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Calcium and iron promote reversible self-assembly of dissolved organic matter into particles

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Abstract Dissolved organic matter (DOM) consists of dissolved molecules, biopolymers, and aggregates with a broad range of molecular sizes. In freshwater and seawater environments, spontaneous self-assembly of DOM forms hydrated particulate organic matter (POM) networks. This conversion from DOM to POM affects carbon transfer through microbial and particle food webs and export to sediments. Particle food webs are based on the direct POM ingestion by zooplankton species. This DOM assembly occurs when the inter-polymer or inter-colloid distances allow chemical (covalent) or physical (e.g., electrostatic, hydrogen) bonds. Here we explore the underlying mechanisms of self-assembly using lake waters with different concentrations of polyanionic DOM with humic content and calcium (Ca) and iron (Fe) crosslinking. We experimentally adjusted the cations by chelating and/or increasing the concentration of calcium or iron. To monitor the self-assembly of DOM, we employed homodynamic laser scattering. Results indicate that DOM self-assembly and physical gel-particle formation result from low-energy Ca^{+2} and Fe^{+3} counterion bonding. It can be readily reversed by Ca and Fe chelators, resulting in the disassembly of the network and dispersion of DOM polymers. Calcium cations appear to promote a higher level of self-assembly, reaching larger hydrodynamic sizes during stabilization, compared to iron cations. Our results indicate that the chemical environmental

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context critically affects the formation of POM from DOM, influencing ecosystem processes such as carbon sedimentation and storage, and providing alternative pathways for heterotrophic consumers (i.e., food webs based on particles).

Keywords DOM self-assembling · POM dispersion · Calcium · Iron · DOM-POM reversibility

Introduction

Dissolved organic matter (DOM) is the main reservoir of reduced carbon in marine and freshwater ecosystems (Cole et al. 2007; Hansell et al. 2009; Tranvik et al. 2009). The organic matter that goes through a filter commonly of 0.2 µm to 0.7 µm is operationally defined as DOM, including small molecules (true solutes), colloids, and polymers of different sizes. These polymers can self-assemble into larger particles (Verdugo et al. 2004, 2012), thereby contributing to the pool of particulate organic matter (POM). About 10–40% of marine DOM polymers (mostly autochthonous acidic polysaccharides) can spontaneously self-assemble following second-order kinetics and form larger and larger aggregates (Chin et al. 1998; Engel et al. 2004). By contrast, this spontaneous and abiotic coagulation process from dissolved precursors to POM has been hardly studied in freshwater ecosystems (Kerner et al. 2003; Asmala et al. 2014; He et al. 2016). In these last ecosystems, the DOM concentration is usually substantially greater than in marine ecosystems and enriched in terrestrial, humic compounds. The pH and cation concentrations are also more variable. The promotion of dissolved to particulate carbon has critical consequences for carbon export to sediments (Grossart and Simon 1998; de Vicente et al. 2009; Iversen and Ploug 2010; Jilbert et al. 2018; Martínez-García et al. 2024) or transfer to higher trophic levels via direct consumption of particles by zooplankton (Passow and Alldredge 1999; Ling and Alldredge 2003; Toullec et al. 2019).

According to the polymer gel theory, DOM polymers can interact with each other via chemical (covalent) or physical bonds (hydrogen, electrostatic bonding, or hydrophobic/hydrophilic interactions, van der Waals forces, among others). These interactions result in the formation of POM networks with features similar to those of microgels (Chin et al. 1998; Kerner et al. 2003; Verdugo et al. 2004; Verdugo and Santschi 2010; Verdugo 2012). These polyanionic POM networks can be stabilized by bi- or trivalent cations (Chin et al. 1998; Verdugo et al. 2004). These cations can form bridges with more than one negatively charged functional group, such as carboxyl (–COOH) or hydroxyl (–OH) groups of humic or fulvic acids. Other interactions, such as hydrophobic forces, can also play a crucial role in the DOM self-assembly without cation interventions (Ding et al. 2008; 2009). The first experimental evidence describing the "salting out" of humic matter from river waters upon mixing with seawater, due to the relative abundance of cations in seawater, was reported by Sholkovitz (1976). Later, Mullholand et al. (1981) found that calcium (Ca^{2+}) and magnesium (Mg^{2+}) additions caused an increase in the rate of POM formation due to rapid physicochemical flocculation. Baalousha et al. (2006) and Pace et al. (2012) noted that an increase in the ionic strength (sodium chloride, calcium chloride, or calcium bicarbonate additions) and pH altered the size and shape of humic acids. Other studies have shown that DOM decreases due to the formation of aggregates with iron, increasing POM (Luider et al. 2003; Jilbert et al. 2018). Hence, physicochemical properties of the DOM (e.g., concentration and composition) and solvent (i.e., water ionic strength, temperature, or pH) appear to play a critical role in the process of spontaneous self-assembling or phase changes (Chin et al. 1998; Ding et al. 2008; Lam and Simpson 2009; Verdugo 2012; Pace et al. 2012). These interactions between organic matter and major cations or trace metals can affect carbon processes such as flocculation and export rates toward the sediments (Iversen & Ploug 2010; Jilbert et al. 2018), microbial degradation (Xu and Guo 2018), and direct transfer to zooplankton (Kerner et al. 2003; Ling and Alldredge 2003; Toullec et al. 2019).

Freshwater ecosystems contribute disproportionately to the global carbon cycle despite their small global surface area. Annual carbon inputs from the terrestrial sources are about 1.9–2.7 Pg C y^{-1}, of which about 0.15–0.6 Pg C yr^{-1} is buried in lake and reservoir sediments (Cole et al. 2007; Tranvik et al. 2009; Mendonça et al. 2017). von Wachenfeldt and Tranvik (2008) observed that about 10% of DOM is flocculated to POM and exported to the sediments in humic lakes. The organic carbon burial in lake sediments builds a carbon pool for long-term storage (Tranvik et al. 2009), estimated to be approximately 200 Pg over the Holocene (Cole, 2013). In lake waters, DOM flocculation appears to be stimulated by light and mediated by the formation of organic particles (von Wachenfeldt et al. 2008; Helms et al. 2013). However, the underlying mechanisms of DOM self-assembly, subsequent sedimentation, and potential disaggregation remain poorly understood in lakes with relevant inputs of terrestrial humic compounds, which differ substantially from those in marine ecosystems, where acidic polysaccharides predominate (He et al. 2016; Liu et al. 2018). Moreover, Kerner et al. (2003) suggested that protozoa can directly uptake microparticles self-assembled from dissolved organic matter. This particle pathway could be more efficient than the microbial loop in incorporating carbon into higher trophic levels, as no carbon losses are associated with bacterial respiration. Indeed, several studies have demonstrated, using stable isotopes, the direct ingestion of allochthonous particulate organic carbon by zooplankton (Pulido-Villena et al. 2005; Cole et al. 2006).

We hypothesized that: (1) DOM polymers with high humic content coagulate and self-assemble spontaneously like acidic polysaccharides, (2) this self-assembling is mediated by cations and is reversible, and (3) specific cations (i.e., Ca^{2+} vs. Fe^{3+}) can differ in their self-assembling potential and stabilization sizes for the same DOM substrate. We performed two sets of experiments. First set to measure self-assembling and disaggregation by cation chelation in two northern temperate lakes with terrestrial inputs and high humic content. The second set to discriminate the DOM self-assembling potential of specific cations by additions of Ca^{2+} and Fe^{3+}. Here, we provide evidence that the DOM polymers self-assemble spontaneously into particles stabilized and interlocked by cationic bridges of calcium and iron. In addition, we explore the differential reversibility/stability in the formation of dissolved and particulate organic matter of these two cations. Calcium cations appear to promote a higher level of self-assembly, reaching larger hydrodynamic sizes during stabilization, compared to iron cations. Stabilization of particulate organic matter can have implications for the export of organic carbon to sediments and its long-term storage in lake sediments, as well as its role as food for zooplankton.

Methods

To perform the DOM self-assembly and disaggregation experiments, we took water from two lakes located at the University of Notre Dame Environmental Research Center in Michigan (USA): Paul (46.25° N, 89.50° W) and Hummingbird (46.24° N, 89.51° W) on August 29th and September 1st of 2006, respectively. These lakes, particularly Hummingbird, have high content of chromophoric (humic) dissolved organic matter and tend to be acidic to neutral, with low to moderate ionic strength (see more details of these lakes in Reche et al. 1999; de Vicente et al. 2010). We measured conductivity and pH at the time of sample collection. To determine fundamental variables, such as dissolved organic carbon (DOC) and cation concentrations, we collected epilimnetic water samples in clean bottles (see Fig. S1, supplementary material). For DOC analyses, we filtered lake water through pre-combusted Whatman GF/F filters (Cytiva, USA) and then poured it into pre-combusted 20 mL glass ampules. The DOC samples were acidified with phosphoric acid (final $pH < 2$), sealed, and stored at 4 °C in dark conditions until analysis, approximately two weeks later. We analyzed DOC using high-temperature catalytic oxidation in a Shimadzu Total Carbon Analyzer (TOC-5050, Shimadzu Corporation, Japan) with a laboratory precision of 0.1 mg/L. We quantified Ca, and Fe concentrations using inductively coupled plasma mass spectrometry (ICP-MS) analysis.

After collection, in a separate procedure, we performed pre-treatment on the GF/F filtered water for subsequent DOM self-assembly experiments (see Fig. S1, supplementary material). We gently filtered water samples through 0.22-μm polycarbonate membrane filters (pre-washed with Milli-Q water to minimize contamination) to remove all particles and microorganisms. In addition, to inhibit any potential biotic processes, we added sodium azide (0.02% final concentration). We send these samples to Friday Harbor Laboratory (University of Washington, WA, USA) in cold and dark conditions to perform the DOM self-assembly and disaggregation experiments during the following week.

DOM polymers self-assembly and disaggregation experiment

We re-filtered the water samples through 0.22 µm polycarbonate membrane filters (pre-washed with Milli-Q water or 0.1 mol/L hydrochloric acid) to establish experimental starting conditions (i.e., filtrates that contained only dissolved colloids and polymers). We monitored self-assembly kinetics by measuring changes in particle size, as determined by hydrodynamic diameter using dynamic light scattering (DLS) spectroscopy, as described in Chin et al. (1998). We used a Brookhaven BI-200SM laser spectrometer (Brookhaven Instruments, NY, USA), equipped with a 632.8 nm wavelength helium–neon laser, to monitor the spontaneous self-assembly of DOM polymers. Scattering fluctuations were detected at a 45-degree scattering angle at 20 °C. The autocorrelation function of the scattering intensity fluctuations was averaged over a 10 min sampling time using a Brookhaven BI 9000AT autocorrelator. Particle size distribution was calculated using the CONTIN algorithm (Provencher 1982). Calibration was conducted using monodisperse suspensions of standard latex microspheres ranging from 50 nm to 6 µm (Polysciences Inc., PA, USA). We present the means of these distributions, referred to as the hydrodynamic diameter, which is the apparent average size of a collection of dynamic, hydrated particles in the solution (Chin et al. 1998). We acquired measurements of the hydrodynamic diameter at the initial time, 12, 36, 56, 84, and 132 h for Hummingbird samples and at the initial time, 10, 19, 43, and 67 h for Paul samples. Then, we fitted the changes over time of the hydrodynamic diameter to the particle coagulation model of Smoluchowski (1916) described by the equation:

$$D_t = D_0(1 + At)^{1/3}$$

where D_t is the average hydrodynamic diameter at time t, D_0 is the initial average diameter, and A the apparent aggregation rate (more details in Supporting information).

To test whether DOM self-assembly to POM was a reversible process mediated by cations, we used chelation agents and then measured again the hydrodynamic size of the resulting polymers. We used ethylenediamine-tetra-acetic acid (EDTA) as a chelation agent for calcium and + 5 methyl amino methyl pyridinone as a chelation agent for iron, since it has been demonstrated that these compounds promote the dispersion of polymeric particles assembled by cations (Chin et al. 1998; Cilibrizzi et al. 2018). In two replicate vials, we added 10 mmol/L EDTA, and in the other two, + 5 methyl amino methyl pyridinone to the assembled DOM from Hummingbird and Paul lakes and monitored the changes in the hydrodynamic sizes of the polymers again. We measured hydrodynamic sizes with a Brookhaven BI-200SM laser, as explained above.

Data exploration procedures revealed that the differences in aggregate size before and after chelation under the different treatments did not meet the linear model assumptions. Therefore, a non-parametric factorial analysis (analogous to analysis of variance, ANOVA) based on aligned rank transformation was applied to determine the effect of chelation on the size of aggregates in the presence of different cations. Briefly, the method consists of aligning responses by removing estimated effects of other factors and interactions, followed by a rank transformation applied to these aligned responses. Subsequently, standard factorial ANOVA (F-tests) can be performed on the aligned ranks, preserving factorial designs and interactions while addressing violations of parametric assumptions such as normality or homogeneity of variances. The non-parametric analysis included the variable lake as a categorical variable. Non-parametric factorial models were fitted with the function art from the R-package ARTool (Kay et al. 2020; R Core Team 2021).

Colloidal organic matter and microgels (Hummingbird Lake) obtained by self-assembly were examined using a scanning electron microscope (SEM). One drop of sample solution was deposited on top of a glass slide and air-dried at room temperature in enclosed petri dishes before imaging (thereby minimizing any perturbation due to dehydration). Images were taken using a Hitachi S-3500N system equipped with an additional energy dispersive X-ray analyzer for elemental microanalysis (Oxford Instruments) model INCA-SIGHT.

Calcium and iron differential effects on the dissolved-particulate dynamics

To determine whether the stabilization of the polyanionic matrix of polymers was similar using calcium and iron, we carried out an additional experiment with water from Hummingbird Lake, which contains a higher concentration of DOM polymers and has a higher ionic strength than water from Paul Lake. We filtered the Hummingbird Lake water through 0.22 µm filters to separate dissolved colloids and molecules from the particulate fraction. Once the self-assembly of DOM polymers reached its maximum size, spontaneously forming particulate organic matter (phase 1), we added 6 mmol/L EDTA to induce POM disaggregation. Then, we monitored by duplicate the changes in the particle sizes under three different treatments (phase 2). The control treatment consisted of a set of vials with 6 mmol/L EDTA that chelated the major cations and inhibited polymerization. A second treatment consisted of adding 1 mmol/L calcium chloride ($CaCl_2$) to monitor polymer re-assembly due to calcium. The last treatment consisted of adding of 1 mmol/L ferric chloride ($FeCl_3$) to monitor re-assembly due to iron. We monitored average particle sizes for a total period of 300 h (phase 1 + phase 2). We performed 5 to 7 measurements at each time in two replicates for each treatment.

In this experiment, we determined the average particle sizes using a Malvern 4700 laser spectrometer (Malvern Instruments, UK) equipped with a 632.8 nm helium-neon– laser and a scattering angle set to 45°. Temperature (20 °C) was controlled using a Peltier cell and external bath acting through the sample cell. Aliquots of 5 ml were poured directly into the scattering cells and then positioned in the goniometer. The particle size distribution was calculated from the scattered intensities autocorrelation function using cumulant analysis (Koppel 1972). We calibrated the DLS spectrometer using standard suspensions of monodisperse latex microspheres 220 ± 6 nm (Duke Scientific Corp.).

We assessed the differences in the re-assembly capacity and stabilization sizes of polymers depending on cation (i.e., Ca vs. Fe) by fitting a linear analysis of covariance (ANCOVA), including the interaction between time and cation treatment as the primary predictor. Then, inter-treatment differences were further assessed with a Tukey post-hoc test. This parametric approach was appropriate here because model assumptions were met, and the continuous structure of the time variable justified the use of ANCOVA rather than a non-parametric alternative. We fitted the ANCOVA model and post-hoc test using the functions lm() and TukeyHSD() from the R-package Stats (R Core Team 2021).

Results

The DOC concentration was more than fourfold higher in Hummingbird Lake (18.2 mg/L) relative to Paul Lake (4.2 mg/L); whereas the ionic strength -measured as conductivity- was about twofold higher in Hummingbird (23.6 µS/cm) relative to Paul (13.6 µS/cm). During this study, both lakes were acidic with a pH of 3.8 in Hummingbird and 5.6 in Paul. Calcium concentration was 1.57 mg/L in Hummingbird and 1.07 mg/L in Lake Paul. Iron concentration was about fourfold higher in Hummingbird (0.55 mg/L) than in Paul (0.13 mg/L).

We observed that DOM polymers/colloids self-assembled spontaneously in both lakes, increasing their hydrodynamic diameters over time and reaching sizes sufficient to be considered particles (Fig. 1). The hydrodynamic average diameter (D_t) of the DOM from Hummingbird Lake with an initial diameter (D_0 in the coagulation model) of 0.65 µm increased to a maximum diameter of ca. 4.5 µm, a size clearly in the POM size range, and stabilization at 56 h (Fig. 1A). The apparent aggregation rate (A in the coagulation model) was 0.99/h, and the variation explained by the model (R^2) was 0.94. Similarly, we observed a significant increase in the hydrodynamic average diameter of the DOM from Paul Lake with an initial diameter (D_1) of 0.14 µm to a maximum diameter of ca. 2.9 µm and an stabilization at 19 h (Fig. 1B). The apparent aggregation rate (A) was 47.1/h, and the variation explained (R^2) by the coagulation model was 0.97. The assembly kinetics (i.e., apparent aggregation rates) and maximum hydrodynamic diameters differed for each lake. To check the reversibility of DOM assembly to POM and confirm that cations mediated the polymers aggregation, we added EDTA, which predominantly chelates calcium, and + 5 methyl amino methyl pyridinone, which predominantly chelates iron, to two different sets of replicates. These additions significantly reduced the hydrodynamic diameters in both lakes (Fig. 1C, D). In the water from Hummingbird (Fig. 1C), the POM hydrodynamic average diameter decreased from about 4 µm at the

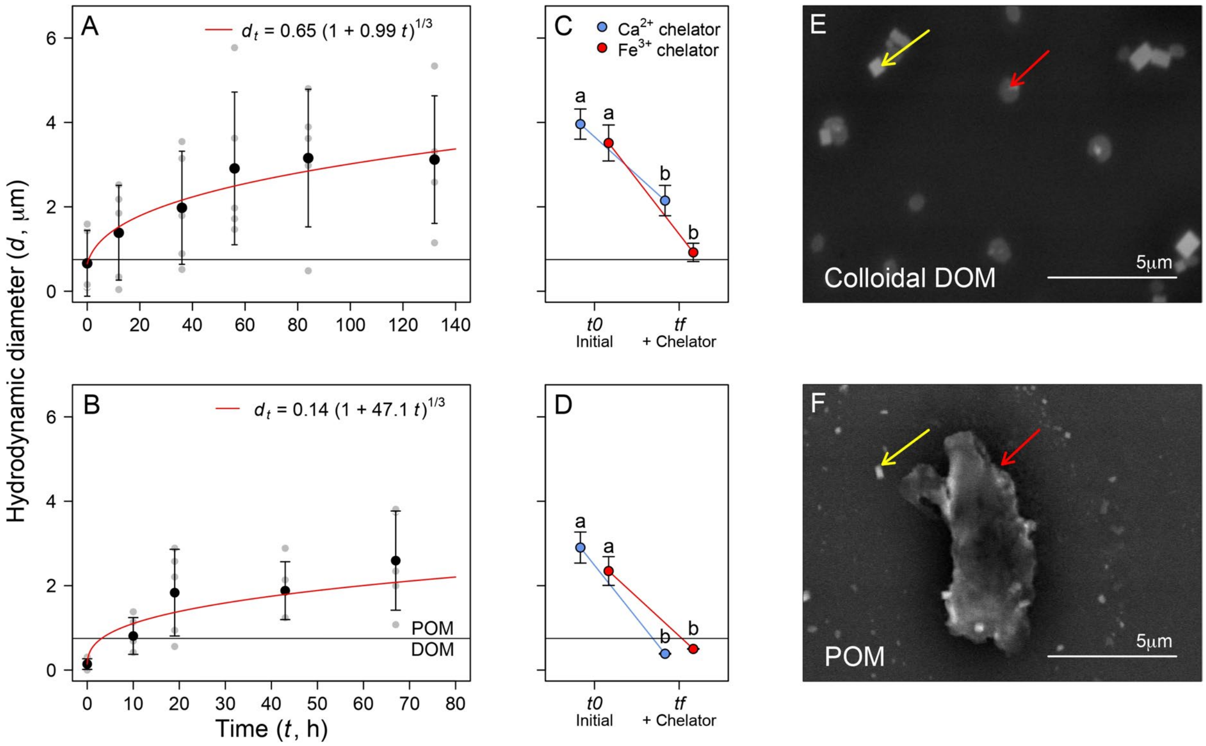

Fig. 1 Self-assembly kinetics of DOM polymers in **A** Hummingbird Lake and **B** Paul Lake. Each black dot is the average ± se (standard error) of five replicates (grey points). The red line is the fit to the semi-empirical coagulation model. Disaggregation changes in the hydrodynamic diameter of POM polymers after the addition of a Ca^{2+} (blue dots) or Fe^{3+} (orange dots) chelator in **C** Hummingbird Lake and **D** Paul Lake. Each error bar corresponds to the average ± se (standard error) of two replicates samples. Same letter (a or b) on the bars denotes no significant statistically differences and different letters (a and b) denote statistically significant differences. Scanning electron microscopy (SEM) images before DOM self-assembly (**E**) and after DOM self-assembly into POM (**F**). Red arrows mark DOM and POM and the yellow arrows mineral crystals. White line is a 5 μm scale

initial time to 1.9 μm and 0.9 μm after calcium or iron chelation. In the water from Paul (Fig. 1D), the POM hydrodynamic average diameter decreased from about 2.9 μm at the initial time to 0.39 μm and 0.50 μm after calcium and iron chelation, hydrodynamic diameters representative of the dissolved phase. Non-parametric factorial analysis (supplementary, Table S1) detected a significant reduction in the hydrodynamic diameter after chelation ($F_{1,128} = 18.22$, $p < 0.001$). The size reduction in the aggregates was not different after calcium or iron chelation ($F_{1,128} = 2.33$, $p = 0.13$). Images from the scanning electron microscopy before (Fig. 1E) and after self-assembly (Fig. 1F) depict these changes in colloidal and particle organic matter (red arrows) sizes. Inorganic crystals (indicated by yellow arrows) were also visible, albeit of varying sizes.

To determine whether the stabilization of the polyanionic matrix of polymers was similar with calcium and iron, we carried out an second experiment. In phase 1 of this experiment, DOM polymers and cations self-assembled to reach a sizeable hydrodynamic diameter larger than 8 μm. Then, in phase 2, after EDTA addition, the hydrodynamic diameter decreased to 3.2 μm (Fig. 2, black dots). We set up three treatments in this phase: control, Ca addition, and Fe addition. The control treatment contained EDTA, and DOM self-assembly was prevented over time, maintaining a size around 3 μm (Fig. 2, black dots). In the Ca^{2+} addition treatment (Fig. 2, blue dots), we observed an increase in the hydrodynamic diameter from ca. 3.2 μm to 5.4 μm compared to the control treatment. We also observed a slight increase after adding Fe^{3+} (Fig. 2, orange dots) from 3.2 μm

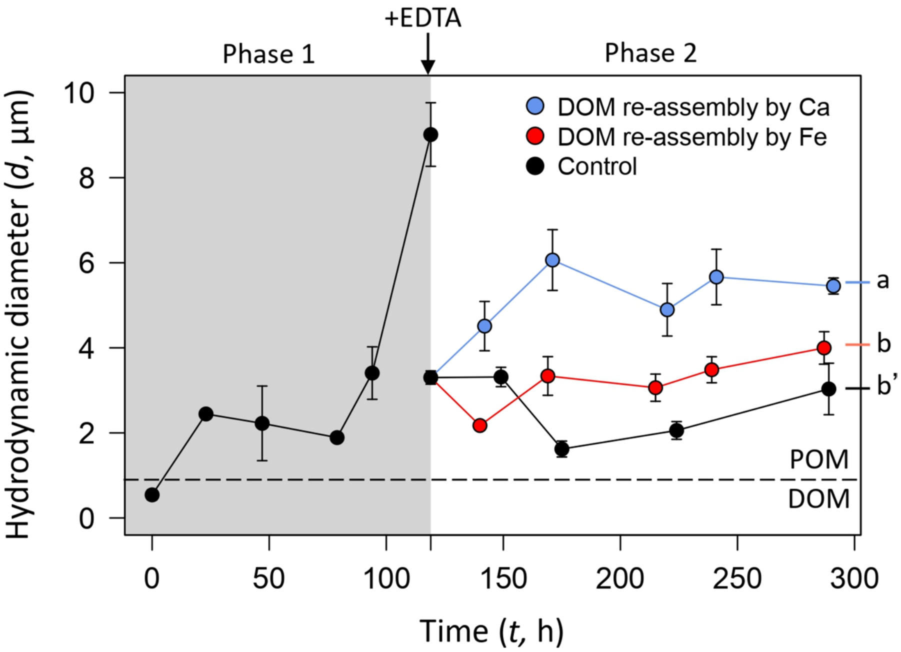

Fig. 2 Changes in the hydrodynamic diameter after DOM self-assembly to POM and POM disaggregation by addition of the chelator EDTA (phase 1, grey zone) and re-assembly (phase 2) by additions of $CaCl_2$ (blue dots) or $FeCl_3$ (orange dots). Black dots denote the control treatment with EDTA. Each point is the average ± se (standard error) of two replicates. Treatments where the responses were not significantly different after post-hoc analysis (P-value > 0.05) are indicated with the same letter in the panel

to 3.9 µm, although we did not detect differences compared to the control treatment. The results of the ANCOVA test indicated a differential response in the re-assembly capacity over time of the organic aggregates depending on the cation type ($F_{2,143} = 5.10$, $P = 0.007$). Post-hoc Tukey tests showed that aggregates re-assembled significantly faster in the presence of calcium than under control conditions (diff = −2514.7, 95% CI [−3026.8, −2002.6], $P < 0.001$), reaching larger hydrodynamic diameters. In contrast, the iron treatment differed only marginally from the control (diff = 494.3, 95% CI [−17.8, 1006.4], $P = 0.061$).

Discussion

We demonstrated that DOM polymers and/or colloids from the study lakes self-assemble into particles of a larger size, fitting the semi-empirical adaptation of Smoluchowski coagulation model (Smoluchowski 1916). Calcium and iron cations mediated this partially reversible process. The rate of self-assembly (A value) differed in each lake, likely depending on DOM ligands (humic substances vs. acidic polysaccharides) and cations dissolved in the water. We observed that re-assembling after the dissociation of the polymer networks also differed depending on the cations involved (i.e., Ca^{2+} vs. Fe^{3+}). These results emphasize the importance of understanding the biophysical mechanisms underlying DOM coagulation in lakes and the drivers involved in DOM self-assembly, stabilization, and POM disaggregation to determine their final fate.

In seawater, Chin et al. (1998) reported DOM polymer self-assembly reaching particle sizes up to 4 µm. They demonstrated that the causal mechanism was mediated by the electrostatic interactions among calcium cations and negatively charged DOM polymers. In marine environments, this process is primarily associated with the aggregation of polysaccharides to form transparent exopolymeric particles with gel-like features, which enhances carbon export toward the seafloor (Passow et al. 2001; Engel et al. 2004; Guo et al. 2020). In contrast, Kerner et al. (2003) found that calcium was not involved in the formation of microparticles, which were smaller than the oceanic particles, with a size range of 0.4 to 0.8 µm. Using phytoplankton exopolymeric substances, Ding et al. (2009) obtained similar results, proposing that hydrophobic interactions were the mechanism for DOM coagulation in the absence of calcium intervention. In contrast, de Vicente et al. (2010) and Martínez-García et al. (2024) found inverse relationships between dissolved divalent cations (Ca^{2+} and Mg^{2+}) and acidic polysaccharides in Mediterranean lakes and reservoirs, suggesting the sequestration of these cations during the particle formation. Indeed, in Fig. 1E, we can appreciate minerals likely acting as internal nucleation points for DOM coagulation and self-assembly. On the other hand, von Wachenfeldt et al. (2008) found indirect evidence that DOM flocculation in humic lakes was mediated by dissolved iron and determined experimentally that POM formation in the water was related to the loss of dissolved total iron and DOC. Reiche et al. (2011) also found the formation of iron-rich particles in an acidic mine lake with high sinking velocity acting as carriers of organic carbon, microorganisms, and iron to sediments. In our study, we found a direct role of both calcium and iron in forming particles with sizes similar to those obtained in marine environments (Fig. 1A, B). The particle size reached in Hummingbird Lake was larger than in Paul Lake, likely due to its higher concentration of DOC and greater ionic strength, which is particularly relevant in the case of dissolved iron concentration. Hummingbird Lake had a higher composition of allochthonous compounds of humic nature than Paul Lake (Reche et al. 1999; de Vicente et al. 2010). Flocculation of allochthonous DOM in boreal lakes was demonstrated to be an important process by von Wachenfeldt et al. (2008) and related to dissolved iron. Similarly, Xu and Guo (2018) demonstrated that the humic and fulvic fractions of DOM significantly coagulated through abiotic self-assembly, although they did not propose an underlying mechanism. Therefore, the polysaccharide or humic nature of DOM polymers can also affect the coagulation mechanism to generate particles.

Our experiment also indicates that DOM polymer stabilization and interlocking by cationic bridges were reversible. The evidence for reversibility is based on cation chelation leading to POM disaggregation to typical DOM sizes in our experiments (Fig. 1C, D). These results are similar to those found previously by Chin et al. (1998) in seawater. On the other hand, Baalaousha et al. (2006) and Pace et al. (2012) demonstrated that the water pH and ionic strength strongly influence the size of the polymeric aggregates and conformational structure. Under high ionic strength, the electrostatic interaction reduces enough to bring the polymers within short distances, thus establishing bridging points among them (intra and intermolecular bridges). This situation results in highly stable microaggregates (Tiller and O'Melia 1993). However, at lower ionic strength, the intense repulsive electrostatic interactions promote disaggregation of polymers. Under this scenario, (non-electrostatic) steric interactions could be predominant, causing DOM self-assembly (Tiller and O'Melia 1993). However, this process could also be due to the presence of high molecular weight molecules with lower charge density or more hydrophobic structures (Ding et al. 2008; 2009; Tiller & O'Melia 1993). Baalousha et al. (2006) suggest that a higher or smaller number of negative charges of the DOM biopolymers can be neutralized depending on the cation type (e.g., Ca^{2+}, Na^{+}). In general, higher valence cations produce a different effect in aggregate size and conformation and, therefore, promote a higher rate of coagulation (Mulholland 1981). However, we did not observe a significantly higher coagulation rate mediated by iron than calcium (Fig. 2, phase 2). In fact, re-assembly by calcium occurred at a significantly higher rate than iron ($F1,143 = 5.10$, $P = 0.007$). This discrepancy could be related to the pH value of Hummingbird Lake, which is usually about 3.8–4.8, and the iron species likely was not Fe^{3+}. Maranger and Pullin (2002) showed that a pH variation promotes a change in the dominant iron species from Fe^{3+} at pH values lower than 2 to ferrous hydroxide ($Fe\ (OH)_2{}^{+}$) at pH values higher than 2, which affects the iron complexation. Therefore, the characteristics of DOM polymers (acidic polysaccharides vs. humic acids) and the magnitude of the ionic strength of the solvent (cation charge) can affect aggregation-disaggregation dynamics. Other studies (Mayer et al. 2006; Kieber et al. 2006; Pisani et al. 2011; Schiebel et al. 2015) have demonstrated the POM and detritus disaggregation mediated by sunlight. However, this mechanism is not plausible in our experiments since they were performed in dark conditions. Changes in pH can also regulate the aggregation-disaggregation of dissolved organic matter from sediments (Reiche et al. 2011).

Regardless, if DOM coagulation is driven by terrestrial, humic acids (von Wachenfeldt et al. 2008; Xu and Guo 2018) or acidic polysaccharides (de Vicente et al. 2009; Martínez-García et al. 2024), the stabilization of POM-sized particles with calcium and iron can increase carbon export to sediments contributing to the long-term carbon storage in lakes and reservoirs (Fig. 3). DOM flocculation mediated by iron in stable aggregates (i.e., mineralization in Fig. 3) could also promote iron limitation in lakes. Vrede

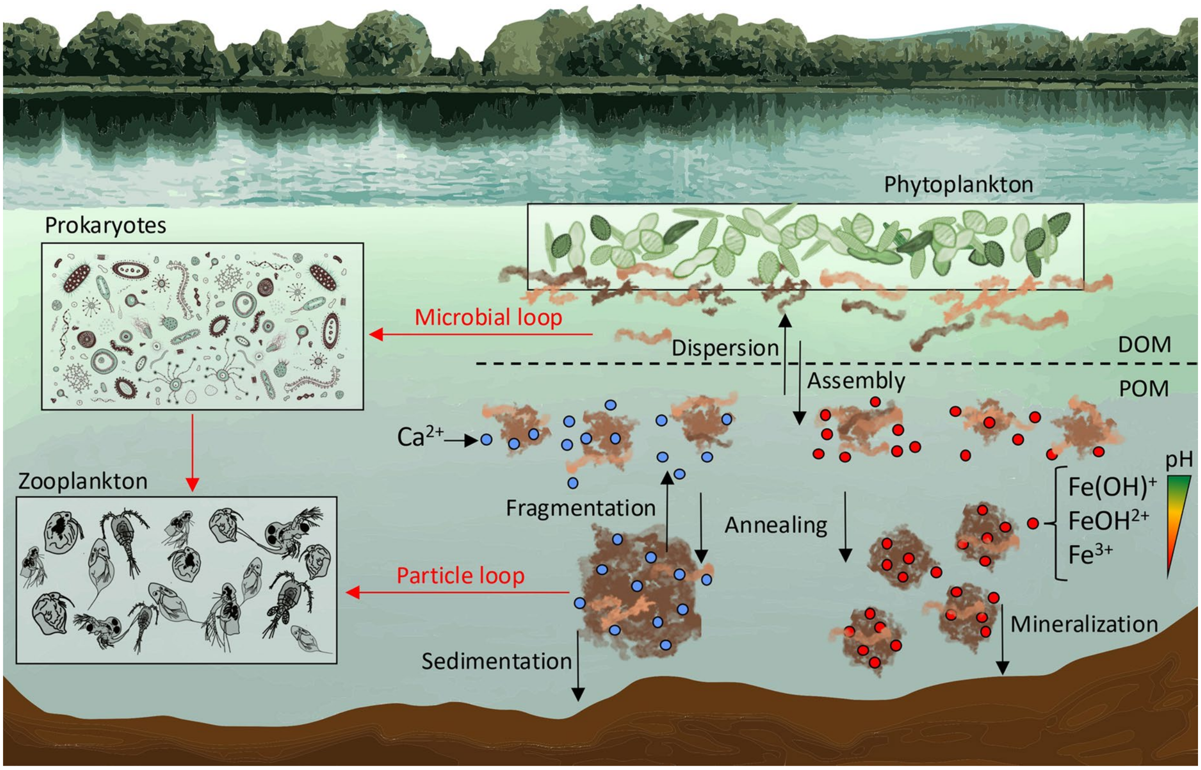

Fig. 3 Conceptual diagram of the relevance of the dissolved organic matter (DOM) self-assembly in particulate organic matter (POM) in aquatic ecosystems. Dissolved organic matter released by phytoplankton (i.e., acidic polysaccharides) or of terrestrial origin (humic and fulvic acids) can coagulate to form particles by the intervention of cations such as calcium and iron. These particles can be stored for a long time in the sediments affecting the global carbon cycle or be directly consumed by protozoa and zooplankton, forming the particulate loop that could be more efficient than the microbial loop

and Tranvik (2006) documented the iron limitation of phytoplankton growth in oligotrophic Swedish lakes. On the other hand, these particles can be consumed directly by protozoan and zooplankton species depending on particle sizes contributing to aquatic foodwebs (Passow and Alldredge 1999; Kerner et al. 2003; Ling and Alldredge 2003; Pulido-Villena et al. 2005; Cole et al. 2006; Toullec et al. 2019). This particle loop (Fig. 3) appears to be energetically more efficient than the microbial loop since, where an intermedia step through bacteria involves C losses by respiration (Kerner et al. 2003). Therefore, future studies on the specific effects of diverse cations on DOM polymer coagulation and POM stabilization, as well as its reversibility, can shed light on the role of these processes in long-term carbon and trace metal sequestration in aquatic ecosystems. Moreover, POM stabilization with different cations will affect its role in carbon transfers in aquatic food webs depending on POM qualitative properties such as stoichiometry (C, N, P ratios), stabilizing elements, and microorganism content.

Conclusions

The experiments performed confirmed that, in freshwater ecosystems with a predominance of terrestrial inputs and humic substances, DOM spontaneously self-assembles to form POM. These results are significant because most previous studies have focused on the ocean, with a predominance of acidic polysaccharides of autochthonous origin (phytoplankton and bacterioplankton) that form transparent exopolymeric particles. Both dissolved calcium and iron cations promote and stabilize the particles, which reach different sizes depending on the initial amount of DOM and available cations.

This self-assembly process is reversible. The particles return to the dissolved phase if the cations are chelated. The addition of Ca^{2+} to disaggregated DOM induced POM formation with significantly larger hydrodynamic size than the Fe^{3+} addition during stabilization.

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Data availability The datasets generated during the current study are available in the supplementary material.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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