Supplementary Information

Exploring environmental nanobiogeochemistry with field-flow fractionation and ICP-MS-based tools: Progress and frontiers

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Section S1

For each soil type (CM and OB), subsamples were collected from a depth of 0–0.15 m. The collected soils were air-dried, and coarse materials were manually removed. Subsamples were manually homogenized to form a composite sample. Plastic pails with an inner diameter of 0.29 m and a height of 0.37 m were each filled with 19 kg (wet basis) of two different soil types. These soils achieved bulk densities of (1.122, and 1.101) $*10^3$ kg m^{-3}, respectively Soils were saturated using ultrapure water, to achieve the field capacity and kept overnight until achieve equilibrium. Rhizon MOM lysimeters with 0.58–0.65 µm pore size were employed to collect soil solutions and subsequently filtered through a 0.45 µm membrane before analysis.The pH of the CM and OB soil solutions were 6.55 ± 0.08 and 8.1 ± 0.3, respectively, with corresponding electrical conductivities of 0.132 ± 0.003 and 0.77 ± 0.08 (at 95% CI). A miniaturized AF4 channel with 300-Da poly(ethersulfone) (PES) membrane and a 700 µm spacer were used to perform the separation. The system was coupled to a UV/Visible diode array detector and quadrupole (Q)-ICP-MS. The carrier fluid was 10 mM $(NH_4)_2CO_3$ adjusted to pH 8.5 and ionic strength 2.126 dS m^{-1}. A sample volume of 0.125 mL was injected and focused for 7 min, with a tip flow rate of 0.1 mL min^{-1}, a crossflow rate of 0.45 mL min^{-1}, and a detector flow rate of 1.02 mL min^{-1}. The membrane was conditioned by the sample 5 times before the measurement to avoid losses due to adsorption.

Section S2

The size fractionation of colloidal particles in pore water samples was conducted with an AF4 instrument (AF2000, Postnova Analytics) online coupled with a UV/VIS detector (diode array detector, SPD-20A Postnova Analytics) and with ICP-MS (Agilent, 7700). The fractionation conditions involved an analytical AF4 channel equipped with a 1 kDa cut-off PES membrane and 500 µm thick channel spacer. The carrier solution consisted of 1 mM NH_4HCO_3, and the injected sample volume was either 0.1 mL or 1 mL, depending on the colloid loading. The sample was focussed for 12 min, with tip flow rate of 0.2 mL min^{-1} and detector flow rate of 1 mL min^{-1}. The flow program used a gradient decrease in cross-flow to achieve separation across a wide range of colloid sizes up to 100 nm, although a high initial cross-flow was sustained for the first 15 minutes, allowing high resolution of the size-range < 30 nm. The initial constant cross-flow was 1.5 mL min^{-1} for 30 min, followed by a linear decrease to 0.2 mL min^{-1} in two minutes, which was kept constant for another 30 minutes.

Discussion regarding the nature of Fe-NNPs based on size-based fractionation: The first peak with high UV absorbance is attributed to humic substances (HS).[1] Despite the lower resolution for small-sized components compared to **Fig. 1**, this statement is supported by the co-elution of Cu, known to have high affinity for HS. In this small sized, humus-rich fraction, Fe and Al likely consist of mononuclear complexes, i.e. iron(III) and Al(III) cations that form stable chelates with humic substances (Rhy soil, Alu soil). In the Zeg soil, the Fe size distribution is slightly larger than the main UV and Cu peak, which suggests that Fe bound to the humic substances might be polymerized, as proven in other studies using. Indeed, oligomers consisting of Fe dimers and trimers bound to soil and aquatic organic matter of this type have been identified

with X-ray Absorption Spectroscopy (XAS) analysis.[2] In the Zeg soil and Rhy soil, the colloids have a narrow size distribution that did not extend beyond approximately 50 nm, whereas in the Alu soil the inorganic colloids are larger mainly present in 5─50 nm range, and extended over the size range presented (> 100 nm). Regression analysis on the Fe particle size distribution determined in pore waters of 11 soils showed that small inorganic Fe NNPs (5─50 nm fraction) prevailed in soils with OC content > 3.5%, while larger inorganic Fe NNPs (50─100 nm) dominated in soils with lower OC content. Organic matter inhibits the growth and crystallization of Fe oxyhydroxides[3] and promotes nanometer-sized Fe by reducing aggregation of the Fe oxyhydroxides. The low but detectable UV signal associated with the predominantly inorganic colloids was corrected for Fe[4], indicating co-elution of HS with the mineral particles. The negative correlation between the fraction of Fe in the large mineral size range (50 – 100 nm) and the humic substances content of the particles (OC/Fe signal) supports the mechanism of Fe-NP growth inhibition by surface-adsorbed humic substances.[4]

In soils with low %OC content and low ionic strength (Alu soil), larger mineral Fe particles probably existed as oxide-clay associations which shifted the Fe oxyhydroxide distribution to the larger size range of clay particles. The colloids in the Zeg soil differed from the other two soils in that colloidal Al and Si particles were absent. In the Rhy soil, the size distribution of Fe was similar to Al and Si (not shown) for the larger inorganic colloids, whereas the size distribution of Fe deviated from Al and Si in the Alu soil. The molar Si/Al ratio was 1.9 (Alu soil) and 2 (Rhy soil), and the Mg/Al ratio was 0.1 (Alu soil) and 0.2 (Rhy soil) in the predominantly inorganic colloids, which is characteristic for phyllosilicates.[5] Illite clay minerals prevail in the topsoil where the soil samples were collected and can occur as nano-minerals in soil solutions.[6] Illite has a Si/Al ratio of 1.7 and Mg/Al ratio of 0.13 with limited structural Fe (i.e. Fe inside the phyllosilicate), so that the Si/Fe ratio is about 9.[5] The lower Si/Fe ratio measured in the inorganic colloids (Si/Fe = 5 in Alu soil and 12 in Rhy soil) suggests Fe oxyhydroxide coatings on illite colloids. Iron oxyhydroxides have a high affinity for clay minerals at this pH due to electrostatic interactions between the positively charged Fe oxyhydroxides and negatively charged clay minerals. With a decrease in size, the Si/Fe ratio decreases, which supports surface coatings of Fe oxyhydroxides on clay, similar to trends for Mn oxides.[7] The size and composition (molar ratios) suggest the presence of clay and oxide clay-associations, but further analysis is needed to univocally determine the speciation in these NNPs. For instance, different Fe species have been distinguished using Fe K-edge XAS analysis to support the various colloids determined from AF4-UV-ICP-MS analysis.[6]

Section S3

Each sample was firstly ultrasonicated in a classic US bath for 5 min, then ultrasonicated using a probe US for 15 min in cycles of 30 s of exposure and 30 s of resting time, at a low power of 7 W. The sample was then passed through 0.7 µm glass microfiber filters. The separation conditions developed for typical fractionation of TiO_2-NPs standards were adapted by using a Novachem® carrier to avoid the aggregation of NPs during in-channel pre-concentration, and to minimize interactions with the membrane. The concentration of Novachem® was 0.05% to limit the amount

of salts and surfactant introduced to the ICP-MS. During the focalisation step, the inlet flow was set to 0.2 mL min^{-1} and outlet flow was set to 0.5 mL min^{-1}. The cross-flow rate and focus-flow rate were adjusted according to the respective demand and nature of the separation and matrix. Inline pre-concentration in the AF4 channel used a 10 mL sample loop and focusing time of 57 min. Elution conditions: exponential gradient method (power 0.2) with cross-flow rate decrease from 0.75 to 0 mL min^{-1} over 40 min of elution. A collision cell with He flow rate of 4.3 mL min^{-1} was used for removing isobaric polyatomic interferences in Q-ICP-MS.

Limit of detection determination obtained for in-channel preconcentration procedure: Titanium isotopes (^{47}Ti, ^{48}Ti, ^{49}Ti) were measured, and the sensitivity of the MALS and ICP-MS detectors were determined using a stabilized standard suspension of TiO_2-NPs . A limit of quantification (LOQ) of approximately 50 µg Ti L^{-1} for MALS was determined as the minimum sample concentration required to accurately fit the data and obtain precise size information. An LOQ of 1 µg Ti L^{-1} was determined to be the minimal sample concentration needed to measure a peak significantly higher than the analytical blank with ICP-MS.

Section S4

AF4 separation was performed with 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonate sodium pH 7 as carrier and using a 10 kDa regenerated cellulose membrane and a 350 µm spacer mounted in an analytical channel. One mL of EPS sample doped with Hg and MeHg (2.5 nM), was injected for 10 min before an elution program involving a linear gradient cross-flow decrease from 2 to 0 mL min^{-1} over 15 min. The AF4 was coupled online to a Q-ICP-MS, with a collision cell employing He to minimize polyatomic interferences.

Section S5

AF4-ICP-MS was performed under the same conditions as for example presented in Figure 5 (section S4), but with the gradient of cross-flow decreased exponentially. The measurements of Ag-NPs by spICP-MS were performed using a dwell-time of 3,000 µs, following the procedure recommended by RIKILT (Institute of Food Safety, Wageningen, NL) and using the data-processing procedure recommended by this institution.

Section S6

This mixed colloid standard was created using Suwannee River Humic Acid (SRHA) Standard III, $FeSO_4 \cdot 7\ H_2O$ (99.4%), $CuSO_4$ pentahydrate (99.999%) and $Pb(NO_3)_2$ (99.9%). The pH was adjusted to 7.5–8.0 using 0.100 M NaOH. The mixture of these constituents leads to the adsorption or binding of Fe to SRHA, and the formation of Fe(III)-SRHA nanoparticle complexes through the oxidation of Fe(II) in the presence of HA. A DOC:Fe ratio of 5 was used to stabilize the Fe oxyhydroxides following the results of a prior study of the impact of DOM:Fe ratio on the size distribution of stable Fe particles.[7]

Triplicate batches of standards were produced on separate days and each batch was measured in triplicate using AF4-UV-ICP-MS to assess reproducibility of the analysis and

standard generation procedure. Three batches were tested on March 15, May 7, and May 20, 2024 (Tables 2 and 3). One of the March 15th batches was discarded due to preparation error. **Fig. 7.** shows the fractograms for the three standard batches measured on May 20, 2024 (1BR1, 1BR2, 1BR3). Fractograms were assessed for consistency in the concentrations of Fe, Cu, Pb and OM in the void, primarily organic (i.e. mainly OM as SRHA with inorganic clusters), and the larger, primarily inorganic fractions. The conditions of fractionation were identical to **Fig. 1.** (Section S1).

Works cited

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