The concentration and size distribution of iron rich colloids in pore waters are related to soil organic matter content and pore water calcium concentration

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Abstract

Iron (Fe) rich colloids in the environment have a high surface reactivity and facilitate transport of oxyanions and trace metal cations in soil. This study was set up to help identify the soil properties that might affect the concentrations of colloidal Fe and its size distribution in pore waters of soils. The pore water was isolated by centrifugation from a collection of 97 topsoils with contrasting properties and land use. The total Fe concentration in the pore waters ranged between 0.2–26 μM (10th–90th percentile) and was largest at low soil solution Ca and high dissolved organic carbon (DOC), indicating that enhanced colloidal stability determines total Fe in solution. Colloidal size distribution (< 100 nm) of Fe in the pore waters of 11 soils was determined with Flow Field Flow Fractionation (FIFFF-UV-ICP-MS), which yielded three different fractions that varied strikingly among soils. The smallest fraction (< 5 nm) of Fe co-eluted with high DOC and Cu, suggesting mononuclear Fe-organic carbon (OC) complexes. This fraction increased with an increasing DOC/Fe ratio in pore waters. The 5–50 nm fraction of Fe prevailed in soils with > 3.5 % organic carbon (%OC), while the 50–100 nm fraction was dominant in soils with low %OC content. This

suggests that natural organic matter inhibits crystallization and growth of mineral Fe particles. The elemental ratios of mineral colloids (> 5 nm) indicated the presence of clay minerals and Fe oxyhydroxides, which was confirmed by SEM analysis. This study thus identified soil %OC and Ca in soil solution as the dominant factors predicting both the concentration and size of pore water Fe colloids with the smallest and, potentially, most mobile Fe colloids occurring in OC-rich soils.

Keywords

colloid-facilitated transport, colloid stability, environmental nanoparticles, Flow Field Flow Fractionation, iron colloids, iron oxyhydroxides, iron speciation, nanovector, soil organic matter, soil pore water

Highlights

- Pore water iron concentrations in topsoils are governed by colloid stability
- Flow Field Flow Fractionation analysis allows to characterize iron colloids in the low nanometer range
- Iron colloid size and composition varies strikingly among soils
- Small iron colloids prevail in organic carbon rich soils due to colloid growth inhibition

Introduction

Iron (Fe) is the fourth most abundant element in the lithosphere and a very common component of soil minerals with total concentrations in European soils ranging 5–32 g Fe_{tot} kg⁻¹ (P₁₀–P₉₀) (Reimann et al., 2014). Iron is released from the lithosphere into surface environments by weathering of primary silicate and sulfide minerals. In oxic environments, the released Fe(II) is oxidized to Fe(III) which, in turn, hydrolyses to form Fe(III) oxides and oxyhydroxides, further commonly referred as oxyhydroxides. The Fe oxyhydroxide crystals are among the smallest mineral particles found in soils and contribute considerably

to the reactive surface area and, hence, to sorption controlled solubility of numerous elements in soils (Schwertmann, 1988).

Iron(III) oxyhydroxides have very low solubility in water, the theoretical solubility of Fe in water at near neutral pH is maximally a few nM (Kraemer, 2004). This is 2-3 orders of magnitude below observed concentrations in the environment. For example, the dissolved ($< 0.45 \mu\text{M}$) Fe concentrations in freshwater streams in Europe range $0.2\text{--}18 \mu\text{M}$ ($P_{10}\text{--}P_{90}$) (Salminen, R., 2005). This higher concentration of Fe in natural waters is due to the formation of organic and mineral Fe colloids (Lofts et al., 2008). Organic Fe colloids consist of iron(III) cations that form stable chelates with organic molecules, most notably humic substances, in the environment. These mononuclear Fe-OC (organic carbon) complexes can dominate total dissolved Fe in acid conditions and at low total Fe concentrations (Karlsson & Persson, 2012). The iron complexes with organic matter are responsible for Fe migration between soil horizons, *i.e.* podzolization process (Lundstrom et al., 2000). At increasing Fe concentrations or at increasing Fe/DOC ratio, mineral Fe oxyhydroxide colloids form (Karlsson & Persson, 2012). These mineral Fe colloids can be electrostatically stabilized in water, *i.e.* the colloids remain suspended by adsorption of the negatively charged humic substances (Karlsson & Persson, 2012; Sjöstedt et al., 2013). The mineral Fe colloids, mainly Fe oxyhydroxides, control the Fe(III) activities and dominate over mononuclear Fe-OC complexes in pH neutral streams (pH 6-8) (Lofts et al., 2008).

Colloidal Fe has important effects on the mobility of nutrients and contaminants in the environment. Colloidal Fe oxyhydroxides might act as a so-called nanovector (Hassellöv & von der Kammer, 2008) for trace metal cations (*e.g.* lead, Pb) and oxyanions (*e.g.* arsenate, AsO_4 and phosphate, PO_4) (Grolimund et al., 1996) due to their high affinity for Fe oxyhydroxides. This colloid-facilitated transport can become the major transport mechanism for strongly sorbing elements in soils (Grolimund et al., 1996) and can represent a rapid transport pathway due to size exclusion. Several mechanisms may explain the presence of colloidal Fe in pore waters of soils. The dispersion of particulates is considered to be the dominant

source of colloids in the subsurface environment (Kretzschmar et al., 1999; Ryan & Elimelech, 1996). Mobile colloidal particles can also originate from *in situ* precipitation of supersaturated solutions (Kretzschmar et al., 1999; Ryan & Elimelech, 1996), *e.g.* under varying redox conditions.

The concentrations and size distribution (or specific surface area) of the colloidal Fe in soil solution need to be known to predict colloid facilitated transport in soils. In general, survey data encompassing solution Fe concentration in a range of contrasting soils are poorly available. A study in a Podzol soil in the UK determined that the Fe concentration in lysimeters beneath the O horizon varied seasonally between 1 and 10 μM and this was mainly related to variations in DOC (Hughes et al., 1990). Other work reported a broad range of soil solution Fe concentrations in 32 agricultural topsoils, from a few μM Fe to about 200 μM Fe, but the Fe concentration did not correlate with soil physical and chemical characteristics (Ammari & Mengel, 2006). The pore water Fe concentrations in 46 Dutch soils ranged 1–55 μM (P_{10} – P_{90}) (de Groot et al., 1998) but no analysis was provided to explain that variation in relation to soil properties, land use or soil classification.

The size distribution of natural Fe oxyhydroxide colloids in soil pore waters is even less reported than the Fe concentrations. Several experimental studies have shown that organic matter and certain anions such as phosphate and silicate inhibit crystal growth and transformation of initially precipitated Fe oxyhydroxides due to occupation of Fe growth sites (Guénet et al., 2017; Mayer & Jarrell, 2000; Voegelin et al., 2010). In addition, colloid size is also increased by aggregation *e.g.* when the pH is close to the point of zero charge (PZC) (Baalousha et al., 2008) or due to flocculation with Ca (J. Bollyn et al., 2016). Measuring particle size in the low nanometer range is analytically challenging because the Fe colloids have a high surface reactivity resulting in sample alteration or loss during analysis. Flow Field Flow Fractionation (FIFFF) is a valuable emerging technique for colloid analysis because of the compatibility of the carrier with natural samples and the high size resolution for a wide particle size range. The FIFFF can detect Fe-OC complexes < 5 nm and Fe oxyhydroxide particles ranging 5–300 nm in an environmentally relevant

background (1 mM NH_4HCO_3 , pH 8.3) with an Fe recovery of at least 70% (Moens et al., 2019). Baken et al. (2016) applied FIFFF to characterize Fe-rich colloids in streams draining catchments with contrasting properties. Several studies have investigated soil colloids with FIFFF (Baalousha et al., 2006; Missong et al., 2018; Neubauer et al., 2013; Regelink et al., 2011, 2013, 2014). However, these studies either investigated only a limited amount of samples or did not focus on Fe oxyhydroxides colloids specifically.

This study aims at identifying the soil chemical and physical factors that affect the Fe concentrations and the colloidal size distributions in pore waters of soils. It was anticipated that Fe colloid concentration and size distribution relates to soil chemical and physical properties, in particular dissolved organic carbon, soil pH and soil Fe content. The pore water is defined as the resident water in soils that are wetted to a soil moisture content relevant for unsaturated soils, *i.e.* near field capacity for practical reasons to obtain sufficient sample volume. The pore waters were obtained by centrifugation, a sampling method that is assumed to have a smaller sampling artifact compared to soil extracts that change the ionic strength and, hence, colloidal stability and for which unknown dilution effects occur. This study started with surveying dissolved ($< 0.45 \mu\text{m}$) Fe concentration in pore waters of soils with contrasting properties followed by a statistical analysis to search which soil factors are mainly involved. In a second step, size fractionation of Fe colloids ($< 100 \text{ nm}$) was made with FIFFF-UV-ICP-MS on a subset of the soils.

Material and methods

Pore water survey

A survey of the pore water Fe concentration was made on 97 different soil samples. This survey consisted of pore water data collected earlier by De Troyer et al. (2014) but for which the dissolved Fe data were not analyzed statistically. Of the 97 soil samples, 69 originated from Belgium and 28 from other EU countries. The land use of the soils was agricultural ($n=87$, 0–10 cm for grassland, 0–20 cm for arable land) and forest or shrubland ($n=10$) that were sampled after litter removal.

Soil solution was collected by centrifugation. The soil sample was placed in a double chamber setup, which consists of a compartment that contains the soil and a second compartment underneath where soil solution is collected due to the applied centrifugal force. The obtained soil solution was filtered through a 0.45 μm membrane filter (polyester, Chromafil Xtra Machery-Nagel), thereby discarding the first droplets. The pore water extraction was done on samples fresh from the field (Set 1; n=30), samples that had been cold stored (4°C) at field moisture for 1.5 yr (Set 2; n=35) and samples that had been air dried and rewetted to field capacity (pF=2.0) and subsequently incubated for 5 weeks at 20°C (Set 3; n=32 of which 10 are non-agricultural soils). The effects of these three treatments on dissolved Fe was tested statistically (see below). The volumetric water content of the soils ranged between 12–49%. Soils were analyzed for pH, texture, organic and inorganic carbon, cation exchange capacity (CEC) and exchangeable cations, total carbon and nitrogen and on a selection of samples the oxalate extractable Fe, Al, Mn was determined. The pore waters were analyzed for dissolved organic and inorganic carbon, specific UV absorbance (SUVA), anions (SO_4 , NO_3 , Cl). The pore waters were acidified to pH 1.0 by adding HNO_3 and were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, PerkinElmer, Optima 3300 DV) for Na, Mg, K, Ca, Fe, Al, Mn and P. For a detailed description of the analysis methods, the reader is referred to the original paper from De Troyer *et al.* (2014).

Soil analysis

Eleven topsoil samples that cover a wide range in soil pH and organic carbon content were selected for colloid analysis. Selected properties were determined on air-dried, 2-mm sieved samples and reported on an oven-dried (105°C) weight basis. Soil pH was measured in 10 mM CaCl_2 (1:5 soil/solution ratio) after 1 h shaking and 30 min settling. Total carbon content was determined with a vario Max N/CN analyser (Elementar Analysensysteme GmbH). Soil organic carbon content (%OC) was calculated as the difference between the total and inorganic carbon content. Carbonate-C content was determined from the pressure

increase after the addition of HCl to the soil in closed containers, with FeSO₄ as a reducing agent to minimize release of CO₂ from organic matter (Loeppert & Suarez, 1996). Ammonium oxalate-extractable aluminium (Al_{ox}), iron (Fe_{ox}) and manganese (Mn_{ox}), a measure of the amorphous Al, Fe and Mn oxyhydroxides in soil (Schwertmann, 1964), were determined in a 1:50 soil to oxalate extractant ratio and the concentrations of Al, Fe and Mn in the extract were analyzed by ICP-OES (iCAP 7400, Thermo Scientific). Total elemental composition of the soil was determined after *aqua regia* digestion (0.5 g soil digested in 1.5 mL of HCl and 0.5 mL of HNO₃) for 2h at 140 °C and Al_{tot}, Fe_{tot}, Mn_{tot} concentrations were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700). The CEC, exchangeable cation concentrations and soil texture was taken from the study of the Troyer *et al.* (2014).

Pore water sampling

The 11 topsoil samples (air-dried and < 2 mm sieved) for FIFFF analysis were incubated for three weeks at field capacity at 20°C in plastic bags that were opened and mixed regularly to avoid reducing conditions. Soil water content at field capacity (pF=2.0) was determined using 100 cm³ soil cores with the sandbox method (Eijkelkamp Agrisearch equipment). The pore water sampling protocol used by De Troyer *et al.* (2014) was modified by excluding the membrane filtration step to better preserve the colloids. The soil pore water was extracted by centrifugation with the double chamber method at 2000 *g* for 15 min and the pore water obtained was subsequently fractionated at < 0.45 µm to remove larger particles from the soil solution by means of additional centrifugation instead of membrane filtration to reduce filtration artefacts (Gimbert *et al.*, 2005). To calculate centrifugation time for size fractionation of colloids, the particle density of Fe-oxides was used (4 g/cm³) and fractionation was done with a runtime of 10 min and gravitational field of 270 *g* (Thermo Multifuge X3R-swinging bucket). The eleven pore waters were

analyzed by FIFFF-UV-ICP-MS, each pore water was extracted in duplicate and analyzed in duplicate. The FIFFF analysis was always done on the same day of solution extraction.

Pore water analysis

Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were measured with a total organic carbon (TOC) analyser (Shimadzu, TOC-L). The elemental composition of pore waters (Na, Mg, Ca, K, Fe, Al, Mn) was measured by ICP-MS determined after *aqua regia* digestion (1 mL of the pore water, 1.5 mL of HCl and 0.5 mL of HNO₃) for 2h at 140 °C. Earlier work with synthetic Fe colloids showed that the ICP detected Fe concentration (< 0.45µm fraction) was larger after digesting than without digestion (Jessica Bollyn et al., 2017), this potentially points to a different atomisation and ionisation efficiency between dissolved standards and Fe colloids in the plasma (Meermann & Nischwitz, 2018). The inorganic anions Cl, NO₃ and SO₄ were analyzed by ion chromatography (Dionex, ICS 2000, AS18 column).

Flow Field Flow Fractionation (FIFFF-UV-ICP-MS) and Scanning Electron Microscopy (SEM)

The size fractionation of colloidal particles in pore water samples was analyzed with Flow Field Flow Fractionation (FIFFF) (AF2000, Postnova Analytics) online coupled with a UV/VIS detector (diode array detector, SPD-20A Postnova Analytics) and ICP-MS (Agilent, 7700). The FIFFF is a flow-based hydrodynamic size separation technique for fractionation of macromolecules and particles. A detailed description of the principle can be found elsewhere (Giddings, 1993a; von der Kammer et al., 2011). Briefly, particle separation is achieved by retention of particles in the FIFFF channel compared to the average flow velocity of a carrier. Retention is caused by balancing the diffusional movement of the particles and a secondary flow (cross-flow), which acts on the particles perpendicular to the carrier flow. The larger the diffusion coefficient, the more the particle cloud extends into the center of the channel. Due to the parabolic flow profile of the carrier, particle clouds in the center of the channel move faster than particle clouds closer to the accumulation wall and therefore small particles elute before larger ones.

183 We previously optimised FIFFF conditions to maximize Fe recovery (Moens et al., 2019). A cross-flow of
184 1.5 mL min^{-1} was set as a constraint to obtain sufficiently high Fe recovery, therefore a relatively wide
185 spacer ($500 \text{ }\mu\text{m}$) instead of high cross-flows are used to have sufficient retention of small particles. A
186 gradient in cross-flow was applied starting with a constant cross-flow of 1.5 mL min^{-1} for 30 min to
187 separate small particles ($< 30 \text{ nm}$) with high resolution, followed by a linear decrease to 0.2 mL min^{-1} in
188 two minutes, which was kept constant for another 30 minutes to separate particles up to 100 nm . The
189 carrier composition was $1 \text{ mM NH}_4\text{HCO}_3$ at pH 8.3, the membrane at the accumulation wall is a polyether
190 sulphone (PES) membrane (Postnova) with cut-off size 1 kDa . Between each sample 10 minutes a rinse
191 step was used (carrier flow 1 mL min^{-1} , focus flow 1 mL min^{-1} and cross-flow 1.5 mL min^{-1}) to reduce sample
192 carry-over. Conversion of retention time to diffusion coefficient and hydrodynamic diameter (D_h) was
193 accomplished by applying FIFFF theory to the constant cross-flow step and calibrating with particle-size
194 standards of known size. Polystyrene standards with D_h 22 nm (NIST) and $50, 100 \text{ nm}$ (Postnova) dispersed
195 in surfactant (0.2% Novachem, Postnova) eluted at elution time 21 min , 43 min and 59 min respectively.
196 Pore waters were measured undiluted and the injected sample volume ranged between 0.1 and 1 mL
197 depending on the Fe concentration. The UV/VIS detector monitored the absorbance at wavelength 254
198 nm . The absorbance, corrected for interference of Fe(III) (Poulin et al., 2014), is used as a proxy for
199 aromatic organic matter (Weishaar et al., 2003). The individual UV readings are corrected for the co-
200 eluting Fe signal obtained from ICP-MS analysis. The ICP-MS monitored in time resolved analysis (TRA) the
201 isotope lines ^{56}Fe , ^{27}Al , ^{55}Mn , ^{28}Si , ^{44}Ca , ^{24}Mg . Elements were measured with He in the collision cell at 5 mL
202 min^{-1} (He mode), except for Si which was measured at high He flow (10 mL min^{-1}) in the collision cell (high
203 energy He mode), each pore water sample ran in duplicates. The ICP-MS calibration slopes were
204 determined offline using certified multi-element standards acidified to $1\% \text{ HNO}_3$. The median signal during
205 focusing was subtracted from the signal during elution before multiplying with the calibration slopes to
206 correct for the background signal.

The size and morphology of colloids present in the pore water of two selected soils of contrasting %OC content were determined by Scanning Electron Microscopy (SEM, Philips XL 30 FG). For the analysis a droplet of the fractionated pore water was air-dried on Lacey carbon film-coated 300-mesh copper grid (Agar Scientific). The samples were taken at the elution times that correspond with high colloidal concentrations to have sufficient particles to detect with SEM. Therefore, the SEM pictures represent the colloids that are dominantly present in the samples.

Fractogram data analysis

Colloid size and composition were determined from the FIFFF fractograms. The colloidal concentration of humic substances (UV signal) and Fe was obtained by integration of the elution profiles in the fractograms. For a quantitative description of particle size, each fractogram was divided in three *ad-hoc* defined fractions: elution time 0–5 min (corresponding size <5 nm) -; 5–45 min (~5–50 nm) and 45–60 nm (~50–100 nm) and numerical integration was used to infer concentrations in the different size fractions. The Fe concentrations in each of the three classes were expressed relative to the total Fe concentration in the fractogram. The smallest size fraction corresponds with the size range of humic substances and is referred to as organic carbon complexes (<5 nm), the distinction between the small mineral colloids (5–50 nm) and large mineral colloids (50–100 nm) is arbitrary but represent both a particle size range of about 50 nm. The colloid composition was inferred from the mean molar element ratios. To obtain an estimation of the amount of humic substances (here assumed to be fulvic acids only) bound to the mineral particles, the Fe corrected UV-signal is converted to a fulvic acid concentration based on the specific UV absorbance (SUVA) of fulvic acids. The SUVA is defined as the UV absorbance of a fulvic acid normalized for dissolved organic carbon concentration and is 37 l g⁻¹ DOC cm⁻¹ (Weishaar et al., 2003). In fractionated samples, there is uncertainty with this approach because the specific absorbance of the natural organic matter (NOM) varies with molecular mass (Weishaar et al., 2003)..

Statistical analysis

Statistical data analysis was performed using JMP® Pro version 14.0.0 (SAS Institute Inc., 2016). All variables except pH were $\log_{10}$ transformed prior to analysis to normalize the distributions, it was visually confirmed that $\log_{10}$ transformation decreased the skewness for most variables. There were 97 observations for pore water Fe. Concentrations below the limit of quantification (LOQ) were replaced by the LOQ (0.14 μM Fe). Pearson correlation coefficients were calculated between pore water Fe concentration and soil and pore water properties. To identify the variables that might predict Fe concentrations in the pore water, a stepwise regression analysis was conducted on the selection of variables that correlated with pore water Fe and that have few missing observations (total N, %OC, CEC, C/N ratio organic matter, soil pH, % clay, % sand, % base saturation, DOC, solution Ca, Al and Mn concentration). Variables that correlated with pore water Fe concentration but with more than 30% missing observations were not considered in the regression analysis, for example Mn_{ox} and SUVA in the survey were not measured in all samples of the dataset. Variables that resulted in minimization of Bayesian Information Criterion (BIC) and maximization of adjusted R^2 were selected and further used in multivariate regression analysis using the ordinary Least Squares Model (LSM). These criteria for variable selection favors models with fewer adjustable parameters. To avoid parameter covariance, the *variation inflation factor* (VIF) was evaluated on the variables in the regression model (Mason & Perreault, 1991). In addition, the effects of the three different soil treatments after sampling, *i.e.* air drying or cold storage, on the pore water Fe concentration was tested by adding dummy variables as additional independent variables to the regression equation. On the dataset from the pore waters that are characterized by Flow Field Flow Fractionation, a similar approach was taken to identify variables that might predict the Fe colloid size distribution. There were 22 observations to describe the Fe colloid size distribution. Pearson correlation coefficients were calculated and a stepwise regression analysis was conducted with the

253 variables: CEC, exch. Ca, Fe_{ox}, Fe_{tot}, %OC, % sand, % clay, pH and pore water DOC, Fe, Ca concentration.

254 The statistical significance was set at the $p \leq 0.05$ level.

255 Geochemical speciation calculations

256 Geochemical speciation calculations of pore water Fe was performed with the Windermere Humic

257 Aqueous Model VII (WHAM)(Tipping, 1994) to predict pore water Fe speciation in the data from the survey

258 based on the measured solution composition. In addition, the environmental Fe binding capacity of

259 organic ligands was modelled to verify measured Fe binding to humic substances in fractograms. More

260 details on the modelling assumptions are given in supplementary information (Table S7).

261 Results

262 Pore water Fe concentrations ($< 0.45 \mu\text{m}$) in contrasting soils

263 Descriptive statistics of selected soil and pore water properties are shown in Table 1 and a complete

264 overview of measured soil properties is given in Table S1. Soil samples of the survey consisted of a very

265 heterogeneous collection of soils as reflected by the large variability of soil chemical properties. The range

266 in soil pH, %OC and clay content were within the 10th and 90th percentile values for European soils

267 (Reimann et al., 2014). Pore water Fe concentrations in the entire dataset ranged 0.2–26 μM (P₁₀–P₉₀)

268 with a median value of 1 μM . Pore water Fe concentration (log transformed values) correlated significantly

269 with a number of soil properties (Table 1 and Figure 1). Significant positive correlations were found for

270 DOC ($r = 0.45$), %OC ($r = 0.41$), pore water Al ($r = 0.65$) and %sand ($r = 0.46$) and negative correlations for

271 pore water Ca ($r = -0.66$), NO₃ ($r = -0.45$) and pH ($r = -0.55$). A full table describing correlations among all

272 soil and pore waters properties is given in Table S2.

273 A multivariate least squares regression model was constructed to test factors predicting the dissolved Fe

274 concentration (μM) in the full dataset ($n = 97$), yielding a model explaining 67% ($p < 0.0001$) of the

275 observed variation by a combination of the variables pore water Ca (mM), soil organic carbon (%OC) and
276 pore water Al (μM) (Eqn. 1):

$$277 \log_{10}(\text{Fe}) = -0.92 \cdot \log_{10}(\text{Ca}) + 0.84 \cdot \log_{10}(\% \text{OC}) + 0.21 \log_{10}(\text{Al}) + 0.37 \quad (\text{Eqn. 1})$$

278 All variables and the intercept were significant ($p < 0.0002$).

279 The regression coefficients indicate the mean increase in $\log_{10}$ Fe per unit increase in $\log_{10}$ of the predictor
280 variable, while other predictor variables are corrected for.

281 The stepwise regression analysis included the variable pore water Al in the regression equation (Eqn.1),
282 which explained 5% additional variation. The relation between Fe and Al might be due to collinearity and
283 has little predictive value. By omitting Al (and Mn) in the stepwise regression analysis, as a result %sand
284 was selected in the regression equation yielding a model explaining 64% of the observed variation ($p <$
285 0.0001) (Eqn. 2):

$$286 \log_{10}(\text{Fe}) = -1.1 \log_{10}(\text{Ca}) + 0.95 \log_{10}(\% \text{OC}) + 0.39 \log_{10}(\% \text{sand}) - 0.11 \quad (\text{Eqn. 2})$$

287 All variables were significant ($p < 0.05$).

288 The dataset consisted of soils with different sample treatments after sampling (*i.e.* fresh, cold stored,
289 dried and rewetted). Storage treatments were not varied independently with soil type, *i.e.* soil types
290 varied within the subsets with different storage methods, but the Fe concentration differed significantly
291 among the treatments (Table S3). Significantly higher pore water Fe concentrations were measured in the
292 fresh samples (set 1) compared to the dried and rewetted (set 3) and the cold stored (set 2) soil samples,
293 which suggest an effect of storage on pore water Fe concentrations. To test the effect of soil treatment,
294 firstly dummy variables were added to the regression model (Eqn. 1) and indicated that the incubated
295 soils (Set 2 and Set 3) have also significantly lower Fe concentrations after correction for soil properties
296 (%OC, Ca and Al) compared to the fresh soil samples. Fresh samples (Set 1) contained on average 4 times

297 higher pore water Fe concentration than dried and rewetted samples (Set 3) and on average 2 times
298 higher pore water Fe concentration than cold stored soil samples (Set 2). The difference in Fe
299 concentration between the dried and rewetted soil samples compared to the cold stored soil samples was
300 also statistically significant ($p < 0.05$). Secondly, to test the effect of storage method, a selection of the
301 fresh samples ($n = 6$) underwent different soil treatments (only the data of the fresh soil samples were
302 included in the survey). The incubated samples have lower pore water Fe concentrations than the
303 corresponding fresh samples, on average 10 times lower for cold stored samples and 4 times lower for
304 dried and rewetted samples (Table S4). Both analysis show an effect of soil treatment, however, the best
305 way to understand the influence of sample storage would be to compare different storage methods for a
306 larger subsets of the same soils. The correlations between pore water Fe and soil and pore water
307 properties for the different soil sample treatments (Set 1-3) separately are given in supplementary
308 information, but not further discussed here (Table S5).

309 The speciation calculation for Fe is done to facilitate the interpretation of the correlations between pore
310 water Fe and soil (solution) characteristics. The geochemical speciation modelling (WHAM VII) showed
311 that pore water Fe mainly occurred as colloids because the pore water Fe concentration exceeded the
312 truly dissolved Fe concentrations, referring here at free ion and inorganic species, which are negligible at
313 the pH of the samples. Using the generic Fe(III) binding parameters in WHAM VII (Tipping et al., 2011), in
314 about half of the samples, the predicted organic complexes of Fe, *i.e.* Fe bound to fulvates, dominated the
315 total pore water Fe concentration (Table S7). In the other samples, the Fe binding capacity of organic
316 substances forming Fe-OC mononuclear complexes was exceeded, which suggests the presence of mineral
317 Fe oxyhydroxide colloids. Such calculations bear uncertainty due to the many model assumptions. For
318 instance, several Fe binding constants for Fe to humic substances in literature and the choice for one
319 another largely affect the outcome (T. Hiemstra & Van Riemsdijk, 2006; Vega & Weng, 2012). The
320 sensitivity analysis shows that for the chosen binding constants, the model predicts more Fe oxyhydroxide

colloids compared to generic NICA-Donnan model. However, the mineral Fe oxyhydroxide colloids might also be underestimated here because the DOC in the dried and rewetted samples consisted of a large fraction of non-humified organic matter (lower SUVA) (De Troyer et al., 2014) which was not corrected for in the modelling. In addition, part of the pore water DOC might be present as surface coatings on mineral particles (see below: FIFFF section) and thus less available to form mononuclear Fe-OC complexes and Fe in clay particles was not taken into account.

Size and composition of pore water colloids

Flow Field Flow Fractionation analysis was performed on the pore waters from eleven soil samples with contrasting chemical properties (Table S9), *e.g.* a range in soil pH (3.4–7.3), organic carbon content (0.9–30 %OC), soil total Fe concentration (Fe_{tot} 23–2000 mmol kg⁻¹) and different soil types. About half of the samples had an organic carbon content > 1.8%, which is the median %OC content for European agricultural soils (Reimann et al., 2014).

The average Fe concentration in the pore waters of these eleven soils (262 μM Fe) was larger than the corresponding average from the survey dataset (10 μM Fe). After correcting for soil properties affecting dissolved Fe, *i.e.* Eqn. 1, this small dataset yielded twofold (average) higher Fe concentration than the survey dataset. This might be related to the fact that these pore waters were not membrane-filtered (< 0.45 μm) but centrifuged and were digested prior to ICP-MS measurement to completely dissolve the colloids or avoid losses due to precipitation of Fe-humic colloids at low pH, yielding larger ICP-MS detected Fe concentrations (as mentioned in Materials and Method section). The most traditional method for measuring total dissolved Fe is that of the survey study *i.e.* based on 0.45 μm filtration and acidification, not based on digestion of solutions. In agreement with the survey data, high pore water Fe concentrations were measured in soils with low Ca ($\text{Ca} < 2 \text{ mM}$) *e.g.* soil G and J and in soils with high OC (%OC > 5% and $\text{Ca} < 10 \text{ mM}$) *e.g.* soil F and D (Table S9).

344 The Fe recovery in the FIFFF analysis is calculated from the amount measured from the start (0 min) to
345 the end (62 min) of the measurement relative to the amount injected and is on average $68 \pm 28 \%$. The
346 unrecovered Fe can be attributed to loss of Fe smaller than the membrane cutoff (1 kDa) or due to particle
347 loss on the membrane. Samples where the Fe concentration is not back at the baseline at the end of the
348 run contain particles that exceed the size separation range of the FIFFF method, which will also explain
349 unrecovered Fe in some samples.

350 The colloid size distribution of Fe and UV absorbance for three contrasting soils is shown in Figure 2, the
351 fractograms of all soils are given in Figure S2. The first peak in the fractogram with high UV absorbance at
352 the small size range (< 5 nm) is attributed to humic substances in the pore water. The Fe that co-eluted
353 with the UV signal in this size range is interpreted as Fe organic matter complexes (see below in Discussion
354 section). The fractions with larger size (5–100 nm) likely consisted of mineral particles because of the
355 association between Fe, Al (Figure S2) and Si (data not shown). The low but detectable UV signal (which
356 was corrected for Fe) indicated co-elution of humic substances in the mineral particles. The UV signal in
357 the mineral particles was converted to an OC concentration and the resulting OC/Fe ratio in the mineral
358 particles ranges $5.7 - 204 \text{ mmol Fe mol}^{-1} \text{ OC}$ (Table S10).

359 In the mineral colloids, the distribution of Fe is closely associated with that of Si and Al. The molar Si/Al
360 ratio in that size fraction ranged between 2 and 3 and the molar Mg/Al ratio between 0.1 and 1.1 (Table
361 S10), which is characteristic for phyllosilicates (Langmuir, 1997). The clay mineral illite prevails in the
362 topsoil in the region of Europe where most samples were taken (Ito & Wagai, 2017) and can occur as
363 nano-minerals in soil solutions (Li et al., 2012; Regelink et al., 2014). Illite has Si/Al ratio of 1.7 and Mg/Al
364 ratio of 0.13 with limited structural Fe (*i.e.* Fe inside the phyllosilicate), the Si/Fe ratio is about 9 (Mermut
365 & Cano, 2001). The low Si/Fe ratio in several of the mineral particles of this study (e.g. Si/Fe down to 3,
366 Table S10) therefore suggest the presence of Fe oxyhydroxides. The co-elution of Fe with Al and Si in

mineral particles might indicate Fe oxyhydroxide coatings on illite or Fe oxyhydroxides colloids that eluted simultaneously with illite particles of similar size.

The size distributions were divided ad-hoc between Fe-OC complexes (<5 nm), and small (5–50 nm) and large (50–100 nm) mineral particles. The relative distribution of Fe over the three size groups was used to categorize soils in three classes depending on dominant Fe colloids (Figure 2). The first group is represented by soil E (pH 5.5 and 3.5% OC) in which the main Fe peak occurs at the small size edge (approximately < 15 nm) and the Al concentration was below the detection limit over the whole size range. In the second group represented by soil D (pH 5.2 and %OC 12%), the mineral colloids consisted of Fe and Al with a very narrow size distribution that did not extend beyond approximately 50 nm, *i.e.* colloidal Fe is dominated by small mineral colloids. The third group represented by soil J (pH 6.6, %OC 1.4%) had a broad size distribution of Al and Fe that extended over the separation range (> 100 nm) and where small mineral particles < 50 nm were less present. The humic substances (UV signal) were largely present in the <5 nm size range in soil E, but the UV and Fe peak do not exactly coincide. In soil J, the humic substances were predominantly present in the large mineral size range (50–100 nm) and in soil D, the UV signal is more or less equally distributed between the size fraction < 5 nm and the small mineral colloid size range (5–50 nm).

The Fe size distribution (< 100 nm) in the pore waters of the eleven soils is summarized in Figure 3. The fraction of Fe-OC complexes was always relatively small compared to the mineral colloids. Single correlation analysis showed that the fraction of Fe as Fe-OC complexes increased significantly ($r = 0.62$, $p < 0.05$) with the DOC/Fe ratio in the pore water. The fraction of large mineral Fe colloids ranged 14–69% with the largest fraction present in soils with low %OC and low pore water Ca (soil G, J). The fraction of large mineral colloids correlated significantly with %OC ($r = -0.68$; Figure 4), DOC ($r = -0.68$), pore water DOC/Fe ($r = -0.51$), Fe_{ox} ($r = -0.46$) and with major cation and anions, *e.g.* Ca ($r = -0.58$) although several of these characteristics were also correlated with each other, *e.g.* %OC with Ca ($r = 0.61$) and with Fe_{ox} (r

= -0.75) (Table S11 and S12). The particle size distribution was not correlated with the pore water Fe concentration. Variables that significantly correlated with the large mineral colloids also correlated with the small mineral Fe fraction but with obviously opposite trends (Table S12).

A multivariate least squares regression model was constructed to test factors predicting the Fe colloid size, *i.e.* expressed as the percentage of Fe in the fractogram that is present in the size range of large mineral colloids (50-100 nm), yielding a model explaining 88% ($p < 0.0001$) of the observed variation as:

$$\log_{10}(\text{large mineral Fe colloids}) = -0.32 \log_{10}(\text{Fe}_{\text{ox}}/\text{Fe}_{\text{tot}}) - 0.22 \log_{10}(\text{exch. Ca}) - 0.26 \log_{10}(\text{DOC}) + 2.4$$

(Eqn. 3)

in which large mineral Fe colloids and oxalate extractable Fe fraction ($\text{Fe}_{\text{ox}}/\text{Fe}_{\text{tot}}$) are expressed as a percentage and the dimensions of all other soil variables as described above.

The SEM analysis was done on pore waters from two soils with contrasting organic carbon content, soil D (%OC 12%) and J (%OC 1.4%) for FIFFF size fractions that contained sufficient amount of colloids (Figure 5). The SEM analyses conducted on fractions obtained from FIFFF analysis at different elution times (and thus particle size) confirmed that very small particles were present in the organic-rich soil and larger particles in the organic-poor soil.

Discussion

Variables predicting pore water Fe concentration in contrasting soils

For practical reasons, soil solutions obtained by centrifugation instead of in situ collected leachates were used to monitor Fe in pore waters. The centrifugation methods might underestimate colloid concentrations because the soil structure is either compacted by centrifugation (J. Bollyn et al., 2019) and/or phase separation methods use filters in which a large number of colloids, even smaller than the filter cutoff are trapped by straining (Moens et al., 2019). Methods that sample percolating water in situ,

for instance suction plates, rhizon samplers and wick lysimeters maintain the limited exchange between mobile and non-mobile water during leaching. However, they underestimate colloids due to trapping of the colloids on the plates (Ilg & Winkler, 2007) or onto the wicks (Shira et al., 2006). As a result, there is no consistent trend between Fe concentrations in soil solutions obtained with centrifugation compared to the in situ sampling methods (Geibe et al., 2006). Dissolved Fe concentrations in pore waters of the European soils range (P_{10} - P_{90}) 0.2–26 μM (Table 1) and cover a similar range as those in European streams (0.2–18 μM for P_{10} - P_{90}) suggesting that similar properties control its total dissolved concentration (Salminen, R., 2005).

Drying and rewetting and cold storage of soils is often done for practical reasons, however these treatments might also mimic seasonal variability in soils (De Troyer et al., 2014), therefore the data of the survey covering the different treatments were pooled. There is no correlation between the amount of Fe in the soil (oxalate extractable Fe concentration, Fe_{ox}) and the pore water Fe concentration. In fact, that is logical, solubility of a mineral is unaffected by the concentration of a mineral in the soil even admitting that this only holds at equilibrium and for truly dissolved species, not colloids. Our finding of the lack of effect of soil Fe is also consistent when data from a previous Dutch study (de Groot et al., 1998) on pore water composition are analysed. No correlation is found between pore water Fe concentrations and Fe_{ox} ($p = 0.33$), a significant but weak correlation was found with Fe_{tot} ($r = -0.32$, $p = 0.03$) but the correlation was negative. Instead the pore water Fe concentration is governed by factors that affect stability of Fe colloids, the correlation analysis indicated an increase in pore water Fe with increase in DOC and with decrease in pore water Ca concentration (Figure 1). The correlation between Fe and Ca is stronger than between Fe and DOC. Multiple regression analysis showed that the effect of DOC is significant after correction for Ca, but the variation explained by DOC (partial $R^2 = 0.12$) is smaller than by Ca (partial $R^2 = 0.43$). Iron in the pore water was predicted to occur as organic complexes and mineral Fe oxyhydroxides. The high Fe concentrations at low pH and high DOC point to Fe-OC complexation because the

environmental binding capacity of humic substances for Fe is highest at low pH (Lofts et al., 2008). These correlations are also observed in European streams (Salminen, R., 2005). Mineral Fe(III) oxyhydroxides have points of zero charge ranging pH_{PZC} 7–9 and are, therefore, positively charged at most of the soil pH range, which provides good electrostatic conditions for adsorption of negatively charged compounds like DOC. Since the pH of the soils from the survey ranged from pH 4.2–7.4 (P_{10} – P_{90}), adsorption of negatively charged DOC and electrostatic and steric stabilization of Fe oxyhydroxide colloids may additionally explain the positive correlation between DOC and Fe concentrations. The zetapotential of organic matter coated Fe oxide colloids decreases with pH, this results in colloid stabilization if the zetapotential increases in absolute value, *i.e.* if sufficient amount of NOM is adsorbed (Chekli et al., 2013). This likely explains why DOC is a better predictor of pore water Fe concentration than pH. The pH is significantly correlated with DOC (and %OC), and, therefore, does not appear as a significant factor for Fe after correction for these soil properties. In the regression equation of the full dataset (Eqn. 1) the variable soil %OC is present instead of DOC likely because the incubated samples (Set 2 and Set 3) have a larger fraction of DOC with low SUVA (less humified) than the fresh samples. The low aromaticity (low SUVA) was attributed to decaying biomass in the dried and rewetted samples and to flocculation of high molecular weight DOM in the cold stored samples (De Troyer et al., 2014). The quality of DOC (*e.g.* SUVA) rather than quantity might be a better explaining variable (Amery et al., 2008). The regression equation solely based on the fresh samples contained DOC instead of %OC as predicting variable (not shown).

The negative trend between Ca concentration and pore water Fe concentration (Eqn. 1 and 2) points to the destabilizing effect of Ca on colloids. It is well known that increasing Ca^{2+} concentration efficiently induces aggregation of high molar mass compounds of NOM, *i.e.* humic substances and mineral colloids coated with humic substances because of charge neutralization and Ca-bridging (Philippe & Schaumann, 2014). Figure 1 shows that at low Ca (< 2 mM) the Fe concentrations were higher than about 10 μM , at high Ca concentrations (> 5 mM) the Fe concentrations are about two orders of magnitude lower. In an

461 experimental study, Ca concentrations < 1 mM were determined as threshold value for stability of NOM
462 coated Fe-oxide colloids (J. Bollyn et al., 2016). In general, the non-agricultural soils of our study had
463 significantly higher average concentrations of pore water Fe (32 μM Fe) compared to the agricultural soils
464 (8 μM Fe). The much lower Fe in the pore waters in the latter soils can be explained by their higher Ca
465 concentration (7 mM versus 1.7 mM) and higher pH (5.9 versus 4.8) resulting from liming and fertilization.
466 The N-mineralization and nitrification likely explains the high solutes concentration (*e.g.* Ca, NO_3) in some
467 of the incubated agricultural samples (Table S3), which explains the negative correlation between Fe and
468 NO_3 and the positive correlation between NO_3 , DOC and Ca (Table S2). Trivalent cations induce humic
469 substance coagulation at lower concentration than divalent cations, nevertheless, the correlation
470 between Fe (and Al) and DOC is positive. The critical coagulation concentration (CCC) of Ca for purified
471 forest humic acids is $10^{-2.5}$ M Ca, the CCC of the trivalent cation Al occurred at more than an order of
472 magnitude lower concentration, i.e. about 10^{-4} M Al (Weng et al., 2002). However, due to low solubility
473 of Fe and Al oxyhydroxides only below pH 4 for Al and below pH 3 for Fe the free ion concentration reaches
474 a level at which coagulation of humic substances can be expected, and surface charge neutralization of
475 humic substances due to complexation occurs only at about pH 4 with Al and below pH 2.5 for Fe (Figure
476 S1). The positive correlation between pore water Al and Fe with DOC is thus due to complexation reactions
477 with humic substances and due to electrostatic and/or steric stabilization of colloidal oxyhydroxides.

478 The strong correlation between Fe and Al concentration might be caused by the complexation of Al and
479 Fe at high DOC or because Fe-and Al oxyhydroxides often occur as mixed minerals. In addition, aluminum
480 is a major component of aluminosilicates and the high concentration of Al compared to Fe is an indication
481 that colloidal aluminosilicates are present in the pore waters. In soil solutions Fe oxyhydroxides and
482 aluminosilicates can be physically associated in aggregates of colloidal dimensions because Fe
483 oxyhydroxides can precipitate on clay minerals or attach to the clay surface (Murphy et al., 1993; Plathe
484 et al., 2010; Regelink et al., 2014) and the Fe oxyhydroxides can be dispersed together with clay particles,

possibly explaining the strong correlation. In the regression analysis excluding Al (Eqn. 2), % sand is the predicting variable for pore water Fe concentration (Eqn. 2). Particles can easily be detached from sandy soils that have little reactive surfaces for colloid attachment, as observed by Regelink *et al.* (2014).

The environmental significance of these findings is that pore water with highest Fe concentrations can be found in the topsoil (high DOC and high %OC) of sandy soils that are not fertilized (low Ca) and have acid pH. This corresponds with the well-known conditions for Fe migration as Fe-OC complexes in the soil profile, *i.e.* podsolization (Lundstrom *et al.*, 2000). New here is that the role of Ca on Fe leaching has not been highlighted when different soil conditions are compared on a large set of contrasting soils.

The size and composition of pore water colloids

Iron in the small size, humus-rich fraction (< 5 nm) likely consisted predominantly of Fe-OC mononuclear complexes because it corresponds to the size range of humic substances, which was supported by the co-elution of Cu, a trace metal with high-affinity to humic substances (Figure S3). In addition, after conversion of the UV signal to a fulvic acid concentration, except for two soils (soil E and G) the Fe/OC ratio in the Fe-OC complexes is below 12 mmol Fe mol⁻¹ OC, which corresponds with the predicted environmental binding capacity of fulvic acids for Fe at pH ≥ 4 (Table S8). In soil E, the Fe colloids are very small (< 5 nm), but the Fe peak is slightly shifted from the UV peak (Figure 2) and the Cu peak (Figure S4), which suggest that in this pore water the Fe bound to the humic substances might be polymerized. Oligomers consisting of Fe dimers and trimers bound to soil and aquatic organic matter have been identified with XAS analysis (Gustafsson *et al.*, 2007; van Schaik *et al.*, 2008; Vilg-Ritter *et al.*, 1999). The discrepancy between predicted and observed (51 mmol Fe mol⁻¹ OC) Fe complexation in this soil is likely due to the inclusion of both mononuclear Fe-organic complexes and polymeric Fe clusters in the < 5 nm fraction. In the fractogram of soil J, there is some overlap of the void peak (elution time of unretarded substances) with the size range of Fe-OC complexes (Figure S3), which results in overestimation of the Fe binding to the

humic substances. For soil G a Fe/OC ratio of 70 mmol Fe mol⁻¹ OC was calculated, which is therefore an artefact. Overlap of the void peak is especially problematic in that soil because the concentration of Fe-OC complexes is low. This pore water was characterized by large colloids and especially the larger particles might contribute to an extended void peak due to an elution mechanism known in FIFFF analysis as steric inversion (Giddings, 1993b). Nevertheless, the FIFFF analysis indicated that the Fe-OC complexes represented generally a relatively small proportion compared to the mineral Fe colloids (Figure 3). The fraction of Fe in Fe-OC complexes increased with DOC/Fe ratio and is not explained by pH after correction for pore water DOC/Fe ratio (not shown). This conclusion does not fully support the results of the geochemical modelling on the large dataset that predicted that in half of the pore waters all Fe is bound to DOC, which might be a consequence of the model assumptions. In addition, as explained, the sample treatment in the FIFFF analysis was different compared to the survey (centrifugation instead of membrane filtration and aqua regia digestion prior to ICP-MS measurement) to better preserve the colloids. Therefore, it can be expected that a larger fraction of Fe in the pore water occurred as mineral Fe colloids because they are less retained.

Single correlation analysis indicated a negative correlation between %OC and the fraction of large mineral colloidal Fe (Figure 4). In the regression equation (Eqn. 3), DOC appeared as predicting variable for large mineral colloidal iron, but this variable is also correlated with %OC (Table S11). Organic matter is known to be an inhibitor of growth and crystallization of Fe-oxides (Schwertmann & Fitzpatrick, 1992; Sjöstedt et al., 2013; Voegelin et al., 2010) and promotes nanometer sized Fe oxides by inhibiting the development of hydroxide nuclei and preventing the aggregation of the Fe oxyhydroxide precipitates. The FIFFF results indicate the mechanism of growth inhibition by surface adsorbed humic substances because of the observed negative correlation between the fraction of Fe in the large mineral size range and humic substances content of the particles (OC/Fe signal) ($r = -0.55$; $p < 0.05$; Table S12). Nanoparticles and amorphous Fe oxyhydroxide phases are thermodynamically unstable and grow spontaneously. Therefore,

in the organic-poor soils, dispersed particles may grow due to Ostwald ripening or crystallize, which usually coincides with increasing crystal size. A clear relation between the effective reactive surface area of soils and the organic matter content was found by Hiemstra *et al.* (2010). A second variable predicting Fe colloid size is the fraction Fe_{ox}/Fe_{tot} , i.e. the more amorphous the soil Fe, the smaller the pore water Fe colloids are. The oxalate extractable Fe fraction is a characteristic of the soil solid phase and an explanatory variable for the size of the Fe colloids in the pore water, this suggests that the pore water Fe colloids reflect the Fe speciation in the soil solid phase. This is in agreement with Regelink *et al.* (2014) who showed that the Fe speciation in colloids of two soil extracts determined by XAS analysis closely resembled the Fe speciation in the bulk soil. The third predictor variable in Eqn. 3 is the exchangeable Ca concentration, which is less straightforward to explain. Figure 3 shows that at low %OC, high colloidal Fe concentrations can occur and that the colloids are large. In these pore waters, the large mineral Fe colloids might be associated with illite particles that were dispersed because the pore water Ca concentration in these soils is low, possibly explaining the role of exchangeable Ca in the regression equation. Iron oxyhydroxides exhibit a high affinity to clay minerals due to electrostatic interactions between the Fe oxyhydroxides and negatively charged clay minerals (Van Riemsdijk *et al.*, 2007). The co-elution of Fe with Si, Al and Mg might indicate the association between Fe oxyhydroxides and clay particles that as a result distribute the Fe oxyhydroxides to the larger size ranges of clay particles (Murphy *et al.*, 1993; Plathe *et al.*, 2010; Regelink *et al.*, 2014). The SEM results (Figure 5) confirmed small particles not associated with clay platelets in the organic-rich soil (soil D), and large particles in the organic-poor soil (soil J).

These findings highlight the importance of organic matter in the size and composition of Fe colloids. In general, small mineral Fe particles can prevail in pore waters of organic rich soils (> 3.5 %OC) and these colloids are coated with organic matter. In organic-poor soils, larger mineral Fe particles occur and probably exist as oxide-clay associations. The low Ca concentration in organic-poor soils can indirectly affect Fe colloid size by promoting dispersion of clay particles.

Conclusion

This study was set up to understand the concentrations and characteristics of Fe oxyhydroxide colloids in soil pore waters because these are considered vectors of nutrients and contaminants in the environment. The concentration of Fe in pore waters is governed by Fe colloid stability because the highest colloidal Fe concentrations occur at lower pore water Ca, resulting in high charge repulsion, and at higher DOC concentrations, that provides electrostatic and/or steric stabilization to Fe oxyhydroxide colloids. The FIFFF analysis indicated that mineral Fe oxyhydroxide colloids dominated in concentration over Fe-OC complexes. In organic rich soils (> 3.5%), mineral Fe colloids are smaller (< 50 nm) in size and have a higher content of humic substances (higher OC/Fe ratio) compared to organic poor soils. This agrees with the role of humic substances in reducing the rate of growth, transformation and ageing of oxyhydroxides in the environment (Schwertmann & Fitzpatrick, 1992; Sjöstedt et al., 2013; Voegelin et al., 2010). In soils with low %OC content and low Ca concentration, Fe colloids are larger (50-100 nm) and possibly occur as Fe oxyhydroxide coatings on the clay minerals rather than structural Fe in these minerals. Future work will focus on the size depended reactivity of the mineral Fe colloids. The smallest mineral Fe colloids identified in organic soils have high specific surface area and are, potentially, most mobile in soil. These small Fe colloids might be vectors for cationic trace metals rather than for phosphate or arsenate oxyanions because of the pronounced competition with the organic matter coating for sorption on the smaller Fe oxyhydroxides. In addition, the Fe co-eluting with clay particles may consists of nano-oxides attached to clay minerals. Hence, the time of elution does not necessarily reflect the size or surface area of the Fe-oxide particle.

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Table 1 Summary statistics of selected soil and pore water properties and pairwise correlations between these properties and pore water Fe concentration. The correlations were made on log-transformed variables except pH.

	Soil properties					Pore water properties				
	pH	%OC	CEC	% sand	Fe _{ox}	DOC	Ca	NO ₃	Al	Fe
			<i>cmol_c kg⁻¹</i>		<i>mmol kg⁻¹</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>μM</i>	<i>μM</i>
Summary statistics										
Mean	5.8	2.6	10	52	56	4	6.7	18	29	10
Median	5.6	1.5	8	55	32	3	6.4	16	5.4	1
P ₁₀	4.2	0.7	2	10	5	1	0.8	1.6	0.1	0.2
P ₉₀	7.4	4.3	19	91	214	8	13	37	80	26
Pairwise correlation with pore water Fe										
r	-0.55	0.41	-0.17	0.46	-0.09	0.45	-0.66	-0.45	0.65	
P	<.0001	<.0001	n.s	<.0001	n.s	<.0001	<.0001	<.05	<.0001	
n	97	97	97	97	32	97	97	65	97	97

n.s: not significant; %OC; organic carbon; CEC: cation exchange capacity; Fe_{ox}: oxalate extractable Fe concentration;
DOC: dissolved organic carbon; n: number of samples

Figure 1 The pore water Fe concentrations ($< 0.45 \mu\text{m}$) of 97 different soils are significantly ($p < 0.0001$) positively correlated with DOC ($r = 0.45$) and significantly negative correlated with Ca ($r = -0.66$). The grey scale of the dots indicates the relation with the DOC concentration.

Figure 2 FIFFF fractograms showing the Fe and UV signal for pore water samples of three contrasting soils. The vertical dashed lines discriminate the three main colloidal fractions, Fe-OC complexes (<5 nm), small mineral colloids (5–50 nm) and large mineral Fe colloids (50–100 nm). From left to right the soils contain an increasing fraction of large mineral Fe colloids.

Figure 3 Bar graph showing the distribution of Fe in complexes with organic carbon (< 5 nm) and small (5–50 nm) and large (50–100 nm) mineral colloids and selected soil characteristics of the samples. The soils are ranked by decreasing fraction of large mineral colloids. The bars represent the means of two pore water replicates extracted for each soil and error bars indicate the standard errors. The standard error on analytical duplicates is less than 5% (not shown).

Figure 4 The Fe colloid size as derived from the FIFFF fractograms, expressed as the percentage of Fe in the fractogram that is present in the size range of large mineral colloids (50-100 nm), decreased significantly ($p = 0.0004$) with increasing soil organic carbon (%OC) content. Note that the x and y axes have log scale.

Figure 5 The SEM analysis on fractions obtained from FIFFF analysis show small particles in the organic-rich soil (soil D) and larger in the organic-poor soil (soil J). From left to right: soil D (fraction obtained from FIFFF elution time: 5-25 min), soil D (elution time: 35-50 min) and soil J (elution time: 40-55 min).

1 **Supplementary material to:**

2 The concentration and size distribution of iron rich colloids in pore
3 waters are related to soil organic matter content and pore water
4 calcium concentration

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Table S1 Summary statistics on survey data of soil properties and pore water composition (< 0.45 µm)

		Summary statistics					
	unit	n	Mean	St. Dev.	Median	P ₁₀	P ₉₀
Soil properties							
pH		97	5.8	1.2	5.6	4.2	7.4
% Sand	%	97	52	30	55	10	91
% Silt	%	97	35	26	28	5	75
% Clay	%	97	13	12	10	3	28
%OC	%	97	2.6	4.2	1.5	0.7	4.3
IC	%	24	1.2	1.7	0.2	0.01	4.3
CEC	cmol kg ⁻¹	97	10	8	8	2	19
Exch. Ca	cmol kg ⁻¹	97	8.4	9.3	6.2	1.7	17
Exch. Na	cmol kg ⁻¹	97	0.07	0.07	0.05	0.009	0.1
%BS	%	97	88	19	95	68	100
C _{tot}	%	97	2.9	4.3	1.7	0.8	4.7
N _{tot}	%	97	0.2	0.3	0.1	0.08	0.4
C/N		97	15	16	11	9	23
Al _{ox}	mmol kg ⁻¹	32	33	31	24	12	71
Fe _{ox}	mmol kg ⁻¹	32	56	80	32	5	214
Mn _{ox}	mmol kg ⁻¹	32	4.7	8.3	2.4	0.02	11
Pore water properties							
DOC	mM	97	3.6	3.7	2.6	0.9	7.7
SUVA	l g ⁻¹ cm ⁻¹	65	19	7	20	8	29
DIC	mM	65	0.6	0.8	0.4	0.1	1
SO ₄ ²⁻	mM	65	1.6	3.8	0.8	0.4	2.1
Cl ⁻	mM	65	3.2	5.3	1.0	0.4	8.4
NO ₃ ⁻	mM	65	18	14	16	1.6	37
Na	mM	65	1.4	1.4	0.9	0.4	3.7
Mg	mM	97	2.1	2.1	1.3	0.3	5.6
K	mM	97	1.4	1.7	1.0	0.1	3.0
Ca	mM	97	6.7	5.8	6.4	0.8	13
Al *	µM	97	29	62	5.4	0.1	80
Fe *	µM	97	10	32	0.9	0.2	26
Mn *	µM	97	19	85	1.0	0.002	40

n: number of samples; (D)OC: (dissolved) organic carbon; (D)IC: dissolved inorganic carbon; CEC: Cation Exchange Capacity; Exch. Ca and Na: exchangeable Calcium and Na concentration; %BS: base saturation; Sand, Silt, Clay content according to USDA classification; tot: total concentrations in *aqua regia*; Fe_{ox}, Al_{ox}, Mn_{ox}: Oxalate extractable concentrations; SUVA: Specific UV Absorbance (Weishaar et al., 2003) corrected for interference of Fe (Poulin et al., 2014). * 10-20% of the data are below the limit of quantification (LOQ), negative values were replaced by the LOQ: Fe 0.14 µM

Table S2 Pairwise correlation between soil properties and pore water composition in the dataset of the survey. All variables except pH were log₁₀ transformed

	soil properties														
	pH	%Sand	%Silt	%Clay	%OC	IC	C _{tot}	N _{tot}	C/N	CEC	Exch.Ca	Mn _{ox}	Al _{ox}	Fe _{ox}	
%Sand	-0.62														
%Silt	0.55	-0.82													
%Clay	0.53	-0.57	0.56												
%OC	-0.48	0.36	-0.23	-0.14											
IC	0.82	0.49	-0.59	0.62	-0.03										
C _{tot}	-0.20	0.31	-0.18	0.07	0.81	0.87									
N _{tot}	-0.28	0.21	-0.07	0.06	0.93	-0.07	0.79								
C/N	0.08	0.19	-0.18	0.03	0.00	0.85	0.50	-0.15							
CEC	0.55	-0.52	0.57	0.76	0.13	0.67	0.36	0.33	0.11						
Exch.Ca	0.42	-0.48	0.38	0.54	-0.18	0.35	0.02	-0.06	0.11	0.54					
Mn _{ox}	0.65	-0.52	0.36	0.52	-0.05	-0.47	0.05	0.19	-0.19	0.40	0.70				
Al _{ox}	-0.12	0.07	-0.04	0.12	0.40	-0.21	0.32	0.54	-0.29	0.25	0.36	0.30			
Fe _{ox}	-0.15	-0.11	0.08	0.07	0.60	-0.40	0.28	0.71	-0.61	0.23	0.25	0.37	0.62		
DOC	-0.48	0.48	-0.43	-0.35	0.51	0.48	0.45	0.37	0.20	-0.23	-0.30	-0.14	0.13	0.19	
SUVA	-0.04	0.13	0.02	0.01	0.23	0.09	0.21	0.23	0.03	0.04	-0.26	0.00	0.00	0.00	
DIC	0.30	-0.14	0.11	0.10	-0.09	0.71	-0.07	-0.07	-0.02	0.15	0.04	0.00	0.00	0.00	
Cl	0.00	0.12	-0.22	-0.12	0.22	-0.42	0.21	0.22	0.06	-0.07	-0.51	0.00	0.00	0.00	
SO ₄	0.03	0.29	-0.21	-0.06	0.37	0.59	0.37	0.34	0.20	0.05	-0.41	0.00	0.00	0.00	
NO ₃	0.18	-0.15	0.18	0.12	-0.26	0.02	-0.26	-0.28	-0.02	0.03	0.14	0.00	0.00	0.00	
Ca	0.35	-0.24	0.27	0.24	0.05	-0.62	0.03	0.16	-0.16	0.18	0.39	0.74	0.36	0.42	
Na	-0.07	0.23	-0.30	-0.21	0.22	0.26	0.23	0.18	0.20	-0.12	-0.16	0.00	0.00	0.00	
Al	-0.74	0.54	-0.47	-0.29	0.36	0.52	0.25	0.26	0.04	-0.34	-0.30	-0.47	0.09	0.02	
Mn	-0.72	0.43	-0.35	-0.36	0.45	-0.21	0.22	0.37	-0.16	-0.40	-0.35	-0.20	0.23	0.32	
Fe	-0.54	0.46	-0.40	-0.29	0.40	0.09	0.26	0.32	-0.02	-0.17	-0.55	-0.57	-0.10	-0.09	

Bold font: significant correlations ($p \leq 0.05$), red font: highly significant correlations ($p \leq 0.0001$)

Table S2 continued.

	pore water properties (<0.45 µm)									
	DOC	SUVA	DIC	Cl	SO4	NO3	Ca	Na	Al	Mn
%Sand										
%Silt										
%Clay										
%OC										
IC										
C _{tot}										
N _{tot}										
C/N										
CEC										
Exch.Ca										
Mn _{ox}										
Al _{ox}										
Fe _{ox}										
DOC										
SUVA	0.16									
DIC	-0.19	-0.21								
Cl	0.33	0.03	0.04							
SO ₄	0.30	0.10	-0.03	0.51						
NO ₃	-0.35	-0.13	0.19	0.07	0.13					
Ca	-0.17	-0.37	0.41	0.00	0.31	0.54				
Na	0.33	-0.16	0.15	0.65	0.57	0.19	0.43			
Al	0.50	0.05	-0.24	-0.04	-0.15	-0.41	-0.44	0.05		
Mn	0.56	-0.08	-0.24	0.14	0.02	-0.30	-0.07	0.22	0.67	
Fe	0.47	0.32	-0.35	0.17	0.13	-0.46	-0.67	-0.11	0.65	0.45

Table S3 Summary statistics of selected soil and pore water properties.

	Agricultural soils						Non-agricultural soils	
	Fresh (Set 1, n=30)		Cold stored (Set 2, n=35)		Dried and rewetted (Set 3, n=22)		Dried and rewetted (Set 3, n=10)	
	Mean	P ₁₀ -P ₉₀	Mean	P ₁₀ -P ₉₀	Mean	P ₁₀ -P ₉₀	Mean	P ₁₀ -P ₉₀
Soil properties								
pH	5.5 ^(A,B)	(4.8-6.8)	6.0 ^(A)	(4.8-7.2)	6.1 ^(A)	(4.2-7.6)	4.8 ^(B)	(3.0-7.4)
Clay (%)	9.0 ^(B)	(1.8-15)	9.9 ^(A,B)	(3.0-17)	20 ^(A)	(4.0-45)	24 ^(A,B)	(0.6-51)
%OC (%)	2.4 ^(A)	(1.1-4.3)	1.4 ^(A)	(0.7-2.4)	5.0 ^(A)	(0.4-20)	2.0 ^(A)	(0.3-5.2)
Organic C/N	11 ^(A,B)	(9-14)	10 ^(B)	(9-13)	12 ^(A,B)	(6-22)	16 ^(A)	(7-30)
CEC (cmol _c kg ⁻¹)	7.8 ^(A)	(2.9-12)	8.4 ^(A)	(3.7-12)	16 ^(A)	(1.9-35)	12 ^(A)	(1.8-35)
Base saturation (%)	96 ^(A)	(86-100)	89 ^(A)	(72-100)	90 ^(A)	(74-100)	52 ^(B)	(7-94)
Fe _{ox} (mmol kg ⁻¹)	n.m.		n.m.		74	(6.4-279)	17	(4.4-34)
Pore water (<0.45 µm) properties								
Fe (µM)	20 ^(A)	(1.2-47)	0.73 ^(B)	(0.14-1.3)	1.2 ^(B)	(0.14-6.3)	32 ^(A)	(0.24-191)
DOC (mM)	3.9 ^(A)	(1.8-7.1)	1.8 ^(B)	(0.80-3.4)	5.0 ^(A)	(1.2-13)	5.6 ^(A)	(0.33-13)
Ca (µM)	4.4 ^(B)	(0.67-11)	8.9 ^(A)	(6.4-13)	8.7 ^(A)	(2.7-22)	1.7 ^(C)	(0.05-6.7)
Al (µM)	18 ^(A,B)	(0.12-57)	28 ^(C)	(0.07-116)	14 ^(B,C)	(0.07-55)	94 ^(A)	(2.9-302)
NO ₃ (mM)	12	(1.2-30)	23	(15-38)				

Values with different superscripts are significantly different within each row (Tukey test, $\alpha=0.05$). n.m: not measured; DOC: Dissolved Organic Carbon; CEC: Cation Exchange Capacity; Fe_{ox}: oxalate extractable Fe concentration; exch Na, exch Ca: exchangeable Na and Ca concentration

8 **Table S4** Effect of soil treatment after sampling on the pore water Fe concentration for a range of soils
9 that underwent all treatments. The limit of quantification (LOQ) for Fe is 0.14 μM .
10

pore water Fe concentration			factor change relative to fresh soil	
Fresh	Cold stored	Dried and rewetted	Cold stored	Dried and rewetted
(μM)				
1.44	0.14	0.14	0.10	0.10
1.27	0.14	0.14	0.11	0.11
18.48	0.14	1.24	0.01	0.07
1.31	0.14	0.14	0.11	0.11
1.55		1.19		0.77
5.73		2.26		0.39
2.32		0.54		0.23
1.65		0.14		0.08

11

Table S5 Pairwise correlations (r) between pore water Fe concentration and soil and pore water properties. All variables except pH were log₁₀ transformed for correlation analysis.

	Fresh (n=30)		Cold stored soils (n=35)		Dried and rewetted (n=32)	
	r	P	r	P	r	P
Soil properties						
pH	-0.29	0.1202	-0.58	0.0003	-0.69	<.0001
%Sand (%)	0.45	0.013	0.66	<.0001	0.25	0.1653
%Silt (%)	-0.44	0.0151	-0.57	0.0003	-0.21	0.2388
%Clay (%)	-0.38	0.041	-0.51	0.0019	-0.15	0.4195
N _{tot} (%)	0.31	0.1009	0.55	0.0006	0.16	0.3716
C _{tot} (%)	0.31	0.0938	0.60	0.0001	0.08	0.6677
%OC (%)	0.32	0.089	0.65	<.0001	0.31	0.081
organic C/N	0.10	0.5875	0.38	0.0258	0.46	0.0082
CEC (cmol _c kg ⁻¹)	-0.17	0.3745	-0.31	0.0689	-0.10	0.5814
Exch Mg (cmol _c kg ⁻¹)	0.00	0.9981	-0.10	0.5777	-0.03	0.8887
Exch Na (cmol _c kg ⁻¹)	-0.05	0.7747	-0.03	0.8672	0.24	0.1801
Exch K (cmol _c kg ⁻¹)	-0.46	0.0111	-0.12	0.4875	-0.28	0.12
Exch Ca (cmol _c kg ⁻¹)	-0.19	0.3183	-0.44	0.008	-0.36	0.0421
Base saturation (%)	-0.16	0.4113	-0.38	0.0246	-0.54	0.0014
Al _{ox} (mmol kg ⁻¹)	.	.	.	.	-0.11	0.5354
Fe _{ox} (mmol kg ⁻¹)	.	.	.	.	-0.09	0.634
Mn _{ox} (mmol kg ⁻¹)	.	.	.	.	-0.57	0.0006
Pore water properties						
Cl (mM)	-0.61	0.0003	-0.08	0.6624	.	.
SO ₄ (mM)	-0.27	0.1429	0.29	0.0921	.	.
NO ₃ (mM)	-0.55	0.0017	-0.10	0.5594	.	.
DIC (mM)	-0.38	0.0375	0.12	0.4837	.	.
Na (mM)	-0.54	0.0023	0.43	0.0107	.	.
Mg (mM)	-0.60	0.0005	0.53	0.0011	-0.23	0.2075
K (mM)	-0.37	0.0523	0.63	<.0001	-0.12	0.5142
Ca (mM)	-0.72	<.0001	0.38	0.025	-0.61	0.0002
Al (μM)	0.57	0.001	0.56	0.0004	0.82	<.0001
Mn (μM)	0.32	0.0835	0.53	0.001	0.37	0.0351
DOC (mM)	0.44	0.0141	0.71	<.0001	0.24	0.1949
SUVA	0.17	0.3834	-0.14	0.4326	.	.

17 Speciation modelling with WHAM VII

18 The model input for the pore waters from the survey is the pH and major cations (Na, Ca, K, Mg) and
19 anions (NO_3 , Cl, SO_4) based on total concentrations measured in pore water for fresh (set 1) and cold
20 stored (set 2) samples. In dried and rewetted samples (set 3) anions were not measured but estimated
21 from the charge balance in set 1 (median ratio monovalent:divalent anions 4:1 and median ratio Cl: NO_3
22 1:2). The temperature was 10°C for set 1 (fresh samples); 4°C for set 2 (cold stored) and 20°C for set 3
23 (incubated at 20°C). Total Fe in 0.45 μm filtered pore water was added with Fe allowed to precipitate as
24 Fe(III)(OH)_3 with $\log K_{\text{sp}}$ 2.7 (Schwertmann, 1991).

25 The modelling relies on several assumptions (Groenenberg & Lofts, 2014) related to reactivity of the
26 organic matter (i.e. fraction of organic matter that is fulvic acids (FA) /humic acids (HA)) and affinity
27 constants for binding of Fe to FA/HA. A sensitivity analysis is given below for two pore waters and shows
28 that the Fe binding constants for Fe to humic substances largely affect the outcome. The model
29 predictions with WHAM VII (this study) largely underpredict the Fe(III) bound to DOC compared to the
30 generic NICA-Donnan model (Milne et al. 2003) and slightly overpredict the Fe(III) bound to DOC
31 compared to the NICA-Donnan model with adjusted binding constants for marine DOC (Hiemstra & Van
32 Riemsdijk, 2006).

33 Water 1: pH 6.6, pore water Fe concentration 18 μM , DOC 2.38 mM.

34 WHAM generic, 65% DOM is FA: predicts 10 % of pore water Fe bound to FA

35 NICA-Donnan generic, 65% DOM is FA: predicts 99 % of pore water Fe bound to FA

36 NICA-Donnan adjusted marine, 65% DOM is FA: predicts 1.2 % of pore water Fe bound to FA

37 WHAM generic, 50% DOM is HA and 50% DOM is FA: predicts 8 % of pore water Fe bound to FA and 6 %
38 bound to HA

39 Water 2: pH 4.1, pore water Fe concentration 7.0 μM , DOC 1.54 mM.

40 WHAM generic, 65% DOM is FA: predicts 17 % of pore water Fe bound to FA

41 NICA-Donnan generic, 65% DOM is FA: predicts < 99 % of pore water Fe bound to FA

42 NICA-Donnan adjusted marine, 65% DOM is FA: predicts 2.5 % of pore water Fe bound to FA

43 WHAM generic, 50% DOM is HA and 50% DOM is FA: predicts 13 % of pore water Fe bound to FA and 8 %
44 bound to HA

45 For the speciation modelling of the pore waters from the survey, the following assumptions were made.

46 The colloidal fulvic acids concentration was calculated from the measured DOC concentration assuming
47 that 65% of the DOM is present in fulvic acids (%FA=65% (Bryan et al., 2002)) and that 1 g of DOC equals

48 2 g of DOC. The generic model parameters for fulvic acids in WHAM VII and affinity constants of ions for

49 fulvic acids that are used for the modelling are given in Table S6 (Tipping, 1994; Tipping et al., 2011). For

50 Al it was assumed that Al activity is controlled by $\text{Al}(\text{OH})_3$ with $\log K_{\text{sp}} = 8.5$. The predicted fraction of Fe-

51 OC complexes compared to the pore water Fe for the data from the survey is given in Table S7.

52

53

Table S6 Generic WHAM model parameters for fulvic acids used in modelling, references to the used affinity constants can be found in Tipping *et al.* (Tipping et al., 2011).

	Fulvic acid for Model VII	
	carboxyl acid sites (A sites)	phenolic acid sites (B sites)
abundance sites (mol per g)	5.20E-03	2.60E-03
Intrinsic proton dissociation constant (log K)	3.7	9.6
distribution term that modifies log K	3.1	4.4
Ion specific parameters	Intrinsic equilibrium	Intrinsic equilibrium
	constants	constants
Binding species:	logKA	logKB = 3.39 logKA - 1.15
Mg	0.99	
Ca	1.13	
Al	2.50	
AlOH	2.57	
Fe(III)	3.12	
Fe(III)OH	3.12	

Table S7 Predicted fraction of Fe-OC complexes compared to the pore water Fe (average $\pm$ SD) and amount of samples in which no Fe oxyhydroxide colloids are predicted.

subset	%FA=65		
	Fe	Fe-OC	samples without Fe(OH) ₃
	(μ M)	(%)	
Fresh (n = 30)	20	54 $\pm$ 39	10
Cold stored (n = 35)	0.7	93 $\pm$ 20	32
Dried and rewetted-A (n=22)	1.2	93 $\pm$ 18	19
Dried and rewetted-NA (n = 10)	32	30 $\pm$ 40	4

n = amount of samples

Uncertainty in the modelling is further also related to the activity control of Al and as a consequence the competition between Fe and Al for binding to FA/HA. The Fe binding capacity of organic ligands (calculated with WHAM VII (Tipping, 1994)), present as Fe-OC mononuclear complexes, is shown in Table S6 for pH 4 and 7 and in equilibrium with soil-Fe and gibbsite that, in turn, buffer the Fe³⁺ and Al³⁺ activity in solution.

64 The relatively low binding capacity for Fe at low pH is due to competition with Al, which has higher
65 solubility than Fe (Tipping et al., 2002). The Al^{3+} activity is not always governed by equilibrium mineral
66 dissolution in more acidic conditions, which results in less competition with Fe for binding to organic
67 matter (Tipping, 2005). Under these conditions, the capacity of fulvic acids for binding Fe can be an order
68 of magnitude higher, *i.e.* about 12 mmol Fe mol⁻¹ OC at pH 4, when the Al activity is controlled by Al
69 sorption/desorption to organic matter (Tipping, 2005). Figure S1 shows the modelled fulvic acid charge as
70 a function of pH due to complexation with Fe and Al in equilibrium with soil-Fe and gibbsite in 1 mM NaCl
71 background.

72 **Table S8** The Fe binding capacity of organic substances forming Fe-OC mononuclear complexes, expressed
73 per molar organic carbon unit, defined as the capacity in the environment at a given pH, with CaCl_2 at 2
74 10^{-3}M as background and in equilibrium with soil-Fe and gibbsite. Calculations are done with WHAM VII
75 at a temperature of 10°C; natural organic matter (NOM) is assumed to consist of 65% of fulvic acids
76 (Groenenberg & Lofts, 2014) and 1 g NOM, fulvic acids or humic acids is composed of 0.5 g OC. The value
77 between brackets is the predicted Fe binding capacity of fulvic acids when the Al activity is controlled by
78 Al sorption/desorption to organic matter at low pH based on the empirical equation of Tipping (2005).

79

Ligand	environmental capacity	
	mmol Fe mol ⁻¹ OC pH 4	pH 7
Fulvic acids	0.90 (12)	0.54
Humic acids	0.70	0.44
Natural organic matter	0.59	0.35

80

81

82

83

84 **Figure S1** Modelled charge of fulvic acids as function of pH resulting from Al or Fe adsorption.

85

Table S9 Summary statistics of soil properties and composition of the pore water for the soils used for FIFFF analysis.

Soil	Soil type*	Land use	Soil							Pore water			
			pH	%OC	%clay	Al _{tot} <i>mmol kg⁻¹</i>	Fe _{tot} <i>mmol kg⁻¹</i>	Fe _{Ox} <i>mmol kg⁻¹</i>	Al _{Ox} <i>mmol kg⁻¹</i>	DOC <i>mM</i>	Al <i>μM</i>	Ca <i>mM</i>	Fe <i>μM</i>
A	Haplic Podzol	heath	3.4	1.9	5	23	23	11 (50)	11 (48)	39	278	0.2	156
B		forest	3.7	14		470	1406	667 (47)	65 (14)	5	125	4	43
C	Histosol	grass	4.5	30	24	1103	462	257 (56)	150 (14)	19	122	20	31
D	Histosol	grass	5.2	12	13	316	184	58 (31)	25 (8)	126	968	10	361
E		grass	5.5	3.5		494	346	89 (26)	20 (4)	12	274	3	211
F		grass	5.7	4.8		466	267	77 (29)	17	19	1137	4	615
G	Vertic Cambisol	wood	5.8	0.9	51	1213	594	28 (5)	24 (2)	3	1507	1	719
H	Haplic Luvisol	arable	6.2	1.0	15	440	293	34 (12)	14 (3)	5	405	2	206
I		grass	6.4	8.3	49	693	339	79 (23)	26 (4)	27	78	28	33
J	Mollisol	arable	6.6	1.4	19	755	304	8 (3)	25 (3)	10	1783	2	484
K	Haplic Luvisol	arable	7.3	1.3	27	395	251	22 (9)	16 (4)	5	40	5	30

Org C: soil organic carbon content; Fe_{Ox} and Al_{Ox}: oxalate extractable Al and Fe, fraction oxalate extractable to total concentration between brackets; DOC dissolved organic carbon; tot: total concentrations in *aqua regia*. * Soil classification according to World Reference Base

Figure S2 FIFFF fractograms of all soils showing the Fe (brown), Al (grey) and UV (black) size distribution

Figure S2 continued.

Table S10 Median molar element ratios of Si, Al, Fe and Mg in mineral particles (5-100 nm) determined over the size distribution window in which the concentrations were above the detection limits (size range where peak concentration occurs). In soil B, I and k the Si concentration was below the detection limit due to the low colloid concentration. The OC/Fe ratio was determined for the mineral size range based on the UV signal that was converted to a fulvic acid concentration, as described above. The error term is the SD of two replicates.

soil	Si/Al	Si/Fe	median molar ratio in particles		Fe/OC mmol mol ⁻¹
			Al/Fe (mol mol ⁻¹)	Mg/Al	
A	1.9	4.5	2.0 ± 0.2	0.10	12 ± 0.97
B			1.4 ± 0.3	0.19	21 ± 2.0
C	2	8.7	3.7 ± 0.4	0.16	9.4 ± 0.69
D	2.1	12	5.2 ± 0.2	0.18	23 ± 0.31
E	2.8	3.4	0.67 ± 0.5	0.18	167 ± 0.83
F	2.6	5.1	2.0 ± 0.1	0.16	91 ± 26
G	1.9	3.4	1.6 ± 0.2	0.14	204 ± 75
H	2.5	4.7	1.9 ± 0.01	0.21	204 ± 142
I			2.6 ± 0.4	1.1	5.7 ± 0.68
J	3.1	10	3.5 ± 0.01	0.25	115 ± 32
k			1.8 ± 0.2	0.14	36 ± 28

Table S11 Pairwise correlation analysis between soil properties and pore water composition for the samples of the FIFFF analysis. All variables except pH were log₁₀ transformed.

	Soil properties											Pore water properties								
	%OC	CEC	Exch Ca	pH	%sand	%clay	Fe _{ox}	Fe _{tot}	Fe _{ox} /Fe _{tot}	Al _{ox}	Al _{tot}	DOC	Fe	DOC:Fe	Al	Mn	Ca	Na	NO ₃	
CEC	0.33																			
Exch Ca	0.21	0.96																		
pH	-0.48	0.52	0.65																	
%sand	0.24	-0.49	-0.57	-0.83																
%clay	0.05	0.86	0.80	0.63	-0.68															
Fe _{ox}	0.80	0.38	0.28	-0.42	-0.10	0.38														
Fe _{tot}	0.28	0.75	0.70	0.19	-0.59	0.87	0.65													
Fe _{ox} /Fe _{tot}	0.75	-0.26	-0.34	-0.73	0.44	-0.41	0.65	-0.16												
Al _{ox}	0.76	0.57	0.45	-0.34	0.15	0.36	0.72	0.60	0.33											
Al _{tot}	0.17	0.93	0.92	0.50	-0.55	0.85	0.36	0.85	-0.39	0.52										
DOC	0.47	-0.19	-0.21	-0.26	0.46	-0.48	0.003	-0.53	0.53	-0.07	-0.41									
Fe	-0.33	-0.12	-0.08	0.04	0.34	-0.17	-0.33	-0.14	-0.29	-0.32	0.05	0.08								
DOC/Fe	0.58	-0.05	-0.10	-0.21	0.10	-0.21	0.26	-0.28	0.60	0.19	-0.33	0.65	-0.70							
Al	-0.19	-0.01	0.03	0.05	0.37	-0.14	-0.34	-0.12	-0.33	-0.13	0.14	0.08	0.89	-0.61						
Mn	0.58	0.55	0.54	-0.06	0.04	0.23	0.59	0.60	0.16	0.59	0.55	0.17	0.08	0.06	0.14					
Ca	0.61	0.81	0.81	0.37	-0.43	0.58	0.50	0.50	0.14	0.52	0.65	0.22	-0.32	0.40	-0.16	0.59				
Na	0.59	0.64	0.53	0.00	-0.14	0.61	0.50	0.37	0.28	0.56	0.51	0.20	-0.18	0.28	-0.05	0.31	0.70			
NO ₃	0.69	0.10	0.00	-0.20	-0.07	0.17	0.36	-0.01	0.47	0.31	-0.03	0.41	-0.33	0.51	-0.07	0.05	0.40	0.75		
Cl	-0.69	-0.09	-0.01	0.17	0.06	-0.01	-0.25	0.11	-0.43	-0.21	0.04	-0.63	0.10	-0.51	-0.08	-0.12	-0.44	-0.64	-0.83	

Bold font: significant correlations ($p \leq 0.05$), red font: highly significant correlations ($p \leq 0.0001$)

Table S12 Pairwise correlation of Fe in the three different size fractions (Fe-I: Fe-OC complexes; Fe-II small mineral colloids; Fe-III large mineral colloids) obtained from the FIFFF fractogram and soil and pore water properties. Significant ($p \leq 0.05$) correlations are indicated in bold. Correlations are based on the eleven pore waters which were analyzed in duplicate (22 datapoints for Fe fractions).

	Fe-I (< 5 nm)		Fe-II (5-50 nm)		Fe-III (50-100 nm)	
	r	p	r	p	r	p
soil properties						
pH	-0.29	0.1981	-0.01	0.9789	0.10	0.6486
%OC	0.23	0.2981	0.56	0.007	-0.68	0.0004
%silt	-0.60	0.0142	0.17	0.5174	0.15	0.5908
%sand	0.07	0.8072	0.02	0.9555	-0.08	0.7770
%clay	-0.10	0.7221	-0.07	0.8111	-0.05	0.8437
Al _{tot}	-0.40	0.0636	0.07	0.7421	-0.07	0.7571
Fe _{tot}	-0.21	0.3416	-0.01	0.9828	0.04	0.8515
Fe _{ox}	0.15	0.4924	0.40	0.066	-0.46	0.0319
CEC	-0.23	0.3336	0.24	0.3134	-0.30	0.1981
pore water properties						
DOC	0.32	0.1446	0.49	0.0248	-0.68	0.0006
DOC/Fe	0.62	0.0022	0.39	0.0722	-0.51	0.0142
Mn	-0.29	0.1946	0.37	0.0893	-0.32	0.1474
Fe	-0.51	0.0157	-0.05	0.8204	0.04	0.8617
Al	-0.63	0.0018	-0.05	0.8198	0.08	0.7111
Ca	0.01	0.9817	0.51	0.0162	-0.58	0.0049
colloid property						
molar Fe/OC particle	0.46	0.034	0.21	0.3492	-0.55	0.0096

Figure S3 The fractogram of Suwannee River NOM reference sample and of soil D, E and J. Iron in the small size, humic-rich fraction (< 5 nm) likely consisted predominantly of Fe-OC mononuclear complexes because it corresponds to the size range of humic substances. In addition, this is supported by the co-elution of Cu, a trace metal with high-affinity to humic substances (soil D). In soil E there is overlap of small polymerized Fe particles with the Fe-OC complexes as evidenced from the Fe peak that is partly shifted from the UV and Cu peak and extends over the size range of humic substances (shown by the fractogram of the NOM reference sample). A second mechanism that might result in overestimation of the amount of mononuclear Fe-OC complexes is observed for soil J. This pore water sample represent pore waters with mainly large mineral particles and the Fe signal is partly due to interference from particles eluting in the void peak. This artefact can occur in samples containing large particles that elute by steric inversion (von der Kammer et al., 2011) and have an important effect on the actual Fe-OC concentration, which would be low given the low UV signal (soil J). The fact that the Fe peak occurs before the UV and Cu peak supports this interference.

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