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Sulfur Cycling and the Sulfurization of Humic and Fulvic Acids in the Sediments of the rivers Rupel (Belgium) and Authie (northern France)

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Abstract

Sulfur cycling and the sulfurization of humic and fulvic acids were compared in recent sediments from two western European rivers (the heavily polluted River Rupel in Belgium and the pristine River Authie in northern France). The sulfurization of humic and fulvic substrates occurs in both sediments irrespective of organic loading, but the sulfur species added to the organic substrate differ. Some sulphurization of fulvic acid by oxidized S was observed in the strongly reducing sediment of the River Rupel. Humic acids were sulfurized in the sediments of both rivers in these segments with prevailing reducing conditions by reduced S.

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INTRODUCTION

The biogeochemical transformations of sedimentary organic matter during early diagenesis play a key role in the behavior of metals and organic pollutants in the aquatic environment. The most important process responsible for a significant fraction of the mineralization of sedimentary organic matter in organic-rich aquatic sediments is bacterial sulfate reduction (Jørgensen 1977, 1982). Dissolved sulfides subsequently react with dissolved metal ions in the sulfate reduction zone, most commonly Fe released from easily reducible minerals such as Fe (oxy)hydroxides (Canfield 1989) and form insoluble sulfide minerals thus immobilizing metals. Therefore, the transformations of sulfur in the sediments, especially bacterial sulfate reduction, govern the concentration and speciation of many elements (Fe, Cd, Zn, Hg, Pb, Mo….) in the sediments and interstitial water, as well as their bioavailability and/or toxicity (Batley 1990). However, the formation of organosulfur compounds is often a competitive process to sulfide formation and may inhibit the immobilization of metals by dissolved sulfide (Brüchert and Pratt 1996, Henneke et al. 1997, Lojen et al. 2004) by binding it to the organic substrate, especially in freshwater environments. The enrichment of organic matter with sulfur (S) during early diagenesis has been amply documented in marine sediments, whereas the importance of these reactions is not as well recognized in freshwater environments. Sulfide minerals are the most abundant sedimentary S fraction in the marine environment, while in freshwater environments organic-bound sedimentary S prevails (Gerritse 1999). In contrast to marine sediments, the availability of sulfide formed during the decomposition of reactive organic matter by bacterial sulfate reduction and the availability of reactive iron in the sediment are not important determinants for the extent of organic S enrichment in freshwater environments (Urban et al. 1999). The only environmental parameters that appear to be relevant for the addition of S to the organic substrate in freshwater sediments are the trophic status of the water body and the sedimentation rate, where only little or no sulfurization is observed in oligotrophic environments (Gerritse 1999).

The stable isotopic compositions of bioactive elements (C, N, S…) in organic substances are commonly used as natural tracers of their origin, formation pathways, and formation rate (Fry et al. 1977, Peterson & Howarth 1987, Habicht & Canfield 1997) since their isotopic ratios depend on the isotopic composition of their precursors and the isotopic fractionation related to each reaction step in the formation process. Bacterial sulfate reduction is related to substantial kinetic isotopic fractionation, producing ^{34}S-depleted sulfide, whereas the residual sulfate pool becomes highly enriched in ^{34}S. In recent aquatic environments, isotopic separations of up to 70‰ between sulfide and

the residual sulfate pool were reported (Canfield et al. 1998, Lojen et al. 2004). The two major end-products (i.e., sinks) of reduced S in aquatic sediments are acid volatile sulfides (AVS), mostly FeS_x ($0.9<x<1.5$), and pyrite (FeS_2), where the AVS pool is often found as a minor constituent of reduced S and is believed to be a precursor which converts to the pyrite during early diagenesis. Pyrite is highly resistant to recycling and is generally considered to be a terminal sink of reduced S, while its S isotopic composition is a good approximation of the $\delta^{34}S$ of the dissolved sulfide and depends on the initial stable isotopic composition of the sulfate pool and the sulfate reduction rate (Habicht & Canfield 1997**,** Butler et al. 2004). The sulfurization of organic substrates is related to an isotopic separation of 4-5‰ between dissolved S species and sulfurized organic matter, while the reoxidation of reduced S species, in turn, produces little or no isotopic fractionation (Fry et al. 1985, Canfield & Thamdrup 1996, Canfield et al. 1998, Cypionka et al. 1998, Amrani & Aizenshtat 2004).

The relation between sulfide formation and the sulfurization of sedimentary organic matter is well studied in marine and estuarine sediments, whereas little information is available for freshwater environments. The present paper compares the distribution of dissolved and sedimentary S species in sediments accumulated on the tidal banks of two western European rivers, the heavily polluted River Rupel (Belgium) and the pristine River Authie (northern France), that differ in pollution levels. Some of the results presented herein have been published before (Leermakers et al. 2005; Lojen et al., submitted) but were not discussed in light of the sulfurization of humic and fulvic acids. Stable isotopic compositions of dissolved sulfate, as well as sedimentary S species, i.e., acid volatile sulfide pool (AVS), pyrite, elemental S (S^0) and sulfur bound to fulvic (FAS) and humic acids (HAS), were used as natural tracers to estimate the formation sequence of sedimentary S species as well as potential agents for the sulfurization of organic substrates.

SAMPLING SITES

The River Rupel is a tributary of the River Schelde, originating at the confluence of the Dijle and the Nete, and joining the Schelde River in Rupelmonde. It receives a large organic matter input through the River Zenne, which transports untreated wastewaters from the city of Brussels. The sampling site was located near the town of Willebroek, about 25 km north of Brussels.

The River Authie flows into the English Channel at the city of Berck (northern France) in an estuary situated on the border between the departments of Somme and Pas-de-Calais. The river is classified as unpolluted although it has a series of water mills with small dams (up to 2 m high) for different uses, such as power plants or fish farming (Hansen et al. 2002). The main activity in

the area is agriculture. Previous studies indicated that the concentrations of Cd, Cu, Ni, Pb, Hg, and Zn in the sediment and water are indistinguishable from the background levels for the area (Billon 2001) and that organic pollutant concentrations, especially pesticides and herbicides, are also very low (Le-Calvez 2002). The sampling site was located at Pont-a-Cailloux about 5 km upstream from the river mouth where slight marine influence can be observed only at exceptionally high tides a few times per year.

The tidal heights were about 5 m at both sampling sites.

MATERIAL AND METHODS

Sample preparation

Sediment samples were taken manually on tidal mudflats at the end of low tide. The sediment surface was still dry at the moment of sampling. Plexiglas tubes with a 10 cm internal diameter and rubber stoppers were used. All manipulations of the sediment (sectioning, weighing, centrifuging, filtering, etc.) were performed in a N_2-filled glove bag or in tightly closed Teflon or glass vessels to minimize the possibility of the oxidation of reduced compounds.

Redox potential (Eh) and pH were determined on site immediately after coring by inserting electrodes into the core through side openings in the tube covered with plastic adhesive tape.

The sediment cores were extruded and cut into 2 cm thick segments, divided into two portions, each packed into a plastic bag, sealed and stored frozen (-24ºC) until further processing. They were used separately for extracting the following species: (1) sedimentary sulfides and for the estimation of the degree of Fe pyritization (DOP); (2) dissolved sulfate and sulfide in pore water, elemental S, and S bound to fulvic and humic acids.

The degree of Fe pyritization was determined as the ratio of pyrite-bound Fe to total Fe (pyritic + HCl – soluble, Berner 1970). The HCl soluble Fe fraction was extracted by eluting reactive Fe from wet sediment in 1 molar HCl at room temperature for 12 hours (Leventhal & Taylor 1990, Roychoudhury et al. 2003).

The extraction scheme for individual S species is presented in Fig. 1 and is described in detail elsewhere. Acid volatile sulfides and Cr-reducible sulfides were extracted following the sequential extraction procedure using 6 molar HCl and hot $CrCl_2$ developed by Canfield et al. (1986) and modified by Billon et al. (2001, 2001a). Dissolved sulfate and sulfide were precipitated after washing the sediment in a deaerated 0.5 M NaCl solution (Henneke et al. 1997). Elemental S was dissolved in a 3:1 methanol + toluene mixture (Henneke et al. 1997), the solvent evaporated, and the residue was treated further as CRS. Humic

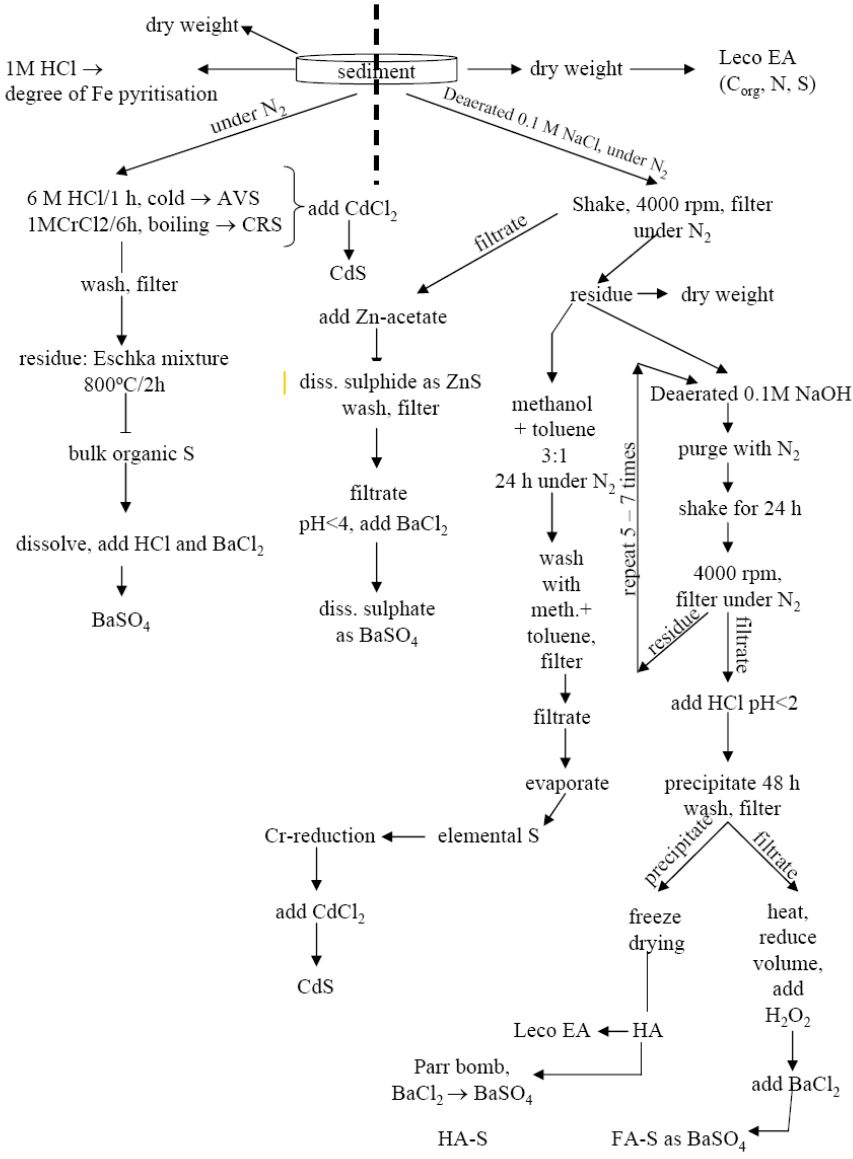

Fig. 1. Extraction scheme for dissolved and sedimentary S species following extraction procedures described by Canfield et al. (1988) and modified by Billon et al. (2001) for AVS and CRS, Henneke et al. (1997) for dissolved sulfate, dissolved sulfide, and elemental sulfur, and Brüchert and Pratt (1996) for sulphur bound to humic and fulvic acids.

substances, which are major constituents of soil organic matter and upland stream sedimentary organic matter, were divided into two fractions that were defined operationally by their solubility in acidic or alkali solutions. Both were extracted from the sediment by digestion in a deaerated 0.1 M NaOH solution (Brüchert and Pratt 1996). Humic acids were precipitated by adding HCl, and S bound to humic acids (HAS) was extracted after decomposition in a Parr bomb (Oxygen bomb 1108, John Morris Scientific) and precipitation with $BaCl_2$. After removing humic acids, the residual solution was evaporated to about 10% of its initial volume. During evaporation, H_2O_2 was added in small portions to convert the FA-bound S to sulfate, which was then precipitated by the addition of $BaCl_2$.

The acid volatile sulfide pool (hereafter referred to as AVS) consisted mainly of solid Fe-monosulfides (greigite, mackinawite, amorphous FeS_x clusters) and dissolved S^{2-} species - H_2S, HS^-, $FeHS^+$, and FeS_{aq} (Rickard & Morse 2005). The chromium reducible sulfur (CRS) was comprised of pyrite, elemental S, and perhaps some Cr-reducible organic S. The organic CRS fraction could not be separated, but as it is supposedly negligible, its presence should not have affected the results (Canfield et al. 1998). The sulfides for isotopic analyses were precipitated by adding excess amounts of $CdCl_2$ to the antioxidant solution containing H_2S liberated during AVS and CRS digestion. The pyrite concentration was then calculated as the difference between the concentrations of CRS and elemental S.

Analytical procedures

The dry weight of the sediment was calculated from the wet weight and the mass difference of the wet and oven dried sediment (60°C to constant weight). Dried sediments were used in elemental analysis to determine the concentrations of organic carbon (C_{org}) and total nitrogen (N) using a Leco 932 elemental analyzer, as well as for the stable isotopic analyses of C_{org} and N. The same apparatus was used to determine the S concentration in humic acid extracts.

Concentrations of all S species precipitated as $BaSO_4$ (dissolved sulfate, HAS, FAS) were determined gravimetrically (Ceseri et al. 1998). Dissolved sulfate was recalculated per ml of pore water and estimated from dry weight analysis (Brüchert and Pratt 1996). The density of pore water was taken to be 1 g cm^{-3} assuming that the error derived from eventual differences in water density could be neglected compared to the analytical error of the gravimetric method, which is ± 2% (Standard methods, 1998).

The concentrations of AVS, CRS, S^0, and dissolved sulfide (previously precipitated as ZnS and re-processed as AVS) were determined with

potentiometric titration of the H_2S – containing antioxidant solution (0.2 M EDTA in 2 M NaOH) with a 100 mg kg^{-1} Cd solution (prepared from the Titrinorm standard $CdCl_2$ solution) using a Metrohm 736 GP Titrino system. The reproducibility of the entire procedure was determined by Billon et al. (2001a) to be better than 8%, mostly due to the natural heterogeneity of the sediment. The concentration differences obtained in replicate samples, however, did not affect the isotopic composition of the sulfide. The concentrations of all sedimentary S species were recalculated per kg of dry sediment.

The stable isotopic compositions of organic C, N, and extracted S species were determined using a Europa 20-20 continuous-flow isotope ratio mass spectrometer (EA-IRMS) with an ANCA SL preparation module. For organic carbon isotopic analysis, carbonate was eliminated by digestion in diluted HCl (1:3) at 50ºC overnight. Samples of sulfur extracted in the form of CdS were mixed with an equal amount of V_2O_5, while a triple amount of V_2O_5+SiO_2 (1:1) mixture was added to $BaSO_4$ prior to analysis.

The results of stable isotope analysis are reported in per mill (‰) as relative δ values defined as

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \; [‰] \qquad (1)$$

where R is the isotopic ratio of heavier to lighter isotopes (i.e., $^{34}S/^{32}S$, $^{15}N/^{14}N$, $^{13}C/^{12}C$) of the sample and standard.

Results are reported in per mill [‰] relative to VPDB (Vienna Pee Dee Belemnite) for carbon, to air for nitrogen, and to VCDT (Vienna Cañon Diablo Troilite) for sulfur. IAEA reference materials and laboratory standards calibrated relative to IAEA calibration materials were used to control the accuracy of the analyses. The precision determined by the replicate analyses of samples was equal to or better than ± 0.1‰ for $\delta^{13}C$, 0.2‰ for $\delta^{15}N$, and 0.5‰ for $\delta^{34}S$. The stable isotopic composition of pyrite was recalculated from the concentrations and $\delta^{34}S$ values of S^0 and total CRS obtained by sequential extraction.

RESULTS

A visual inspection of the sediment cores revealed that the River Rupel sediment was virtually homogeneous, dark grey, and of a gelatinous texture, while that from the River Authie was characterized by alternating layers of different colors (yellowish-brown to dark grey).

The analysis of physicochemical conditions (pH, Eh) in the sediment immediately after coring revealed considerable differences between the two sampling sites (Fig. 2) although both sediments were sampled under similar conditions at rising tide before the sediment surface was submerged under water. Eh became negative immediately below the surface in the sediment from

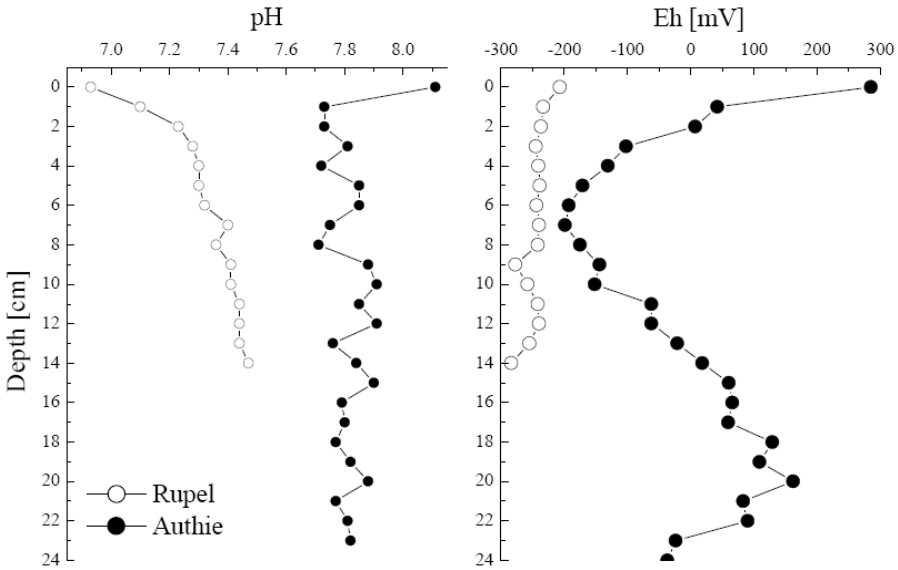

Fig. 2. pH and Eh in the sediments of the River Authie and the River Rupel.

the River Rupel and decreased to values as low as –290 mV relative to the $Ag/AgCl_2$ electrode. The gelatinous texture and high organic loading obviously prevented the diffusion of oxygen into the deeper layers of the sediment during low tide, therefore Eh remained negative throughout the sediment column. In the River Authie sediment, Eh fluctuated between negative and positive values with sediment coloration differences corresponding roughly to changes in redox potential. The River Authie sediment was coarser compared to that of the River Rupel and intensively bioturbated with open and filled-in tubes and lenses of mm to cm size, distinctly differing from each other in density, color, and grain size. Therefore, oxygenation at low tide affected a much thicker sediment layer. The increase of Eh below 7 cm may also be related to the upward invasion of fresh oxygenated water due to the rising tide at the time of sediment sampling.

The River Rupel sediment was characterized by higher C_{org} and N loading in the upper 5 cm of the sediment column, while below that depth, both sites

showed approximately the same C_{org} and N concentrations (Fig. 3). The isotopic analysis of sedimentary organic matter showed no significant changes in $\delta^{13}C$ with depth and values typical of terrestrial or freshwater organic litter (Deines 1980). The $\delta^{15}N$ values, however, indicate that the origin of sedimentary organic matter differed in the two rivers. While the $\delta^{15}N$ values of between 5.5 and 7.5 noted in the sediment of the River Authie are common in soils, the low $\delta^{15}N$ values (around 2‰) in the upper sediment segment of the River Rupel indicate the presence of a considerable fraction of poorly treated domestic and industrial particulate organic waste (Rogers 2003, Sweeney et al. 1980, Van Dover et al. 1992).

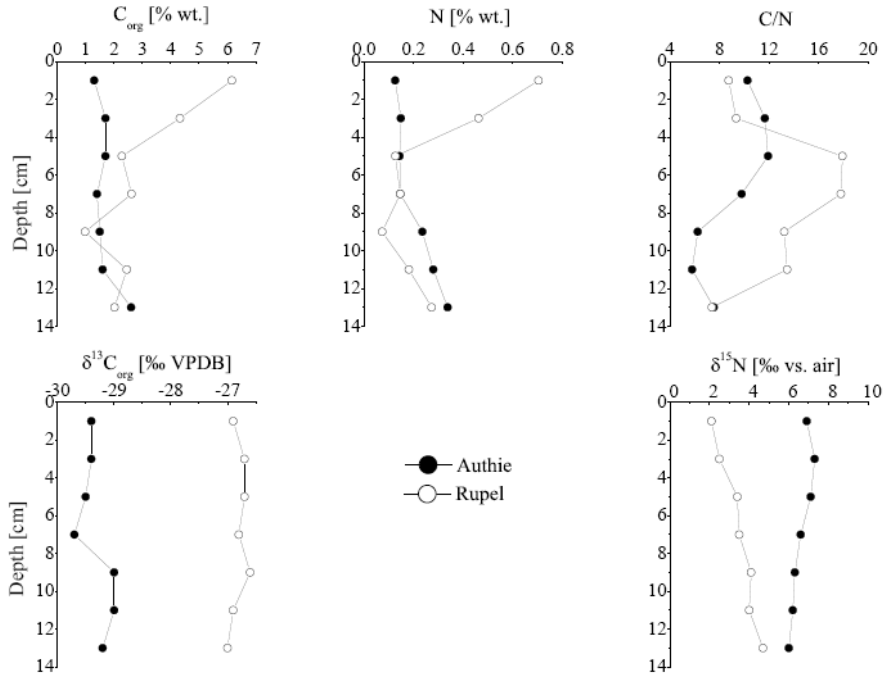

Fig. 3. Concentrations and stable isotopic compositions of sedimentary organic C and N in the River Authie and the River Rupel sediments.

The concentration of dissolved sulfide (Fig. 4) in the pore water of the River Rupel sediment was below the detection limit throughout the sediment column, while in the River Authie sediment sulfide generally increased although the concentration vs. depth profile scattered a lot. Similar to sulfide, sulfate concentrations were higher in the pore waters of the River Authie

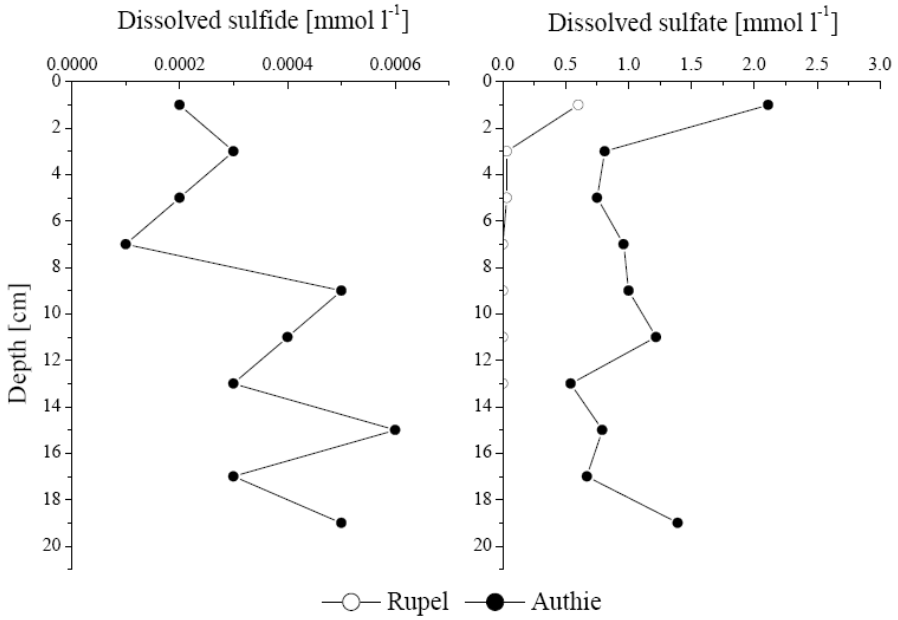

Fig. 4. Concentrations of dissolved sulfate and sulfide in the pore water of River Authie and River Rupel sediments.

sediment, where they remained stable around 1mmol l^{-1} after an initial decrease from about 2 mmol l^{-1} 1 cm below the sediment/water surface. It is noteworthy that the sulfate concentration in the River Authie water was much lower (0.03 mmol l^{-1}) indicating that the river sediment acts as a sink for sulfate. The River Rupel sediment contained much less sulfate (about 0.5 mmol l^{-1}) in the uppermost sediment segment, while not far below this depth sulfate was completely exhausted (i.e., it was below the detection limit).

In contrast to the dissolved S species, sedimentary S was much more abundant in the River Rupel than in the River Authie sediment (Fig. 5a). The total sedimentary S concentration (the sum of all analyzed species, Fig. 5b) in the River Rupel sediment exceeded that of the River Authie sediment by up to thirtyfold, which resulted from the extreme abundance of FAS. The concentration of S in the humic acid extracts, determined by elemental analysis, was lower in the River Rupel than in the River Authie sediment, but it increased continuously with depth (from 0.4 to about 0.8% wt. at a depth of 13 cm), while in the River Authie sediment, the S concentration in humic acids increased from approximately 1 to 1.6% wt. in the upper 7 cm of the sediment column, followed by a continuous decrease down to 0.75% wt. at a depth of 19 cm.

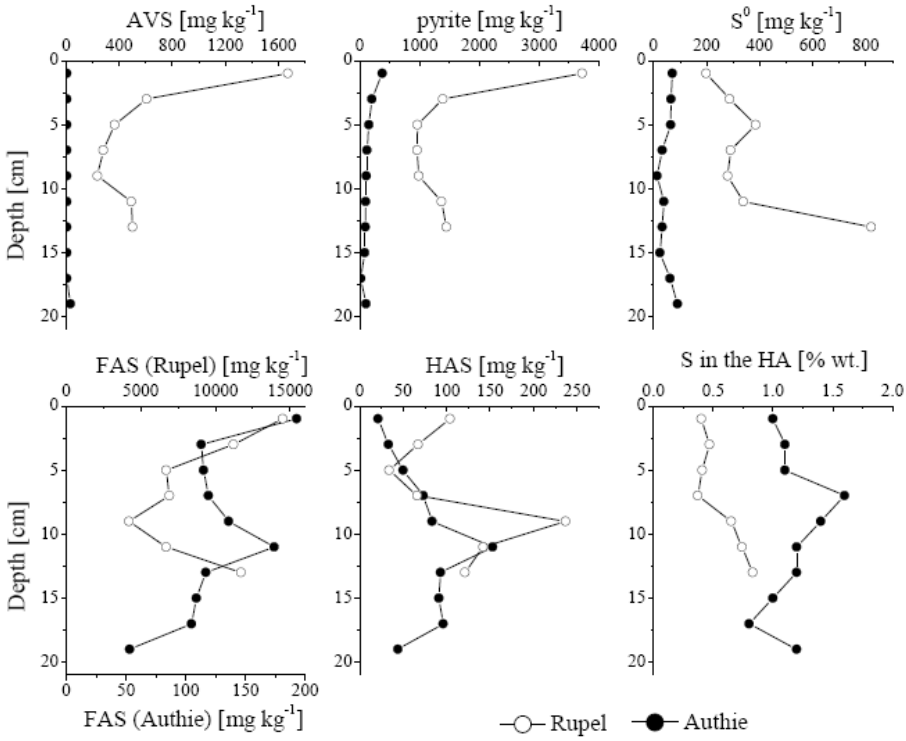

Fig. 5a. Concentrations of sedimentary S species: AVS pool, pyrite, elemental S, total S bound to fulvic acids (FAS), total S bound to humic acids (HAS), and S concentration in humic acid extract in River Authie and River Rupel sediments.

The distribution of sedimentary S species with depth also differed at the two sites (Fig. 5b). Organic S comprised between 72 and 83% of total S in the River Rupel sediment, while in that of the River Authie inorganic S forms (AVS pool, pyrite, and S^0) were more abundant and accounted for 30 to 70% (on average 48%) of total sedimentary S.

The degree of Fe pyritization is higher in the upper few cm of the River Rupel sediment (Fig. 6) and reached up to 70%, but then it dropped quickly and remained stable around 30% below a depth of 5 cm. In the River Authie sediment, the degree of Fe pyritization was low (≤ 10%) throughout the sediment column.

The stable isotopic composition of dissolved sulfate and sedimentary S species at the two sampling sites is shown in Fig. 7. The $\delta^{34}S$ value of dissolved sulfate in the river water (not shown in the figure) was -2.4‰ in the River

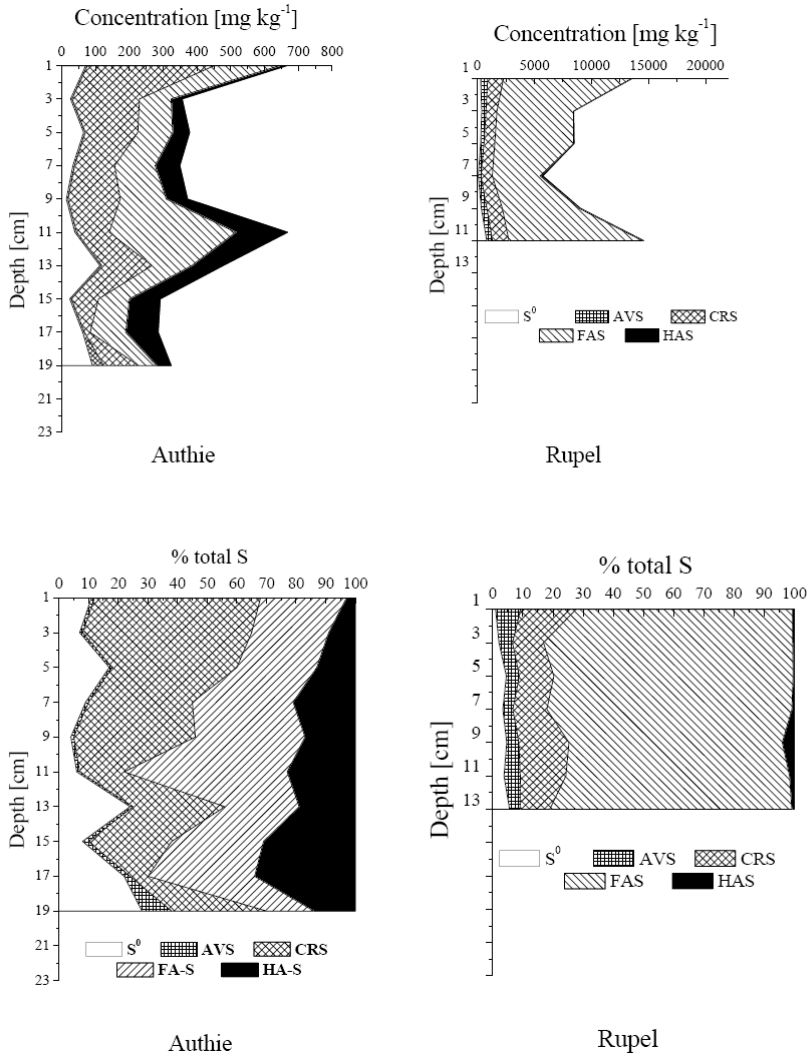

Fig. 5b. Relative fractions of sedimentary S species in River Authie and River Rupel sediments.

Authie and -4.9‰ in the River Rupel. The $\delta^{34}S$ value of sulfate in the interstitial water of River Authie sediment increased up to 18.4‰ in the 16-18 cm depth segment, whereas in the River Rupel sediment sulfate was present only in the topmost sediment layer at a $\delta^{34}S$ value of -1.9‰.

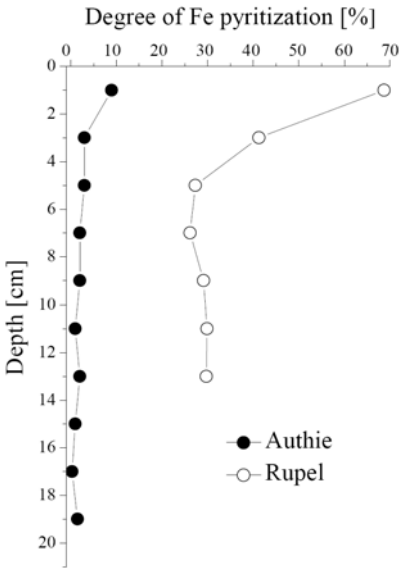

Fig. 6. Degree of Fe pyritization (DOP) in River Authie and River Rupel sediments.

Pyrite was the most ^{34}S depleted S species at both sites with $\delta^{34}S$ values of between -15 and -20‰ VCDT.

In the River Authie sediment, S^0, HAS, and FAS exhibited similar $\delta^{34}S$ values that generally ranged between -10 and 0‰ throughout the sediment column, while dissolved sulfate was the most ^{34}S enriched species.

In the River Rupel sediment, FAS is by far the most ^{34}S enriched species, followed by sulfate (only one value was measured at the 0-2 cm depth segment), HAS, and the AVS pool.

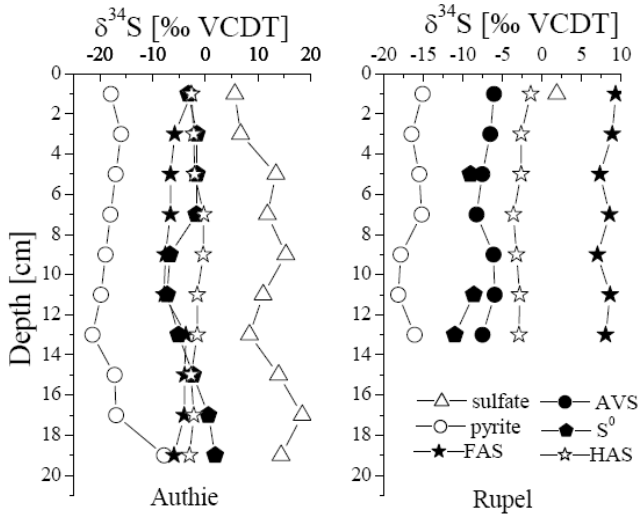

Fig. 7. Stable isotopic composition of S species in River Authie and River Rupel sediments.

DISCUSSION

Inorganic S species

In environments where partial reoxidation of reduced S might take place by bioturbation and hydrodynamic flow, such as tidal flats, pyrite is commonly formed by the reaction of monosulfide (FeS) and intermediate reduced sulfur as elemental S or polysulfides: $FeS + S^0 \rightarrow FeS_2$ (Berner 1970, Middleburg 1991, Billon 2001). The amount of pyrite formed in the sediment is, however, influenced by several factors (Neumann et al. 2005, and references therein): (1) the supply of dissolved sulfide, (2) the AVS $\rightarrow$ CRS conversion rate, (3) the availability of elemental S, (4) the availability of reactive organic matter, (5) the availability of reactive Fe, (6) the availability of an oxidant, which limits the transformation of FeS into pyrite. In contrast to pyrite, AVS is an operationally defined group of dissolved and solid monosulfidic substances, that at present is regarded as a "black box" (Meysman & Middelburg 2005) and is generally considered to be a precursor of pyrite, although this assumption might be false. AVS most probably only constitutes a part of the temporary reservoir for sulfur between burial as pyrite and sulfate in the oxic part of the system (Rickard & Morse 2005).

At both sites, the production of sedimentary sulfides is most intense just below the sediment/water interface, as explained by the highest concentrations of AVS and pyrite in the uppermost sediment segment.

A comparison of the concentrations of C_{org} and S revealed that C_{org} was positively correlated with both total reduced inorganic (as the sum of AVS, pyrite, and elemental S) and organic S species (as the sum of HAS and FAS) in the River Rupel sediment, indicating that S cycling is closely related to the fluxes and decomposition of sedimentary organic matter. The degree of Fe pyritization was very high at the sediment-water interface (70%), but then it dropped and stabilized at about 30% below a depth of 5 cm. This indicates that neither the availability of reactive Fe, nor the availability of S^0 (which also increases with depth in the upper 5 cm) could limit the production of pyrite in this segment. Pyrite production was obviously limited by the limitation of sulfide production since dissolved sulfate was entirely exhausted in the uppermost 3 cm of the sediment column.

No correlation was observed between C_{org} and any of the S species in the River Authie sediment. It is interesting to note that, in fact, two redox boundaries were noted in the sediment column (Fig. 2): one at a depth of 2-3 cm and another at approximately 20 cm. According to the Eh fluctuation, two zones of reduced sedimentary S species accumulation occur: one just below the sediment surface and another below 15 cm, where concentrations of AVS, pyrite, and S^0 increased. In between there is a zone of S recycling with a more or

less stable pyrite concentration, but fluctuating S^0 concentrations that are attributed to disproportionation processes that yield equal amounts of dissolved sulfide and sulfate (Thamdrup et al. 1993**,** Habicht & Canfield 1997, Cypionka et al. 1998, Böttcher et al. 2001). These can then be incorporated into organic matter, secondary sulfides, or recycled in the bacterial sulfate reduction process. Irrespective of the positive Eh values, reduced S species were found throughout the sediment column, indicating that the reduced S minerals produced in the reducing environment can at least partly sustain the downward intrusion of oxygen at low tide and the upward intrusion of oxygenated river water at rising tide. The concentration of dissolved sulfide in the interstitial water increased slightly with depth, but remained below 1 μM throughout the sediment column (Fig. 4). This indicates that sulfate reduction occurred continuously. The degree of Fe pyritization remained low (Fig. 6) throughout the sediment column, indicating that the reactive Fe supply could not limit the production of sulfide minerals. Nevertheless, AVS was present only in trace amounts and slightly increased below a depth of 17 cm. At the same time, a considerable fraction of elemental S (exceeding that of AVS and in some depth segments even pyrite) was present in the sediment. This implies that pyrite formation is limited by the lack of AVS substrate due to the intensive recycling (reoxidation) of sedimentary monosulfides as the most labile reduced S fraction in the sediment.

S in humic and fulvic acids

Generally, the concentrations of both the analyzed organic S fractions in the sediments of the eutrophic River Rupel far exceeded those in the River Authie sediment, which was attributed to the trophic status of the environment (Urban et al. 1999).

Similarly to the inorganic reduced S species, FAS accumulated in the sediments of both sampling sites at the redox boundary, while HAS only accumulated at the sediment surface in the River Rupel. The sediment zone of HAS accumulation in the River Authie was located deeper in the oxidized part of the sediment column.

FAS concentration generally decreased below the sediment surface at both sites although not continuously (Fig. 5a). It was not possible to estimate whether the total concentration of fulvic acids decreased with depth or whether they were just becoming depleted in S. Nevertheless, clear FAS enrichment was observed in the River Authie sediment between 3 and 11 cm, while in the River Rupel it was noted below 9 cm. The stable isotopic composition of S added to the FAS pool in these segments was calculated from the slope of the line $([FAS]/[FAS_0] - 1)$ vs. $(\delta^{34}S\text{-}FAS \times [FAS]/[FAS_0])$, where index 0 indicates the concentration in the uppermost section of the analyzed sediment segment

(Sayles & Curry 1988). It was estimated that in the River Authie sediment the $\delta^{34}S$ of added S was about -7.5‰, while in the River Rupel sediment it was about +8.5‰.

The difference in $\delta^{34}S$ of added S in the two environments indicates that there was sulfurization with different S species. No data on the $\delta^{34}S$ of the AVS pool in River Authie sediment is available, but the $\delta^{34}S$ value of sulfides (between -20 and -15‰, Fig. 5a) is considered to be a good approximation for the stable isotopic composition of the dissolved sulfide pool (Butler et al. 2004). The addition of S from the dissolved S pool to organic substrates is related to an enrichment of 4 - 5‰ (Amrani & Aizenshtat 2004). This indicates that the S (with a $\delta^{34}S$ value of around -7.5‰) added to the fulvic substrate in the River Authie sediment could not be derived from the sulfide pool, nor from the residual sulfate, but most probably came from the partly reduced intermediate species formed during the diagenetic recycling of sulfur. This finding concurs with observations made by Francois (1987).

In the River Rupel sediment, however, the S added to the fulvic substrate is highly enriched in ^{34}S in comparison to all the analyzed sedimentary species, including sulfate. This means that it could only have been derived from residual sulfate pools that were highly enriched in ^{34}S or the partly reduced products of its recycling. This would mean that the sulfurization of fulvic acids was proceeded by oxidized S as was reported for the estuarine sediments of the rivers Authie and Seine (Billon et al. 2002). Nevertheless, since the S concentration in fulvic acid was not determined, these results must be viewed with caution. Another possible explanation would be that increased $\delta^{34}S$ in the FAS in the River Rupel was also related to the higher fraction of detritic biosynthetic S in this sediment segment (Brüchert 1996). No indication of this phenomenon, however, could be found in the C_{org} concentration or its $\delta^{13}C$ vs. depth profile. Currently, it is impossible to prove or disprove either of the proposed hypotheses.

The sulfurization of humic acids could be detected directly by analyzing the S concentration in the humic extracts (Fig. 5a). It was found that humic acids were sulfurized in the upper 7 cm of the River Authie sediment column and below 7 cm in that of the River Rupel. The $\delta^{34}S$ of the added S (calculated in the same way for FAS) was +3.5‰ and -3.3‰ for river Authie and Rupel sediments, respectively. Considering the stable isotopic composition of sulfate and inorganic reduced S species in both sediments (Fig. 7), HA sulfurization occurred in River Authie sediment due to (re)oxidized intermediate S species, whereas in the Rupel River sediment the $\delta^{34}S$ of added S was close to that of the AVS and S^0 pool. Anderson and Pratt (1995) demonstrated in an examination of a wide range of modern and ancient sediments that organic sedimentary S is generally enriched with ^{34}S by around 10‰ in comparison with the pyrite S

pool. The current results show that the enrichment in both analyzed environments was higher, reaching up to 20‰, which indicates that although the diagenetic sulfurization of humic substrates was demonstrated, there is a considerable fraction of biosynthetic organic S present in the sediment.

In conclusion, the results show that sulfurization of organic substrates occurs in both sediments, irrespective of the organic loading, although the sulfur species added to the organic substrate may be different.

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