

## Linking allochthonous dissolved organic matter and boreal lake sediment carbon sequestration: The role of light-mediated flocculation

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### *Abstract*

We measured flocculation of dissolved organic carbon (DOC) in the water from a humic lake (DOC = 14.9 mg C L<sup>-1</sup>) and from an adjacent mire (DOC = 25.7 mg C L<sup>-1</sup>), in in situ enclosure experiments with different light regimes. Light stimulated the formation of organic particles in both waters, and organic particle formation was observed at all incubation depths, even in the dark controls. Production of phytoplankton biomass was negligible, and allochthonous DOC was the most important precursor of the sinking particles. 8–22% and 25–60% of the loss of DOC in lake and mire water, respectively, could be accounted for by flocculation. Depth-integrated flocculation based on the enclosure experiments was 14.7 mg C m<sup>-2</sup> d<sup>-1</sup>. Lake-water DOC concentration and water color has been increasing during the last decade, and sediment trap studies show that gross sedimentation of organic carbon also increased. Thus flocculation of allochthonous DOC, stimulated by light, constitutes a pathway for the sequestration of carbon in lake sediments.

Inland waters receive large amounts of organic carbon from their watersheds, but only about half of the organic carbon reaches the sea (Algesten et al. 2003; Cole et al. 2007). Most of the loss occurs in lakes (Algesten et al. 2003; Cole et al. 2007), primarily because of mineralization into CO<sub>2</sub> followed by evasion to the atmosphere (Kling et al. 1991; Cole et al. 1994) and retention in sediments (Molot and Dillon 1997). Lake sediments are a considerable sink of organic carbon in boreal ecosystems. A study in boreal Finland suggests that carbon sequestration at the landscape level is greater in lake sediments than in forests and terrestrial soils (Kortelainen et al. 2004). Dissolved organic carbon (DOC) is the dominating fraction of organic carbon in most lake waters (Wetzel 2001), and in the unproductive forest lakes with high loads of allochthonous organic carbon typical of the boreal zone, particulate organic carbon (POC) generally constitutes less than 3–10% of the total carbon (Wetzel 2001; Kortelainen et al. 2006). Because most of the organic carbon is dissolved, transformation of DOC into a gravitoidal state, i.e., coagulation

and flocculation into sinking POC, appears to be an important prerequisite for allochthonous carbon sequestration in lake sediments. However, the extent and mechanisms of such transformation processes are currently not well understood.

Solar radiation, especially in the ultraviolet (UV) range, has a multitude of effects on the organic matter in aquatic systems, such as changes in structure, molecular weight, and optical properties (Bertilsson and Tranvik 2000). Solar radiation induces cleavage of high-molecular-weight DOC into a variety of photoproducts and inorganic carbon (Mopper et al. 1991; Moran and Zepp 1997; Bertilsson and Tranvik 2000), and increasing bioavailability of dissolved organic matter (DOM) to bacteria (Lindell et al. 1995; Wetzel et al. 1995). It has also been indicated that DOC can be transformed to particles by photochemical reactions. Irradiation of DOC has been reported to stimulate particle formation, involving iron as catalyst (Gao and Zepp 1998). Similarly, intense irradiation of humic water cleaved humic molecules into fragments, but also within hours resulted in a precipitate of brownish particles (Backlund 1992). Based on these observations we hypothesize that light-mediated flocculation of DOC into POC relocates organic carbon from the water column to the sediments.

We conducted enclosure experiments and sediment trap campaigns, demonstrating flocculation of DOC into POC under natural conditions, and showing that sunlight plays an important role in the transformation from a dissolved or

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colloidal state to macroscopic gravitoidal particles. We used enclosure experiments to decipher the effect of sunlight on DOC flocculation. Water from a humic lake and from an adjacent mire was used to unravel whether flocculation is greater for the interstitial mire DOC, which had previously not been exposed to sunlight. Sediment traps were deployed in the lake to gauge the carbon sedimentation in the lake for comparison to light-mediated flocculation.

## Methods

*Enclosure experiments*—Lake water from Skärshultsjön in southern Sweden (57°16'N, 14°52'E) and the drainage from an adjacent *Sphagnum*-dominated mire (57°11'N, 14°48'E) that constitutes a major inlet to the lake were used in the experiments. Both the lake and the mire were sampled 23 June 2003. Skärshultsjön is a small (area: 0.26 km<sup>2</sup>; maximum depth: 14 m; mean depth: 3.6 m) oligotrophic lake dominated by allochthonous humic DOC, with DOC concentrations typically between 16.8 and 21.5 mg C L<sup>-1</sup> (Algesten et al. 2005; von Wachenfeldt and Tranvik 2008) in recent years. Lake water was sampled at the surface (0–0.5 m) and mire water was collected at the mire outlet, directly where the mire forms a small stream. Water was kept dark at 10°C in polyethylene cubitainers until further processing.

Prior to experiment start, the water was sieved through a plankton net (200 µm) followed by filtration through a polypropylene filter cartridge (5 µm) using a submersible pump resulting in the removal of most of the phytoplankton and other gravitoidal particles. Subsequently, gastight enclosures (0.45 × 0.45 × 0.1 m, length × width × height) made of UV-transparent Plexiglas (Ršhm GS 2458, transmittance is >90% at all wavelengths >300 nm [Denward and Tranvik 1998]) were completely filled with filtered lake or mire water. The enclosures were sealed with butyl rubber stoppers equipped with gastight valves. The enclosures were exposed to natural sunlight in a lake depth gradient that simulates the extinction of light in the upper water column (Langenheder et al. 2006). To achieve this, the enclosures were vertically suspended in the center of an adjacent clear-water lake (Klintsjön [57°13'N, 14°70'E]; absorbance at 430 nm = 0.001 cm<sup>-1</sup>). The enclosures were attached to ropes connected to a floating frame and anchors in the sediment.

The lower light attenuation in Klintsjön enabled us to position the enclosures in a smooth depth gradient of light extinction and shifts in spectral properties typical of lakes in the area. The enclosures were incubated along a depth gradient of 0, 0.25, 0.5, 1.0, 2.0, and 2.5 m, where the 2.5 m enclosure served as a dark control and was covered with aluminum and black polyethylene bags. Attenuation of light at 2 m represents the attenuation of the upper 0.25 m and 0.10 m of the humic lake water and the mire water, respectively. Depth attenuation of light is reported in Langenheder et al. (2006). Accumulated incident total solar radiation during the incubations was 88,157 kJ m<sup>-2</sup> (18,641 kJ m<sup>-2</sup> d<sup>-1</sup>) for the lake and 105,030 kJ m<sup>-2</sup> (17,505 kJ m<sup>-2</sup> d<sup>-1</sup>) for the mire enclosures (data from

Swedish Meteorological and Hydrological Institute, weather station at Växjö airport, 25 km from the lake). Integrated UV-A radiation during the incubation, assumed to constitute 5% of the total radiation, was 4408 and 5033 kJ m<sup>-2</sup> for lake and mire respectively (Langenheder et al. 2006). Lake-water and mire-water enclosures were incubated (starting on 24 June 2003) for 113.5 and 144 h, respectively. Samples were taken initially and at the end of the experiment.

Aliquots of sample (250–460 mL) were filtered on precombusted glass fiber filters (Whatman GF/C) for determination of suspended POC at the start of the experiment. Samples for total organic carbon (TOC) and DOC were stored in the dark at 4°C in sterile 14-mL polypropylene vials. DOC samples were filtered (diameter 25 mm, 0.2 µm, Gelman Supor) using acid-rinsed equipment (Norrman 1993). TOC and DOC were measured by combustion on a Shimadzu TOC-5000 total organic analyzer after acidification with HCl and purging for evasion of inorganic carbon. Instrument error was <2% error with triplicate injections. Samples for optical characteristics (absorbance and fluorescence) were filtered (Whatman GF/C) and stored for less than 3 weeks in the dark in glass scintillation vials (4°C) prior to analysis.

Samples for optical characterization were room-tempered (20°C) and absorbance was measured on a Perkin Elmer Lambda 40 UV-VIS spectrophotometer corrected against Milli-Q water. Absorbance at 250 nm is more sensitive for detection of small changes in colored allochthonous DOC compared to bulk DOC measured on the Shimadzu TOC analyzer. Thus, we report data for both A<sub>250</sub> and DOC. Fluorescence was measured on a scanning spectrofluorometer (FluoroMax 2, Jobin-Yvon, excitation 220–445 nm, emission 230–600 nm) with integration time of 1 s and slit widths of 5 nm. The instrument was calibrated daily against Milli-Q Raman area (excitation 350 nm, emission 380–420 nm) and corrected for Rayleigh scattering, Raman scattering, lamp spectral properties (McKnight et al. 2001), and inner filter effects (Mobed et al. 1996; McKnight et al. 2001). The fluorescence index, defined as the ratio of the emission of 450:500 nm at excitation at 370 nm, was calculated. A low fluorescence index is associated with allochthonous organic matter sources and a high index with autochthonous sources (McKnight et al. 2001). The fluorescence index ratio might vary somewhat between different instruments. Thus, the absolute fluorescence index values may not be directly comparable between studies.

For analysis of chlorophyll *a* (Chl *a*), water samples (50–125 mL) were filtered onto 25-mm precombusted glass fiber filters. Chl *a* in the retained particles was extracted with ethanol (95%, room temperature overnight in the dark) and the extract was measured on a spectrophotometer at 665 and 770 nm with correction for pheophytin (Jespersen and Christoffersen 1987). pH was measured using a standard electrode (PW9420, Philips). Oxygen concentrations were measured with an oxygen electrode (Delta Handy Oxyguard).

In order to concentrate the flocculated matter we applied the following procedure: First, flocs were allowed to settle

Table 1. Formation of POC at the end of the experiment and initial and final values of DOC, Chl *a*, O<sub>2</sub>, and pH for lake and mire water in enclosures positioned along a solar gradient (0–2 m and dark control) in a clear-water lake. ±, standard deviation; nd, no data available.

	Lake					Mire				
	POC (mg C L <sup>-1</sup> )	DOC (mg C L <sup>-1</sup> )	Chl <i>a</i> (µg L <sup>-1</sup> )	O <sub>2</sub> (%)	pH	POC (mg C L <sup>-1</sup> )	DOC (mg C L <sup>-1</sup> )	Chl <i>a</i> (µg L <sup>-1</sup> )	O <sub>2</sub> (%)	pH
Initial ( <i>n</i> =6)	0	14.9 ± 0.4	5.5±3.8	77±5	5.5	nd	25.7±1.9	1.2±1.4	78±5	4.3
0 m	0.38	12.7	3.0	80	5.7	1.59	20.2	0	23	4.5
0.25 m	0.25	12.8	4.4	98	5.7	1.36	21.5	0	47	4.4
0.5 m	0.33	13.4	0.0	92	5.6	0.72	22.8	0	57	4.3
1 m	0.26	13.0	4.4	90	5.5	1.01	24.0	0	61	4.3
2 m	0.26	12.9	13.2	90	5.6	0.78	24.0	4.7	64	4.2
Dark	0.15	13.1	0.5	75	5.3	0.38	25.6	nd	50	4.3

in the enclosures for 3 h before further processing. Then, supernatant water was carefully siphoned off, and aliquots of water (220–730 mL) were filtered (Whatman GF/C) for determination of suspended POC at the end of the experiment. Flocculated matter was carefully transferred together with filtered lake water to 2-liter polycarbonate bottles, where the POC was allowed to settle for another 3 h. Next, the supernatant water was again cautiously removed in order to reduce the water volume prior to filtration of the flocculated matter. Finally, the flocculated matter of each enclosure was filtered onto several parallel filters (Whatman GF/C) using a filter manifold with 10 funnels. The filters for both suspended organic carbon (OC) and flocculated matter were stored at –20°C. Filters were then freeze-dried and kept in a desiccator before weighing, and were stored at –20°C until analysis of organic carbon and nitrogen (NA 1500, Carlo Erba Instruments). Flocculated matter was corrected for changes in suspended POC initially and at the end of the experiment to assure monitoring of newly formed particles by flocculation. Based on this correction, we report the new formation of POC during the experiment (Table 1).

To estimate the areal depth-integrated POC formation in the epilimnion, we plotted the flocculation rates against incubation depths and performed a triangular integration of the area under the graph. We used the value for the dark enclosure to integrate from 2.2 m to the average depth of the lake (3.6 m).

Samples for analysis of total iron in the water were filtered (precombusted Whatman GF/C), preserved with concentrated HNO<sub>3</sub> (final concentration 1%), and stored in acid-washed polypropylene bottles in the dark at 4°C. Total iron was measured on an inductive coupled plasma mass spectrometer (J&N Chemistry).

*Sediment traps*—To assess the in situ sedimentation rate, sediment traps were deployed in the profundal zone of the humic lake Skärshultsjön at the same location (with a lake-water depth of 13 m) for all years (1994 and 2003–2005). The trap used in 1994 and 2003 consisted of tube holders, carrying two glass tubes each (diameter 40 mm, height 190 mm), attached to a rope anchored in the sediment and to a buoy attached 1 m under the water surface. This kept the holders in a vertical position at a depth of 4 m. In 2004

and 2005 a similar sediment trap design was used with a holder for six polycarbonate tubes (diameter 46 mm, height 400 mm) where three tubes were used for estimation of settled organic carbon.

Sediment traps were deployed for 7 d in summer 2003 (26 June–03 July). In 2004 (15 July–05 August) and 2005 (07 May–28 May) the traps were deployed for a 21-d period. In 1994 sedimentation was monitored throughout the stratified period of the lake, from 17 May to 26 September. Traps were harvested with 13–35-d intervals and epilimnion depth varied from 3.50 to 5.25 m. There was no visible inflow of surface water into the lake during the study period in 1994.

Settled matter was filtered onto preweighed precombusted glass fiber filters (Whatman GF/C), which were processed for determination of POC as described earlier for flocculated matter. Lake-water samples for DOC and POC were treated as described for enclosure samples. Analysis was similar in all years, except that POC in 1994 was determined on a LECO CHNS 932 elemental analyzer (Leco), and DOC samples were kept in precombusted 40-mL glass vials with Teflon-lined screw caps prior to analysis.

## Results

*Enclosure experiment*—During the enclosure experiment, particles were formed at all depths in both lake and mire water, and the extent of particle formation decreased with decreasing photosynthetically active radiation (PAR) (Fig. 1). Likewise, DOC concentration in both lake and mire water decreased compared with initial concentrations, and the decrease in DOC was greater in superficial enclosures than in deeper enclosures (Table 1). Based on our measurements, 8–22% and 25–60% of the decrease in DOC could be accounted for by POC formation for the lake and mire, respectively. The absorbance at 250 nm (A<sub>250</sub>), which was used as a measure of allochthonous colored organic matter (Tranvik in press), declined along with the concentration of DOC in the enclosures. Absorbance can be measured with greater precision than DOC concentration, facilitating detection of small differences. Because humic allochthonous compounds dominate the DOC, we focus on A<sub>250</sub> rather than DOC in the following.

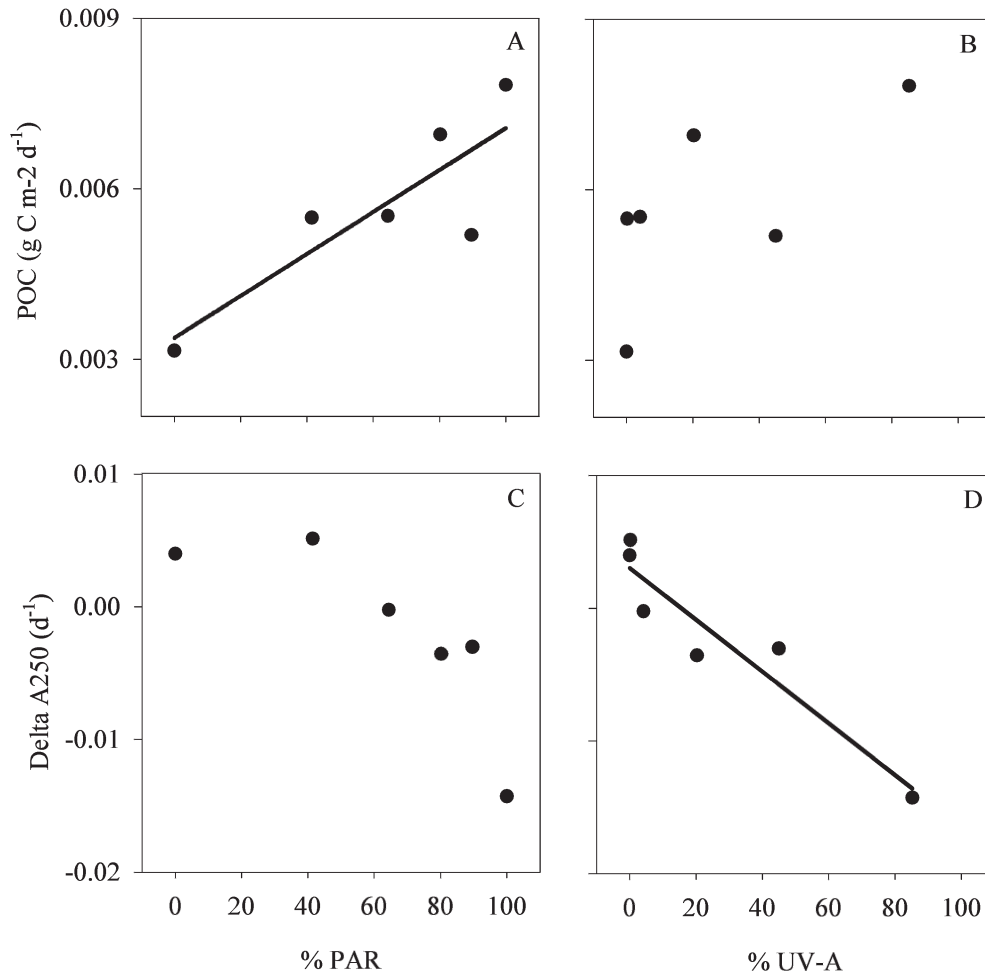


Fig. 1. Relationship of particle formation and loss of absorbance ( $\Delta A_{250}$ ,  $\text{cm}^{-1}$ ) for lake water with light (PAR and UV-A). Significant ( $p < 0.05$ ) linear relationships are indicated by slope lines and  $R^2$  is presented in Table 2.

POC formation decreased with increasing incubation depth as the incident solar radiation was attenuated, whereas humic absorbance showed an opposite pattern (Figs. 1, 2; Table 2). In the mire water, the changes in both A250 and POC were significantly related to the attenuation of both PAR and UV-A (Fig. 2; Table 2), but in the lake water only linear regressions between PAR and POC and between A250 and UV-A were significant (Fig. 1; Table 2). Because PAR attenuation was a significant predictor of the depth distribution of particle formation in both lake and mire water, the effect of sunlight will in the following be demonstrated in terms of % PAR, not % UV-A. The specific absorbance ( $A_{250} : \text{DOC}$ ,  $\text{mg C}^{-1} \text{cm}^2$ ) for the lake was initially  $54 \pm 1$  and at the end of the experiment 58, 61, 59, 62, 64, and 64, for enclosures at 0-, 0.25-, 0.5-, 1.0-, 2.0-, and 2.5-m depth, respectively. The specific absorbance for the mire was initially  $54 \pm 4$  and at the end of the experiment 58, 58, 56, 54, 56, and 53 for enclosures at 0-, 0.25-, 0.5-, 1.0-, 2.0-, and 2.5-m depth, respectively.

As derived from the slopes of the regression of % PAR and POC formation (Figs. 1, 2), the effect of PAR on particle formation was five times stronger in mire water

( $\text{POC} = 20.0 \times 10^{-5} \times \% \text{ PAR} + 0.0054$ ) than in lake water ( $\text{POC} = 3.7 \times 10^{-5} \times \% \text{ PAR} + 0.0034$ ). Loss of A250 from lake and mire water was related to POC formation (Fig. 3). This pattern was consistent for both lake and mire and was statistically significant for mire (Fig. 3B).

The fluorescence index indicates an allochthonous origin of both the lake-water and mire-water DOC, i.e., close to the value (1.4) suggested to represent the terrestrial end member of DOC (McKnight et al. 2001). In the lake water it decreased during the incubation from an initial value of  $1.30 \pm 0.02$  to a final value of  $1.22 \pm 0.02$  and there was no pattern of loss in relation to light attenuation. For the mire the index remained at  $1.19 \pm 0.02$ , with no change during the experiment.

Phytoplankton biomass, as reflected by Chl *a* concentration in the water (Table 1), was not related to light and POC formation. Hence, phytoplankton did not appear to be an important component of the POC formed during the experiment. Oxygen saturation was generally high in lake water and lower in mire water at the end of the incubation with no clear correspondence to POC formation, and pH was stable throughout the experiment (Table 1).

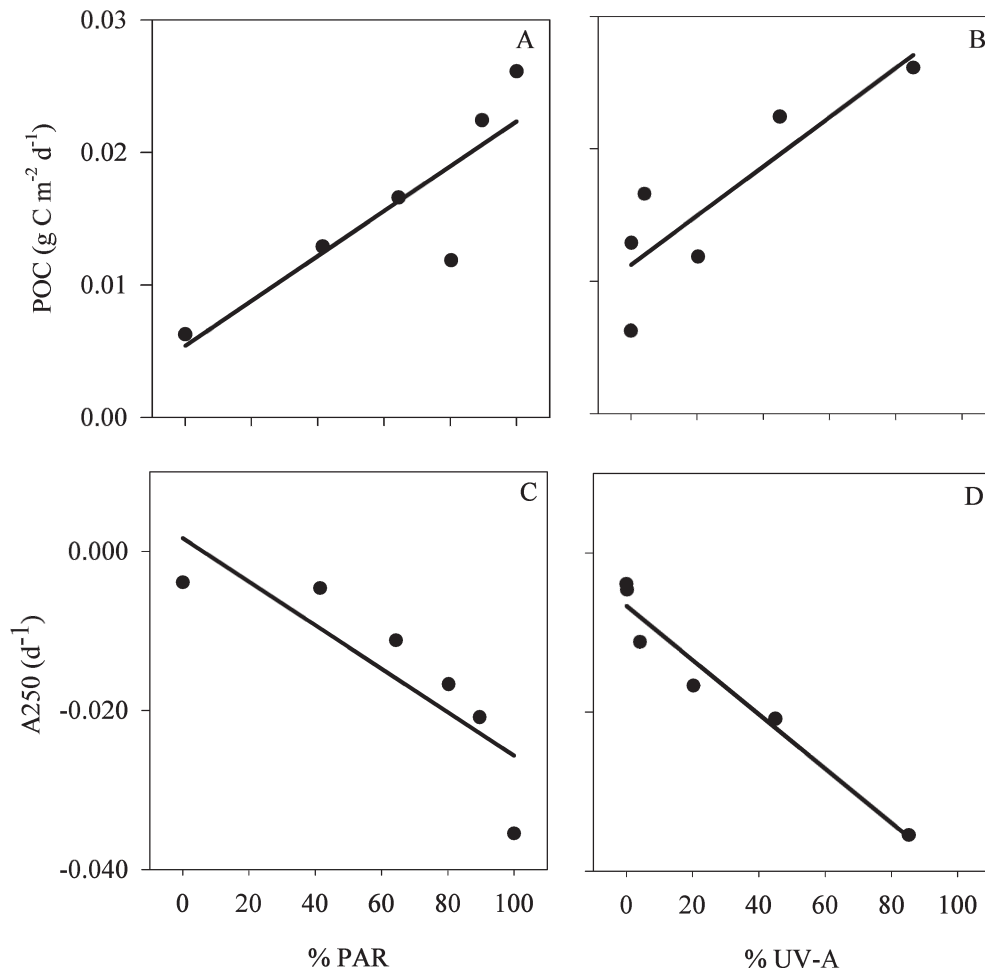


Fig. 2. Relationship of particle formation and loss of absorbance ( $\Delta A_{250}$ ,  $\text{cm}^{-1}$ ) for mire water with light (PAR and UV-A). Significant ( $p < 0.05$ ) linear relationships are indicated by slope lines and  $R^2$  is presented in Table 2.

Because of storage overnight before filtration, the temperatures at the start of the incubation were  $16.6^\circ\text{C} \pm 0.2^\circ\text{C}$  in lake and  $13.8^\circ\text{C} \pm 0.3^\circ\text{C}$  in mire enclosures. Water temperatures at the end of the experiment were  $20.0^\circ\text{C} \pm 0.6^\circ\text{C}$  and  $20.1^\circ\text{C} \pm 0.5^\circ\text{C}$  for lake and mire enclosures, respectively. Water temperature for the surrounding water (Klintsjön epilimnion) ranged from  $17.6^\circ\text{C}$  to  $19.1^\circ\text{C}$ , with

uniform temperatures throughout the experiment at all depths.

We observed a loss of dissolved total iron from the incubated lake and mire waters, which decreased with increasing incubation depth. Hence, the loss of iron from solution was related to organic particle formation in both lake and mire water (Fig. 4A,B). The concentration of

Table 2. Linear regressions ( $R^2$ ) for formation of POC ( $\text{mg C m}^{-2} \text{d}^{-1}$ ), % PAR, % UV-A, and change in absorbance at 250 nm ( $\Delta A_{250}$ ,  $\text{d}^{-1}$ ). na, not analyzed.

	Lake			Mire		
	POC	PAR	UV-A	POC	PAR	UV-A
POC	1	0.72*	0.46	1	0.74*	0.75*
PAR	0.72*	1	na	0.74*	1	na
UV-A	0.46	na	1	0.75*	na	1
$\Delta A_{250}$	0.63	0.65	0.89**	0.76**	0.73*	0.96***

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .



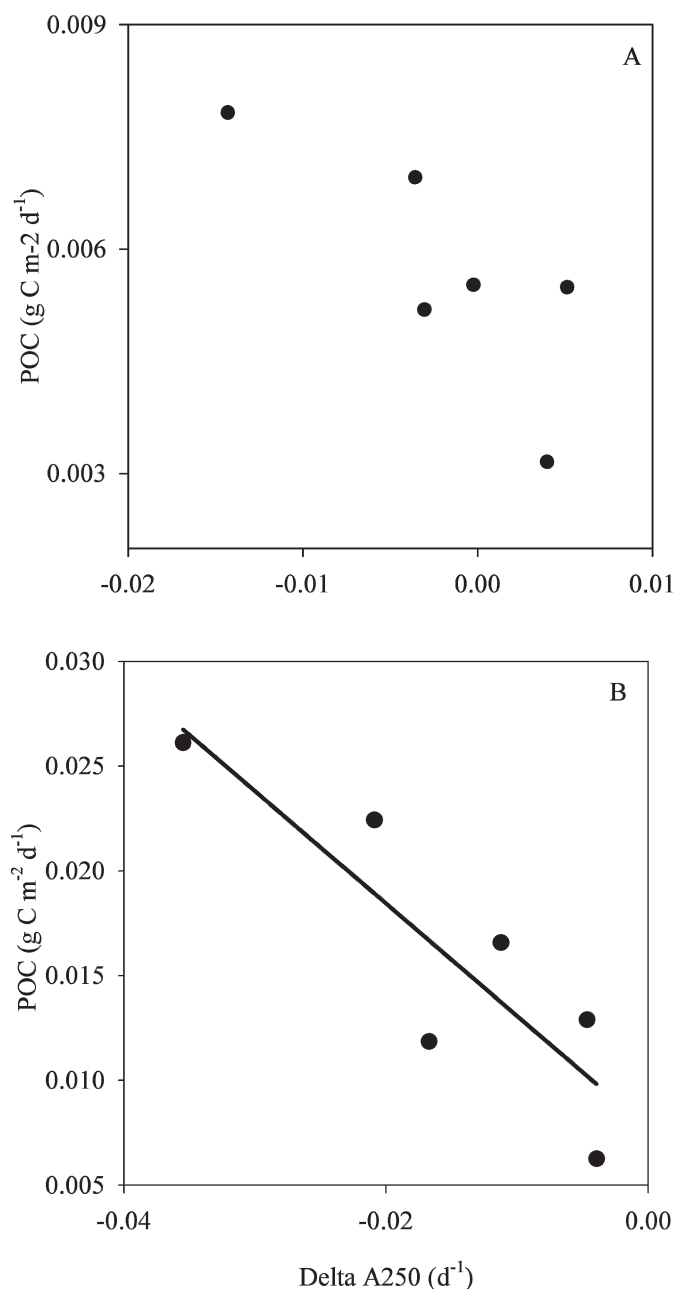


Fig. 3. (A) Relationship between the formation of POC and the change in absorbance at 250 nm ( $\Delta A_{250}$ ,  $\text{cm}^{-1}$ ) in the lake water. The linear regression with the presented data was not significant using the alpha value of 0.05 as threshold ( $R^2 = 0.63$ ,  $p = 0.06$ ). (B) For mire water, there was a significant linear relationship for decrease in absorbance ( $\Delta A_{250}$ ,  $\text{cm}^{-1}$ ) and POC formation ( $R^2 = 0.76$ ,  $p < 0.05$ )

dissolved Fe at the end of the experiment also coincided with the attenuation of PAR in both lake and mire water (Fig. 4C,D).

The depth-integrated flocculation for Lake Skärshultsjön was  $14.7 \text{ mg C m}^{-2} \text{ d}^{-1}$  expressed per unit lake area down to the average depth of 3.6 m of the lake. This rate of total particle production in the water column corresponds to a daily loss of 0.03% of the standing stock of DOC in the

epilimnion. Further, POC derived from depth-integrated flocculation ( $14.7 \text{ mg C m}^{-2} \text{ d}^{-1}$ ) estimated in the enclosures could comprise 10% of the organic carbon settling into the sediment traps deployed during the same time period in 2003. The flocculation rate estimated in the enclosures suggests that 11% of the standing stock of DOC would flocculate and settle on an annual basis.

*Sediment traps*—During 132 d (May–September 1994) of sediment trapping in Skärshultsjön, the accumulated sedimentation increased linearly (Fig. 5). The rate of DOC loss was constant during the study period (Fig. 5). The POC concentration in the water column did not change ( $0.7 \pm 0.2 \text{ mg C L}^{-1}$ ) and there was no relationship between organic carbon sedimentation and POC concentration. When the sediment traps were deployed, Chl *a* in the lake water was 6, 5, and  $1 \mu\text{g L}^{-1}$  in 2003, 2004, and 2005, respectively.

Organic carbon sedimentation rates derived from sediment traps varied between  $16.7$  and  $61.0 \text{ mg C m}^{-2} \text{ d}^{-1}$  during 1994 and were substantially higher during 2003–2005 ( $150$ – $420 \text{ mg C m}^{-2} \text{ d}^{-1}$ ) (Fig. 6A). The sedimentation rates appeared positively related to surface-water DOC concentrations (Fig. 6A). Our measurements of increasing DOC concentration during the 1994–2005 period in Skärshultsjön are supported by the increasing absorbance at 420 nm ( $A_{420}$ ) observed by Swedish Lake Monitoring ([www.ma.slu.se](http://www.ma.slu.se)), reflecting an increase in allochthonous DOC in Skärshultsjön (Fig. 6A,B).

## Discussion

*Allochthonous DOC as a precursor of settling organic matter*—Flocculation of allochthonous DOC into sinking particles constitutes a pathway for the relocation of carbon from the terrestrial environment into lake sediments. Some of the organic carbon may be retained for a long time in the sediment, rather than being subject to photochemical and biological mineralization during continued residence downstream in the water of lakes, rivers, and the sea. We demonstrate that the rate of flocculation of DOC in a boreal lake may explain 10% of the total gross sedimentation (including resuspension), and show that exposure to sunlight generates organic flocs in the water (Figs. 1, 2). Loss of short-wavelength absorbance (250 nm) in the dissolved phase, indicative of allochthonous humic compounds (McKnight et al. 1997; Tranvik and Bertilsson 2001), accompanied the formation of POC in both lake and mire water (Fig. 3). This is consistent with allochthonous, humic components of the lake-water DOC being precursors of organic particles formed in the water. The allochthonous origin is further indicated by the low fluorescence index of the lake water (1.3), similar to the DOC draining from a *Sphagnum* mire in the watershed (1.2), and below the allochthonous end member value (1.4) suggested by McKnight et al. (2001).

Moreover, the flocculation patterns were similar in the *Sphagnum* mire drainage and in the lake water (Figs. 1–4). Phytoplankton biomass (indicated by suspended Chl *a*, Table 1) was low because of initial filtration of the samples,

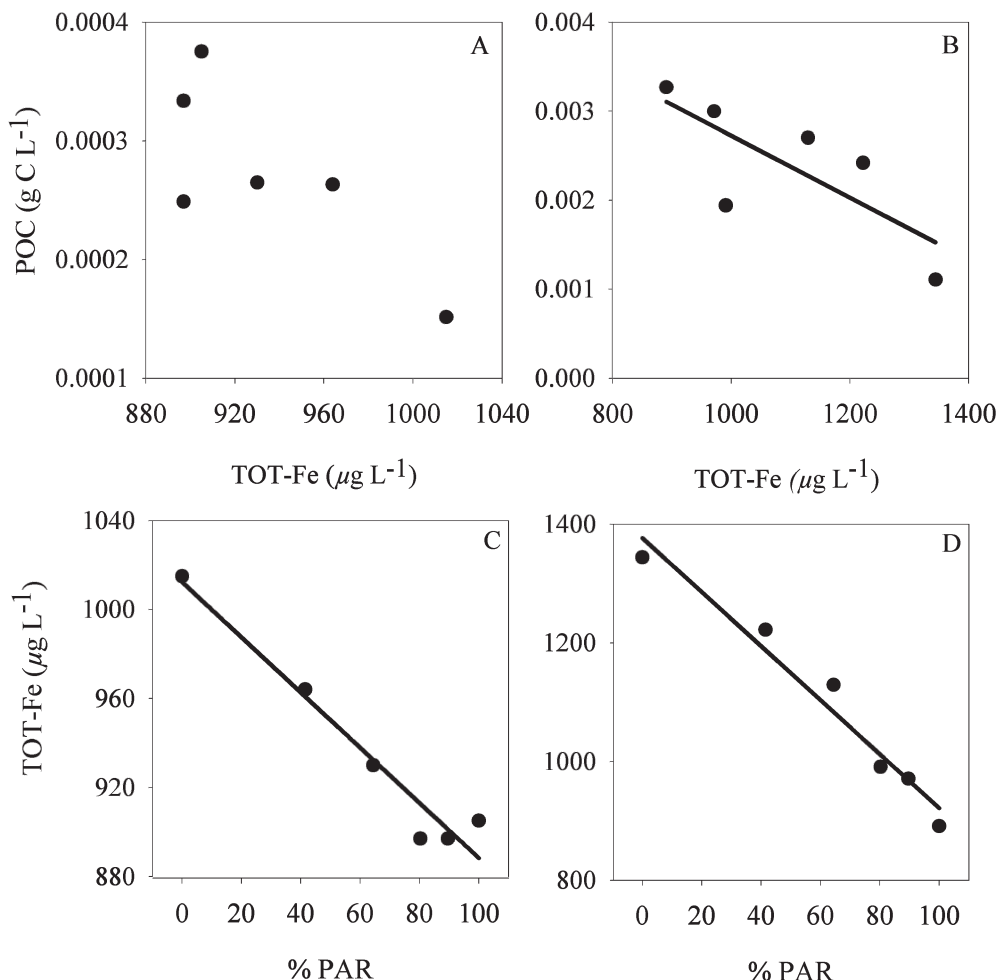


Fig. 4. Linear regressions for loss of dissolved total iron and formation of POC in the enclosures at the end of the experiment for (A) lake water (not significant,  $R^2 = 0.63$ ,  $p = 0.06$ ) and (B) mire water ( $R^2 = 0.70$ ,  $p < 0.05$ ). Linear regressions of concentration of dissolved total iron and light (PAR) in the enclosures at the end of the experiment for (C) lake water ( $R^2 = 0.95$ ,  $p < 0.001$ ) and (D) mire water ( $R^2 = 0.96$ ,  $p < 0.001$ ).

and was virtually absent in the mire drainage. It did not show any consistent patterns with POC formation, such as decreasing along with the decreasing POC formation with increasing depth and decreasing light. POC formation was also high in mire drainage enclosures where no Chl *a* could be detected (Fig. 2; Table 1). Chl *a* ( $\mu\text{g}$ ) per g OC in the flocs for mire and lake enclosures was  $9 \pm 7$  (average  $\pm$  SD) and  $20 \pm 16$ , respectively. Assuming that Chl *a* constitutes 3% of the phytoplankton carbon content (Riemann et al. 1989), phytoplankton biomass was on average  $285 \pm 225$  and  $670 \pm 532$   $\mu\text{g C}$  per g flocculated OC in mire and lake enclosures, respectively. Although we cannot exclude phytoplankton as a source of particles during the enclosure experiments, phytoplankton biomass in the flocs comprised much less than 1% of the settling carbon. Our data strongly support that allochthonous DOC is an important precursor of organic matter in boreal lake sediments, independent of indigenous primary production, but triggered by exposure to sunlight.

*The relationship between DOC flocculation and light—* The formation of particles was attenuated down the water column along with the attenuation of light (Figs. 1A,B, 2A,B; Table 1). The light climate at a depth of 2.0 m depth in the clear-water lake Klintsjön resembles the light climate at 0.45 m depth in the humic Skärshultsjön. 1% of PAR reaches down to 2.2-m depth in Skärshultsjön and deeper than this light is beyond detection. The enclosures at 2.5 m were covered and received no light at all, hence representing light-independent flocculation. Thus, we used the dark enclosure to calculate light-independent flocculation between 2.2- and 3.6-m depth (average lake depth). Light-independent flocculation rates were 24–39% of the flocculation rates compared to the enclosures at the surface (Table 1). When integrated over depth, flocculation in the light-exposed region (0–2.2 m) represented 70% of the total integrated flocculation in the epilimnion. Hence, even if flocculation was stimulated by light, processes independent of light comprised 30% of the POC formed in the lake-

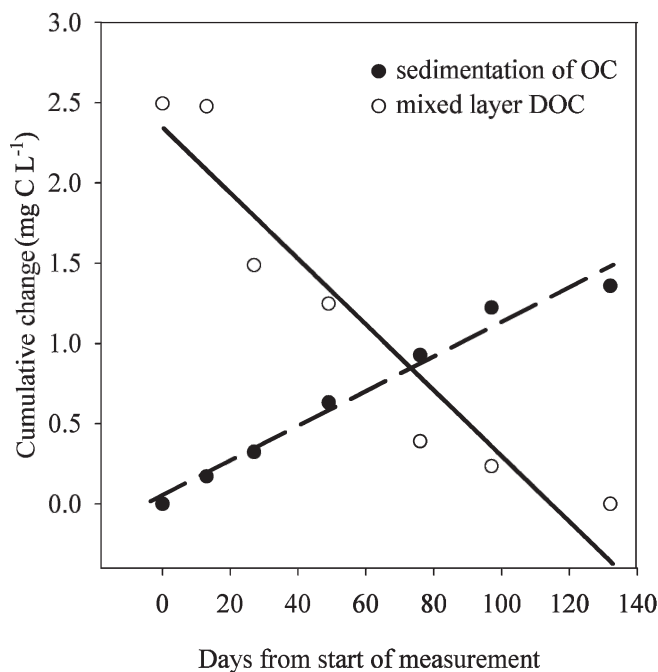


Fig. 5. Particles retained in the sediment traps (linear regression,  $R^2 = 0.94$ ,  $p < 0.001$ ) and the change in concentration of lake-water DOC in the mixed layer (linear regression  $R^2 = 0.91$ ,  $p < 0.001$ ) in 1994.

water column. Depending on the prevailing light climate, lake-water circulation, and stratification in the lake, the importance of solar-mediated flocculation may vary. Incubation in enclosures at fixed depths excludes vertical mixing. Within the upper mixed layer of the lake all DOC should be exposed to sunlight to some extent, causing flocculation of the fraction of DOC most prone to transformation into particles upon light exposure. Stratification may determine the amount of DOC molecules exposed to sunlight; however, stratification could not cause the flocculation in the enclosures. The enclosure experiment demonstrates the role of sunlight as an inducer of flocculation, but does not address physical mixing, stratification, and resuspension. Hence, our enclosure experiment may have resulted in a conservative estimate of light-induced flocculation. Moreover, mixing and turbulence enhances the rate of collision of colloids, which is a prerequisite for flocculation (Kepkay 1994). The limited turbulence in the containers as compared to the open lake water possibly further reduced the estimated flocculation, suggesting that extrapolation to in situ conditions should be made with caution.

The decreasing POC formation with decreasing light appeared linear throughout the PAR gradient (Figs. 1A, 2A). In contrast, there was a tendency for a nonlinear relationship between PAR and the loss of A250 in both lake and mire water (Figs. 1C, 2C), suggesting that absorbance loss declined more rapidly with depth than PAR. On the other hand, the loss of A250 and the UV-A attenuation were linearly related in both mire and lake water (Figs. 1D, 2D). This is consistent with previous findings (De Haan 1993; Moran and Zepp 1997; Bertilsson

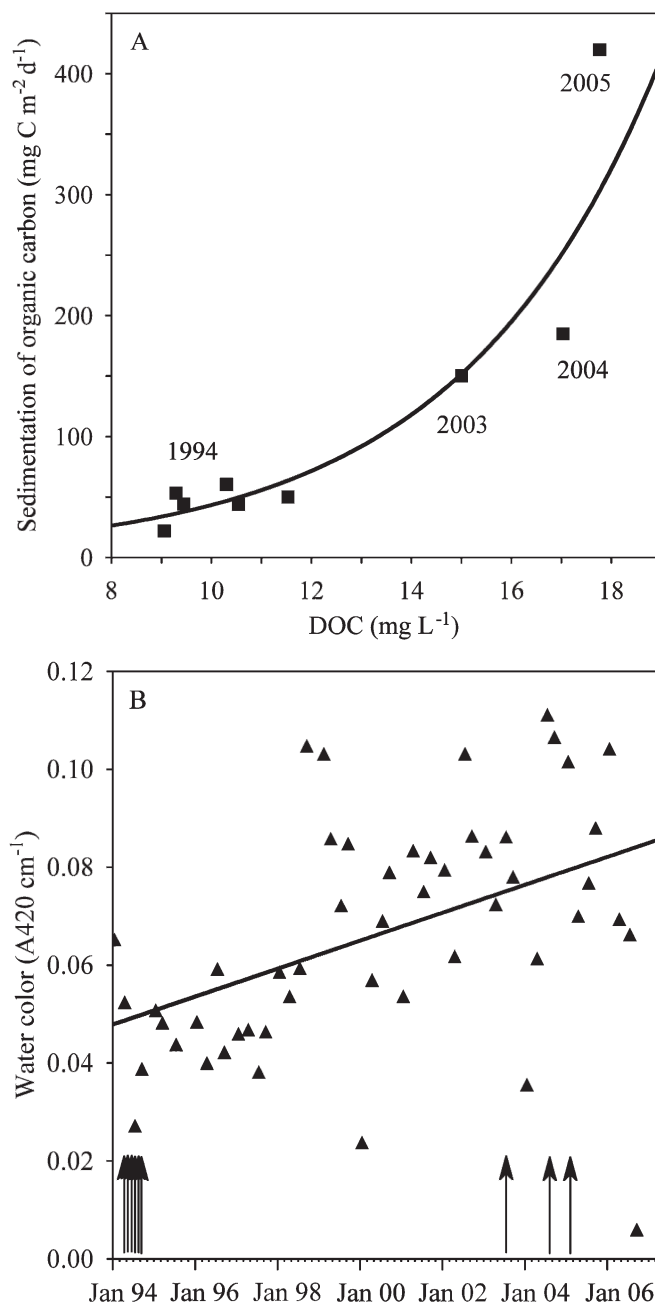


Fig. 6. Rate of POC sedimentation vs. water column DOC concentrations in Skärshultsjön (A) based on data from the years of 1994 and 2003–2005. The curve represents the best nonlinear fit (sedimentation =  $3.5 \times e^{0.25 \times \text{DOC}}$ ,  $r = 0.87$ ,  $p = 0.0019$ ). Water color (absorbance at 420 nm) in Skärshultsjön (B) over the period from 1994 to 2006 (linear regression,  $R^2 = 0.20$ ,  $p < 0.001$ ,  $n = 51$ ). Arrows indicate periods where sediment traps were deployed in the lake.

and Tranvik 2000) that UV-A explains a major fraction of photo-bleaching and photochemical transformations of DOC into low-molecular-weight compounds and  $\text{CO}_2$ . Hence, it reflects that the loss of A250 in our study is to a large extent related to other previously documented photochemical transformations. There was an increase in specific absorbance (A250:DOC) in the lake enclosures



and a minor increase in the mire. Specific absorbance did not change with incubation depth in either lake or mire water, indicating that the decreases in DOC and in chromophoric DOC followed each other.

The mire water had a 72% higher concentration of initial DOC compared to the lake water (Table 1), and the rate of POC formation was 2- to 5-fold higher at all depths (Table 1). PAR had a five times higher effect upon flocculation of the DOC from the mire, as derived from the regression slopes (Figs. 1A, 2A; Table 2). Accordingly, the extent of flocculation is not linearly dependent on DOC concentration. Mire water, previously not exposed to light, may be more susceptible to light-induced flocculation. This suggests that DOC that is newly imported to lakes may be more reactive, with components susceptible to flocculation being successively exhausted during residence in the water column.

*The role of iron*—The formation of POC increased with the loss of total iron from the water (Fig. 4A,B), implying that Fe settled out together with POC. Mire drainage water had previously not been exposed to sunlight and consequently the initial DOC and Fe concentrations were high and could result in more vigorous POC formation (Fig. 4B). Loss of Fe was also correlated to light (PAR; Fig. 4C,D) in both lake water and mire drainage. In oxic waters, Fe(III) forms a complex with DOC that stays in solution. Exposure to UV light results in the formation of organic carbon colloids and free Fe(II) (Gao and Zepp 1998). Fe(II) is oxidized into Fe(III) by photochemically produced  $H_2O_2$  and the redox cycling (Fenton's reaction) is thereby complete (Gao and Zepp 1998). Possibly, as iron is repeatedly undergoing redox cycles, the configuration and affinity of DOM components in the water are changed, and colloids may gradually grow into gravitoidal particles as observed in the enclosures (Figs. 1–4). The enclosures never reached anoxic levels (Table 1), so if redox cycling were the only catalyst for flocculation, a light-induced Fenton reaction would likely be the cause. Because the reaction is of a second order, flocculation should not be linearly related to light (Figs. 1, 2), as in our case (Stumm and Morgan 1996; Gao and Zepp 1998). Irrespective of a possible Fenton reaction, available iron in the water could form co-precipitates with DOC (Fig. 4). The co-precipitates will settle out and eventually end up in the sediments (Tipping and Woof 1983; Kortelainen et al. 2004).

*Sediment traps*—We measured sedimentation in the studied humic lake in 1994 and during 2003 to 2005 (Fig. 6A). From May to September 1994, sedimentation corresponded to a loss of 1.4 mg organic C  $L^{-1}$  from the lake water above the sediment traps. Simultaneously, DOC decreased with 2.5 mg C  $L^{-1}$  in the epilimnion (Fig. 5). Accordingly, the sedimentation of POC corresponded to more than half of the loss of DOC. During the study period the sestonic POC fluctuated between 0.40 and 0.94 mg C  $L^{-1}$  (average 0.70 mg C  $L^{-1}$ ), independently of the decrease in DOC.

In 2003, sediment traps were deployed simultaneously with the enclosures experiment. During this period, the

flocculation in the enclosures corresponded to 11% of the sedimentation estimated from the sediment traps. Although the connection between the experiment with confined samples in containers and sediment trapped in the open water column should be made with caution, the results call attention to a possible role of flocculation of DOC in the formation of sediment. Factors that complicate the comparison include the lower turbulence in the confined samples, resulting in fewer interactions of colloids and thus possibly an underestimate of in situ flocculation (Kepkay 1994), as well as sediment resuspension in the lake but not in the containers, possibly resulting in overestimated sedimentation (Håkanson and Jansson 2002). Nevertheless, the suggested role of allochthonous DOC as an important precursor of settling organic matter corroborates a previous study, estimating that the import of allochthonous POC to boreal lakes can explain only a fraction of the sedimentation of allochthonous POC in these lakes (von Wachenfeldt and Tranvik 2008).

The sediment traps were deployed at the lower end of the epilimnion (4 m from the lake surface) in the profundal zone (at a lake depth of 13 m) and the lake was strongly thermally stratified. The dominating wind direction is from the west and the lake is narrow in the east–west dimension, with only 200 m from shore to shore at the position of the sediment traps. Hence resuspension from the sediments was most probably minimal. In 1994, there was no visible inflow to the lake during summer, suggesting that there was no significant import of terrestrial organic matter. Some of the POC in the traps could originate from resuspension of sediment matter and thus explain the higher settling of OC in situ than in the enclosures.

In the enclosure experiments, the rate of flocculation was higher in mire drainage water than in lake water, which may partly be attributable to a higher susceptibility to particle formation of DOC not previously exposed to sunlight. There was no increase in Chl *a* concentration in the lake during the trap deployment in 2003–2005. Thus, phytoplankton could not exclusively make up the observed increase in sedimentation. A possible explanation for the higher rates of sedimentation in 2003–2005 than in 1994 may be because of a similar effect of different residence time, i.e., during 2003–2005 a higher input of DOC may have resulted in higher sedimentation rates. The DOC levels of lake waters in some regions are increasing. Possibly, a wetter future climate, as predicted for the studied region (Palmer and Ralsanen 2002), results in shorter retention time and less loss of DOC because of in-lake mineralization (Dillon and Molot 1997; Schindler et al. 1997; Tranvik and Jansson 2002). However, there was no significant increase in annual precipitation in this area (1994–2006, daily measurements, Swedish Meteorological and Hydrological Institute; precipitation monitored at Berg [57°12'N, 14°15'E], Växjö [56°85'N, 14°53'E], and Rörvik station [57°24'N, 14°69'E]). Recovery from acidification may also be a plausible contributor to the increase in DOC (Monteith et al. 2007). As derived from absorbance data from Swedish Lake Monitoring, the water color of Skärshultsjön has increased over the last 13 yr (Fig. 6B). Consequently there is an increasing import of organic

carbon of terrestrial origin from the surrounding catchment. As lake-water DOC concentration increases, sedimentation is also augmented (Fig. 6A) and the imported organic carbon will to a large extent be relocated from the water column to the sediment.

Global radiation, and thus PAR, has not increased in the study region, at least not for the period 1999–2007, according to the Swedish Meteorological and Hydrological Institute. Further, Chl *a* did not increase throughout the study period, and thus changes in settling of phytoplankton cannot explain the increasing sedimentation. Water color increased from 1994–2006 (Fig. 6B); however, no increasing trend in TOC was visible in this lake. On the other hand, the ratio of A420:TOC increased significantly ( $R^2 = 0.42$ ,  $p < 0.001$ ) from 1994 to 2006 (Swedish Lake Monitoring). Thus, the contribution of the colored fraction of the TOC seems to have increased, which could be an indication that the fraction of allochthonous OC has been increasing in this lake. If this additional allochthonous OC has not been exposed to sunlight previously, it is probably very prone to flocculation and sedimentation (*see* results for mire water). This may help to explain the relatively higher increase in sedimentation than in water color.

In order to gain information about the fate of flocculated organic matter in the sediment, we estimated the rate of organic carbon burial in a sediment core profile of organic carbon and water content. Assuming a sedimentation rate in the range of 1–3 mm yr<sup>-1</sup>, which is typical for boreal Swedish lakes (Håkanson and Jansson 2002), about 60–170 mg OC m<sup>-2</sup> d<sup>-1</sup> are buried in Skärshultsjön sediment. Further, respiration rates are low in the profundal sediments of Lake Skärshultsjön, averaging at 20 mg C m<sup>-2</sup> d<sup>-1</sup> (Algesten et al. 2005). These numbers return a burial efficiency of organic carbon (buried : [buried + respired] carbon) in the range of 70–90%. Even when assuming a very low sedimentation rate (0.5 mm yr<sup>-1</sup>), OC burial efficiency is at 60%. Alternatively, we calculated OC burial efficiency based on the median of the sediment trap data (buried : trapped OC), but also in this case OC burial efficiency was over 60%. Even if these numbers may be regarded only as coarse estimates of OC burial efficiency, they indicate that a significant fraction of the organic carbon settling onto the sediment surface of Skärshultsjön is sequestered over geological timescales. Allochthonous DOC is considered to be recalcitrant to degradation (McKnight and Aiken 1998). Hence, the flocculation and relocation of allochthonous DOC to the sediment may be an important mechanism for the sequestration of organic carbon in boreal lake sediments.

This study shows that a substantial fraction of the sediment of a boreal lake could originate from allochthonous DOC. The water draining from a mire in the watershed is subject to extensive flocculation upon exposure to sunlight. Increasing import of DOC from adjacent wetlands could therefore result in enhanced flocculation followed by sedimentation, relocating organic carbon from the water column into the sediments. Sediments contribute a minor fraction of the total respiration in such lakes (Algesten et al. 2005), also suggesting that flocculation followed by sedimentation

may be a mechanism for long-term carbon sequestration, rather than simple spatial relocation of the mineralization. Boreal sediments are known to hold a substantial fraction of the organic carbon in the landscape (Kortelainen et al. 2004). However, almost all of the organic carbon transported to lakes is in the dissolved and colloidal state, and hence not initially prone to sedimentation. The flocculation demonstrated here, partly dependent on light exposure, provides a mechanism for such repositioning. Hence, a significant fraction of the sediments could originate from allochthonous DOC, creating the brown, loose, “fluffy” sediments rich in allochthonous carbon.

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