [37], who observed minimal interference from SOM when using 1,2,4-trichlorobenzene to extract LUFA 2.2 and RefeSol 06-A, compared with samples spiked with PE, PP, and PS. Further < 1 µm non-plastic particles would also be removed by the inclusion of a filtration step. For SEM analysis, the contribution of particulate organic matter to the contamination was shown to be negligible, while NPs could be easily identified, showing that an organic matter oxidation step is not necessary for this application.

For NP filtration, many studies in both soil and water have used various membrane filters [5, 11, 19, 20, 36], while recovery of the individual step or the filter material is not always reported. The recovery of our two filtration methods reflected the SEM images of the filters (Fig. SI5). The PTFE membrane filters are multilayered, and many pores are smaller than the 1 µm average, whereas the stainless steel filters are a single layer, and the pores are very evenly spaced and sized. These findings are consistent with Albignac et al. [34], who found that stainless steel filters (albeit with a pore size of 5 µm) exhibited the lowest retention of NPs of various sizes in comparison to membrane filters, attributing this to the pore structure of the filters. Furthermore, Li. Q et al. [47] investigated polyvinylidene fluoride (PVDF), nylon, quartz, and glass membranes for retention of NPs and found that the inorganic membranes had a much lower retention, attributing this to hydrophobic interactions between the polymer membranes and the NPs. This may also play a role in the difference in recovery between the PTFE filters and the stainless steel filters. Li. Q et al. [47] found 68 – 87% recovery for NPs in a procedure comprising glass membrane filtration and cloud point extraction for NP-spiked water samples, indicating that the single-layer stainless steel filters may be superior as a higher recovery was achieved. We therefore recommend stainless steel mesh filters for NP extraction to avoid underestimations of particle concentrations.

Finally, ultrafiltration was used to remove sucrose and other dissolved impurities from the samples and decrease the volume for further analysis. Previous work involving the analysis of NPs in water samples has often used ultrafiltration to concentrate samples, although recovery has not always been reported [5, 20]. However, of the studies which reported recoveries, 60% has been shown when using 50 kDa PES membranes [34], or 48% using tangential flow ultrafiltration [35], and 60% overall recovery in a procedure comprising membrane filtration and ultrafiltration with a 100 kDa PES membrane, including six washing steps of the ultrafiltration device with ultrapure water [19, 36]. Therefore, our optimised procedure with a recovery of 74% improves recovery relative to other methods, highlighting that avoiding stirring is a critical component for improving NP recovery when using these devices, and that washing the membrane with ethanol may be critical to improve recoveries.

Evaluation of the optimised extraction method

When combining all the optimised extraction steps, an expected recovery of 19% was calculated from the individual steps. However, the experimentally measured recovery was 1.4%, suggesting that further losses occurred during the procedure which could not be elucidated in the recovery tests of the individual extraction steps. These losses are therefore likely associated with transfer between the extraction steps through pipettes and intermediate vessels, and due to filtration and ultrafiltration performance in the presence of soils. While our initial tests of filtration and ultrafiltration were carried out in the absence of soil to estimate their performance, soil matrices can complicate these processes through fouling of the membrane or blockage of filters, which may lead to lower recoveries. Additionally, the NPs could agglomerate with or adsorb to soil particles, causing further losses of NPs. When testing the suitability of our extraction method for NP characterisation using SEM, we saw that the spiked soil samples had a lower concentration of NPs than the spiked water sample (SI 10), giving qualitative support to this theory. Despite the low recovery, we were able to detect NPs in spiked soil samples to which our method was applied using SEM. The particles remained isolated and it was possible for size and shape to be determined.

It is important to note that our recovery tests with freshly spiked soil and well-defined PS NPs cannot fully represent the behaviour of NPs which have been present in soil over a longer period. NPs present in environmental soils are likely to undergo chemical and physical aging, consist of a range of polymer types, shapes, and sizes, and interact with the soil matrix [33, 48]. These interactions may include sorption of natural organic matter or organic pollutants [49, 50], attachment to soil surfaces [51], and biofouling [52], which can significantly alter the particle behaviour through altering surface charges and hydrophobicity [33, 52]. These processes could decrease particle extractability. For example, for MPs, aged particles have been shown to be extracted from water with lower recoveries than pristine particles [53]. Therefore, while spiking is necessary for recovery calculations, these limitations should be considered when interpreting the effectiveness of extraction methods.

Only five previous studies have proposed extraction methods for NPs in soil (Table 3). Wahl et al. [11] used water to extract NPs from soil, purified the samples using membrane filtration and AF4, and identified NPs by Py-GC-MS. However, they did not quantify NPs, characterise them by microscopy techniques, or validate their extraction method. While this method does retain individual NP particles, the authors recommend further clean-up steps to reduce the interference from non-plastic particles, which is particularly relevant for microscopy techniques. Furthermore, AF4, though effective, is not widely accessible and can only be applied in highly specialised laboratories. Foetisch et al. [9] identified individual NPs in soil using STXM-NEXAFS but here

Table 3 Comparison of different methods for the extraction and analysis of nanoplastics

Study	Extraction & clean-up	Particle integrity preserved?	(Potential) application to multiple polymer types?	Size & shape?	Quantification by Py-GC-MS?	Recovery	LOD for Py-GC-MS (µg/g)
This study	TSPP → density separation → filtration + ultrafiltration → SEM/ Py-GC-MS	✓	✓	✓	✖	1.4% (PS)	257(PE), 211(PP), 227 (PS). Determined taking recovery into account.
Foetisch et al. [9]	TSPP → density separation→ oxidation → ultrafiltration → SEM/ STXM-NEXAFS	✓	✓	✓	✖	?	?
Monikh et al. [33]	Water, SDS, TMAH → density separation with ammonium sulphate	✓	✓	?	✖	77–82% (PS). No downstream analysis.	?
Wahl et al. [11]	Water → membrane filtration → AF4 → Py-GC-MS	✓	✓	?	✖	?	?
Li. Z et al. [10]	TMAH → flocculation → DCM leaching → Py-GC-MS	✖	✓ (PS and PMMA)	✖	✓ (PS and PMMA)	72.5–87.2% (PS) 80.5–83.2% (PMMA)	0.02 (PS) Not reported for PMMA
Li. P et al. [23]	TMAH → centrifugation → DCM leaching→ Py-GC-MS	✖	✓	✖	? (Includes MPs)	76 – 101% (PS), 75 – 93% (PMMA)	2.3 (PS), 4.8 (PMMA), 12.1 (PET), 9.4 (PVC), 29.2 (PE), 18.1 (PP)

the authors were also not able to test the recovery of the extraction method or quantify NPs. Monikh et al. [33] developed a method to extract Eu-doped PS NPs from soil, achieving 77–82% recovery. However, their workflow did not include any method to concentrate, size-separate, or prepare the samples for downstream microscopic or chemical analysis, for example by SEM or Py-GC-MS. Li. Z et al. [10] were the first to provide a quantitative analysis of NPs in soils using Py-GC-MS, but only of PS. During method testing, the authors also showed potential application of their method for poly(methyl methacrylate) (PMMA) quantification, though this was not tested in environmental samples. Their method was not applied for analysis of other, more common, polymer types such as PE and PP in environmental samples. Because the method relied on the solubility of PS (and PMMA) in DCM at low temperatures (60 °C) which is not applicable for PE and PP, it is unclear if it could be applied for further polymer types. Li. P et al. [23] developed an extraction method for small MPs and NPs of various polymer types with samples dissolved in DCM analysed by Py-GC-MS, using only NPs for recovery testing during method validation. But, due to the inclusion of small MPs < 150 μm in the analysis of soil samples, the mass concentrations found will be dominated by MPs, making it impossible to determine the concentration of NPs as there is no size differentiation. Furthermore, while some studies have achieved quantification of NPs in water using Py-GC-MS, these methods cannot be transferred to soils due to the significantly more complex matrix [19, 20, 36]. As there are significantly fewer non-target particles in water matrices than soil matrices, the extraction methods in water do not include any step to remove mineral particles e.g. via density separation, instead using a combination of filtration to 1 μm, ultrafiltration for concentration, and OM digestion. Without pre-extraction and removal of mineral particles, this would not be possible for soils. In contrast, our study is the first to propose an extraction method for the analysis of NPs with the potential for application to multiple polymer types while maintaining particle integrity, where the individual steps and full method have been tested for their recovery and applicability to downstream analysis techniques, representing a significant advancement towards the analysis of NPs. The advantage of this approach is that imaging techniques allow for size and shape data for NPs to be determined, which allows for a more complete dataset once methods for detecting mass concentrations of NPs have sufficiently advanced. Furthermore, the method was designed to be easy to implement in a wide range of laboratories, expanding the accessibility of NP analysis.

Application to Py-GC-MS and microscopy methods

Py-GC-MS was evaluated for its compatibility with our extraction method and its feasibility for analysis of soil-extracted NPs. Accordingly, there are different ways to prepare samples for Py-GC-MS analysis. Xu et al. [20] suspended NPs extracted from environmental water samples in methanol. DCM has been used to suspend NPs at room temperature [19, 36], while others have used DCM at high temperatures to extract thermoplastic polymer types from soils [23, 54]. We opted to dissolve the samples in a 1:1 v/v mixture of 1,2,4-trichlorobenzene and *p*-xylene containing 100 mg/L butylated hydroxytoluene, which is used to dissolve PE, PP, and PS [37, 42]. Although this excludes other common polymers found in soils such as PA, PET, and PC, this facilitated sampling handling and ease of applicability as there was no need for pressurised liquid extraction. Furthermore, dissolving the samples is preferable to having NPs in a suspension, as it is not possible to be certain these are homogeneously mixed when subsampling.

The instrumental LODs determined in this study are comparable to others in the field, which typically range from 0.001 to 0.081 μg in DCM or 1,2,4-trichlorobenzene/*p*-xylene matrices [10, 36, 37, 42], indicating that the Py-GC-MS method used in this study achieves high sensitivity and analytical performance. However, given the low recovery, the method in its current form is not yet suitable for quantitative analysis of NPs in soil. Even with 100% recovery, the method LOD would still be high, in the range of ca. 3 μg/g. Li. P et al. [23] had a comparable method LOD in their work which included analysis of small MPs and NPs < 150 μm (2.3, 4.8, 12.1, 9.4, 29.2, and 18.1 μg/g for PS, PMMA, PET, PVC, PE, and PP, respectively), but this likely would not be high enough for analysis of NPs, as Li. Z et al. [10] found PS NP concentrations in soil only between 0.16 and 0.73 μg/g. In contrast, Li. Z et al. [10] had a method LOD and LOQ of 0.02 μg/g and 0.07 μg/g for Py-GC-MS analysis of PS NPs in soils. However, their approach is optimised for PS detection through lower pyrolysis temperature [55], and benefits from the use of DCM as a solvent, allowing for larger sample volumes to be analysed (the low boiling point allows the sample to be transferred multiple times and evaporated quickly, so 350 μL can be analysed) but limiting the application to PS and PMMA as most other polymers are not soluble in DCM at the temperature used (60 °C). In contrast, our proposed quantification method is designed for applicability to three polymers by using a higher pyrolysis temperature, while minimising interferences from non-target compounds with a thermal desorption step and a lower split ratio. However, our sample volume is limited by the high-boiling-point solvent, as only 30 μL can be used to avoid overloading the column. This highlights the challenge of designing methods for multiple polymer types, requiring specialised solvents that limit the sample volume, and introducing trade-offs in the yield of target pyrolysis products. Furthermore, our method LOD is limited by dilution during the extraction process: only 24 mL of sample is taken from the original 1 L extract for the density separation. While NPs are assumed to be homogeneously distributed through the extract, only the top layer was collected after 18 h of settling to avoid collecting large particles. The volume is also limited by centrifuge tube volumes for the density separation. Together with the variability observed during the transfer step to Py-GC-MS (87% ± 41%), these factors currently limit the application of Py-GC-MS for quantification of NPs in soil at environmentally relevant concentrations using our extraction method. Future methodological improvements could lower effective detection limits by increasing the processed mass of soil, processing larger subsamples after the initial extraction, decreasing dilution factors during the transfer step to Py-GC-MS, and investigating strategies for analysing higher analyte volumes per pyrolysis cup.

Methods intended for imaging of NPs face difficulties in comparison to methods designed for purely mass-based quantification through Py-GC-MS, as they require more sample purification as well as focus on maintaining the integrity of the individual particles, leading to lower recoveries. In contrast, in mass-based analysis, digestion-based extraction of NPs and leaching of plastics directly from solid samples is possible. This allows to eliminate more interferences from NOM or mineral particles before analysis and yields higher recoveries, albeit at the expense of size and shape information of the particles and a complete dataset. Some studies have increased the sample volume analysed by serially transferring the sample into analysis cups with multiple drying steps in between [10, 20]. However, this option increases the risk of contamination from open samples evaporating under a fume hood. Improvements to future particle-preserving NP extraction methods from soil should focus on optimising the initial particle extraction and increasing sample volumes to increase detection sensitivity, as well as work on improving the method and instrumental LOD. Additionally, testing for multiple soil types will help to elucidate the impact of different soil textures, pH, and organic matter content on the recovery of the extraction procedure.

In contrast to mass-based analysis with Py-GC-MS, our method would be well suited to qualitative analysis of single particles using microscopy techniques such as SEM, AFM-IR, and STXM-NEXAFS. This requires a deposition on a membrane such as Si or Si_3N_4, which can be achieved through drop deposition or deposition by centrifugation [9] instead of dissolving in a solvent. Despite the low recovery, it should still be possible to detect NPs as their expected number concentration in the environment is high [4, 5]. In our proof-of-concept tests with spiked soil extracts, spherical PS NPs were identified in SEM images and the samples contained very few non-target analyte particles, showing the suitability of our method for microscopy techniques. For spiked samples with defined NP shape and size, SEM would be sufficient for NP identification. Environmental samples would require additional techniques to confirm NP identity due to heterogeneity in size and shape. For example, STXM-NEXAFS and AFM-IR have been used to identify NPs in environmental samples, providing information on size, shape, and polymer type [9, 28]. While these techniques are not practical for high-throughput applications, they can provide a first understanding of the properties of NPs in soils.

Conclusions

Our findings confirm the difficulty of extracting NPs from soil, particularly across a wide range of polymer chemistries, and show that current extraction methods which preserve the integrity of NPs are not yet sufficient for fully quantitative measurements of NPs in soil. While particle-preserving methods have significant advantages, potentially allowing determination of size and shape of polymers alongside polymer chemistry and mass concentration, they require very clean samples, making them labour-intensive and vulnerable to particle losses. Given these challenges, combining NP extraction methods which preserve particle integrity with Py-GC-MS is hampered by a high method detection limit, as shown by our feasibility assessment, so that only heavily contaminated samples are likely to have detectable concentrations of NPs. However, our method is the first recovery-tested extraction protocol for NPs in soil that not only preserves particle integrity but also incorporates the necessary purification and concentration steps to produce clean samples suitable for downstream analysis. We demonstrated very clean samples which could be analysed qualitatively by SEM. This makes the method well suited for other advanced microscopic techniques such as STXM-NEXAFS or AFM-IR, enabling size, shape, and polymer type characterisation of soil NPs.

Abbreviations

AF4	Asymmetric Flow Field-Flow Fractionation
AFM-IR	Atomic Force Microscopy–Infrared Spectroscopy
DCM	Dichloromethane
DLS	Dynamic Light Scattering
HEPA	High-Efficiency Particulate Air (filter)
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
LUFA	Landwirtschaftliche Untersuchungs- und Forschungsanstalt (standard soil)
MP	Microplastic

MPs	Microplastics
NEXAFS	Near Edge X-ray Absorption Fine Structure
NOM	Natural Organic Matter
NP	Nanoplastic
NPs	Nanoplastics
NPOC	Non-Purgeable Organic Carbon
PA	Polyamide
PC	Polycarbonate
Pd	Palladium
PE	Polyethylene
PET	Polyethylene Terephthalate
PES	Polyethersulfone
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
PVDF	Polyvinylidene Fluoride
Py-GC-MS	Pyrolysis Gas Chromatography–Mass Spectrometry
RSD	Relative Standard Deviation
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
SOM	Soil Organic Matter
SS	Stainless Steel
STXM	Scanning Transmission X-ray Microscopy
TMAH	Tetramethylammonium Hydroxide
TSPP	Tetrasodium Pyrophosphate
µFTIR	Micro-Fourier Transform Infrared Spectroscopy
µRaman	Micro-Raman Spectroscopy

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-026-00172-x.

Supplementary Material 1

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Author contributions

Hannah Forsyth: Conceptualisation, Methodology, Investigation, Validation, Formal analysis, Data curation, Writing – original draft, Visualisation. Chiara Gnoffo: Methodology, Investigation, Formal analysis, Writing – review and editing. Sehui Oh: Methodology, Investigation, Writing – review and editing. Kaori Sakaguchi-Söder: Methodology, Investigation, Formal analysis, Writing – review and editing. Denise M. Mitrano: Conceptualisation, Resources, Writing – review and editing. Alberto Frache: Conceptualisation, Resources, Methodology, Supervision, Project administration, Funding acquisition, Writing – review and editing. Moritz Bigalke: Conceptualisation, Methodology, Resources, Writing – review and editing, Project administration, Funding acquisition, Supervision.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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