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Isotope alteration caused by changes in biochemical composition of sedimentary organic matter

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Abstract Stable carbon (C) and nitrogen (N) isotope ratios of sedimentary organic matter (OM) can reflect the biogeochemical history of aquatic ecosystems. However, diagenetic processes in sediments may alter isotope records of OM via microbial activity and preferential degradation of isotopically distinct organic components. This study investigated the isotope alteration caused by preferential degradation in surface sediments sampled from a eutrophic reservoir in Germany. Sediments were treated sequentially with hot water extraction, hydrochloric acid hydrolysis, hydrogen peroxide oxidation and di-sodium peroxodisulfate oxidation to chemically simulate preferential degradation pathways of sedimentary OM. Residue and extracts from each extraction step were analyzed using elemental analyzer-isotope ratio mass spectrometry and solid-state ^{13}C nuclear magnetic resonance spectroscopy. Our results show that stable C and N isotope ratios reacted differently to changes in the biochemical composition of sedimentary OM. Preferential degradation of proteins and carbohydrates resulted in a 1.2‰ depletion of ^{13}C, while the isotope composition of ^{15}N remained nearly the same. Sedimentary $\delta^{15}N$ values were notably altered when lignins and lipids were oxidized from residual sediments. Throughout the sequential fractionation procedure, $\delta^{13}C$ was linearly correlated with the C:N of residual sediments. This finding demonstrates that changes in biochemical composition caused by preferential degradation altered $\delta^{13}C$ values of sedimentary OM, while this trend was not observed for $\delta^{15}N$ values. Our study identifies the influence of preferential degradation on stable C isotope ratios and

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provide additional insight into the isotope alteration caused by post-depositional processes.

Keywords Biochemical composition · Organic matter · Stable carbon and nitrogen isotope ratios · Preferential degradation · Sediment

Introduction

The biogeochemical cycling of organic carbon (OC), inorganic carbon and nutrients in aquatic ecosystems is affected by the properties of sedimentary organic matter (OM), which determine the degradation and burial of OM (Herczeg et al. 2001). Stable carbon (C) and nitrogen (N) isotope ratios, along with the C:N of sedimentary OM, serve as proxies for inferring the biogeochemical dynamics and for reconstructing paleo-environments in lakes and reservoirs (Meyers 1997). Because photosynthesis has multiple inorganic sources and pathways, aquatic and terrestrial sedimentary OM are different in original isotope composition (Meyers and Ishiwatari 1993; Ogrinc et al. 2005; Teodoru et al. 2013). Changes in environmental properties in sediments, such as redox conditions, microbiology and thermodynamics, can lead to a shift of the original isotope composition of sedimentary OM (Arndt et al. 2013). By interpreting depth profiles of stable C and N isotope ratios of OM in sediment cores, changes in lake productivity, trophic state, external discharge or water levels in freshwater or coastal ecosystems can be tracked (Brenner et al. 1999; Gu et al. 1996; Lamb et al. 2006; Woodward et al. 2012).

These isotope signals must be interpreted with caution, however, since early diagenesis may cause additional alterations in stable isotope ratios of sedimentary OM that could modify the original isotope signal (Meyers and Lallier-Vergès 1999; Schelske and Hodell 1995). Previous studies indicate that both aerobic and anaerobic degradation of OM can alter stable C and N isotope ratios in sediments (Freudenthal et al. 2001; McArthur et al. 1992). Similarly, enrichment of ^{15}N in residual sedimentary OM has been observed after microbial degradation of algae-derived OM (Altabet et al. 1995; Saino and Hattori 1980). Organic components with distinct stable isotope ratios exhibit different degradation kinetics in sediments (Fabiano et al. 1995; Guillemette et al. 2016; Hatcher et al. 2014; Hayes 1993; Hayes et al. 1990; Mahmoudi et al. 2017). Therefore, changes in their biochemical composition are often accompanied by alterations in stable isotope ratios during early diagenesis of sedimentary OM (Hatcher et al. 1983).

Organic geochemists have long studied mechanisms of early diagenesis-induced alterations in stable C and N isotope ratios of sedimentary OM. Macko and Estep (1984) examined the influence of microbial activity on the distribution of sedimentary stable C and N isotopes, testing microbial isotopic fractionation factors in different substrates. Their results demonstrated that changes in the biochemical environment of the sediment could mediate microbial isotope alterations. Lehmann et al. (2002) investigated diagenetic alterations in stable isotope ratios in anoxic incubation experiments and also hypothesized systematic effects of microbial activity and preferential degradation on stable C and N isotope ratios. Sun et al. (2016) argued that changes in the isotope composition of sedimentary OM represented the rate of OM decomposition rather than the consequence of early diagenesis in a high elevation alpine lake. In contrast, a 12-week in-site incubation experiment on the biochemistry of estuarine plants showed no significant stable C and N isotope alterations during decomposition (Lanari et al. 2018), thus indicating that isotope alterations may require more time.

It remains difficult to distinguish microbially mediated isotope fractionation from preferential degradation of individual organic components when isotope compositions of bulk sedimentary OM are altered during early diagenesis. Inconsistent results for the influence of microbial activity on stable isotope ratios are likely related to varying post-depositional environments (Freudenthal et al. 2001; Holmes et al. 1999; Lehmann et al. 2002). These unresolved issues make it difficult to assess the influence of preferential degradation on the bulk stable isotope ratios during early diagenesis. Researchers usually consider two or more mechanisms to explain alterations in stable isotopes during early diagenesis (Freudenthal et al. 2001; McArthur et al. 1992). Hence, studies of changes in biochemical composition associated with preferential

degradation but without bacterial effects would advance mechanistic understanding of the diagenetic alteration of sedimentary stable isotope ratios.

New insights into correlations between biochemical compositions and stable isotope ratios of sedimentary OM could provide information to support studies of diagenetic alteration of stable isotope ratios. In previous studies, early diagenesis of OM was described using chemical analysis such as solid-state ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectroscopy (Hatcher et al. 2014; Mao et al. 2011), or by determining stable isotope composition (McArthur et al. 1992). Combined ^{13}C-NMR and stable isotope analyses can provide information to explain mechanisms involved in early diagenesis of OM in lacustrine and estuarine sediments (Krull et al. 2009; Longbottom and Hockaday 2019). It is widely accepted that components of sedimentary OM are preferentially degraded in the following order: proteins, carbohydrates, carbonyls, lipids, lignins and chars (Arndt et al. 2013; Burdige 2007; Last and Smol 2006; Middelburg 2018; Wakeham et al. 1997).

We aimed to distinguish the effects of preferential degradation caused by microbial metabolism during early diagenesis of OM on sedimentary stable isotopes as indicators of the paleo-environment and paleolimnology. We performed a sequential extraction procedure and joint isotope and ^{13}C-NMR analyses of the sedimentary OM to simulate and clarify effects of preferential degradation. We hypothesized that preferential degradation can change the biochemical composition of OM and thus cause post-depositional alteration of stable C and N isotopes. Because of preferential degradation, stable C and N isotopes were expected to change asymmetrically, since differing degrees of microbial activity may be involved in the early diagenesis of OM.

Materials and methods

Field site and sediment sampling

This study was conducted in the Hassel and Rappbode pre-dams of the Rappbode Reservoir System in the Harz Mountains, Germany (51.74° N, 10.89° E, Fig. 1), which came into service in the 1960s to avoid heavy sediment and nutrient loads in the main reservoir. The Hassel pre-dam is a eutrophic water impoundment (Rinke et al. 2013) with a surface area, water capacity, maximum depth and water retention time of 0.288 km^2, 1.64 million m^3, 14 m and 65 days, respectively (Dadi et al. 2015). The Rappbode pre-dam is an oligotrophic impoundment with a surface area, water capacity, maximum depth and water retention time of 0.218 km^2, 1.66 million m^3, 17 m and 52 days, respectively (Friese et al. 2014). These systems have been the subject of previous studies of isotopes in the water column (Barth et al. 2017).

Sediments were collected with a simple grab sampler in April 2011 in the Hassel and Rappbode pre-dam reservoirs. The pH of sediments was 7–8. To simplify the simulated degradation system, sediments used for sequential fractionation in this study were an equal mixture of the top 20 cm material from 10 sampling points (5 from each pre-dam, Fig. 1). Aliquots of freeze-dried samples from the 10 sampling points were mixed by continuously rotating the material in a closed barrel. The homogeneity of the sample was confirmed by repeated analysis until the coefficient of variation in total C or N dry weight was less than 5%.

Sequential fractionation of sediments

The fractionation procedure consisted of four steps: (i) hot water extraction, (ii) hydrochloric acid (HCl) hydrolysis, (iii) hydrogen peroxide (H_2O_2) oxidation, and (iv) disodium peroxodisulfate ($Na_2S_2O_8$) oxidation (Fig. 2). In principle, hot water extracts proteins and carbohydrates that have high bioavailability (Haynes 2005; Leinweber et al. 1995; Sparling et al. 1998), while HCl preferentially hydrolyzes most amides, nucleic acids, polysaccharides and certain carboxyl compounds (Paul et al. 1997; Paul et al. 2006). Subsequently, both H_2O_2 and $Na_2S_2O_8$ oxidize aliphatic and aromatic organic compounds (Jagadamma et al. 2010; Mikutta et al. 2005; von Lützow et al. 2007). However, H_2O_2 is more effective in removal of alkyl compounds, while $Na_2S_2O_8$ tends to remove O/N-alkyl compounds (Helfrich et al. 2007). The C and N stable isotope ratios and biochemical compositions of all residue generated in this fractionation procedure were analyzed.

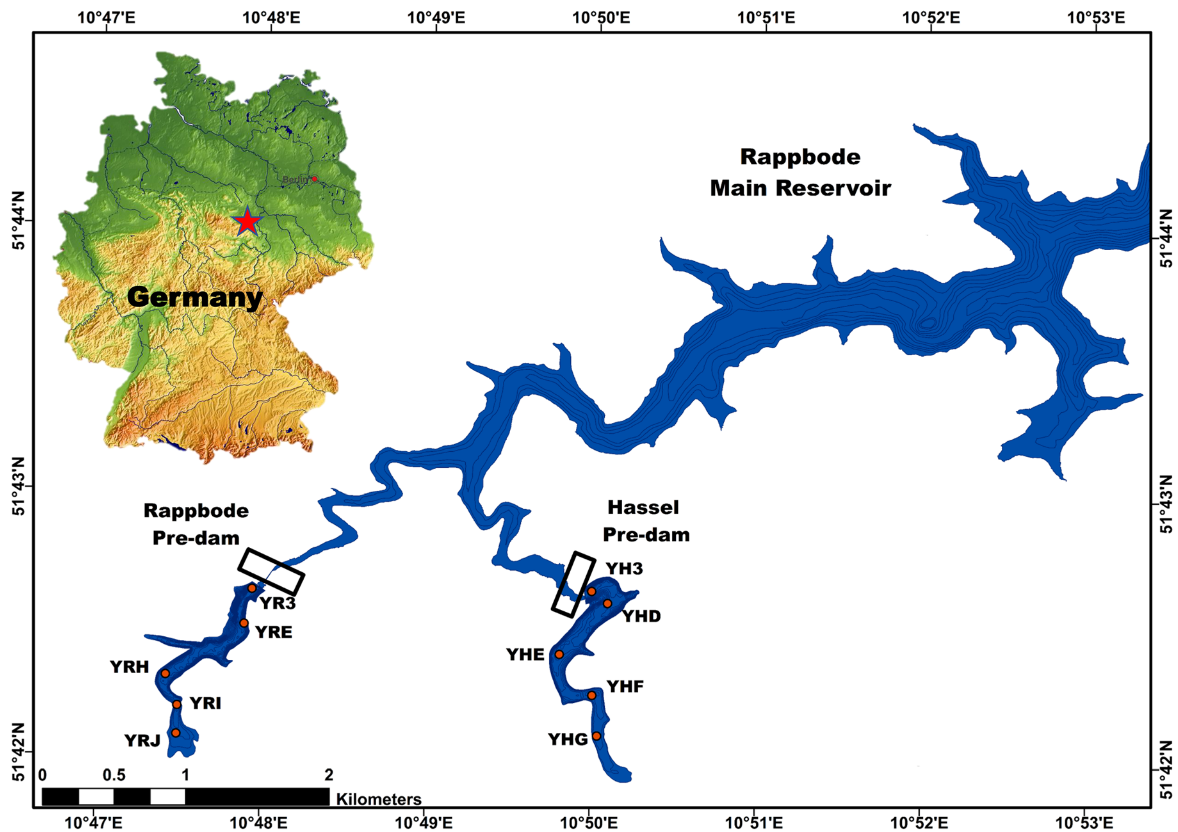


Fig. 1 Map of the Rappbode Reservoir System with sampling sites (red points) and location in Germany (top left). The geological data come from ATKIS1 DGM50 M745; Geobasisdaten© Vermessungsverwaltungen der Bundesländer und BKG (www.bkg.bund.de); The map was created using ArcMap (ESRI 2014). ArcGIS Release 10.2. Redlands, California USA). (Color figure online)

Extraction with hot water

Hot water extraction of sediments was modified following the method of Ghani et al. (2003). Three replicates were prepared for the extraction of sedimentary OM. Aliquots of dry and ground sediments (3 g) were suspended in 30 mL of distilled water in a 50 mL polypropylene centrifuge tube and mixed on a shaker for 30 min at 200 rotations per minute (rpm). The suspension was centrifuged at 3500 rpm at 20 °C for 20 min (Heraeus Labofuge 400R, Heraeus Quarzglas GmbH & Co. KG, Bitterfeld-Wolfen, Germany). The supernatant was collected, and the residue was suspended in 30 mL of distilled water. The residue and water were mixed on a vortex shaker (Multifunction Vortex Mixer Set VM-10, Witeg Labortechnik GmbH, Wertheim, Germany) for 30 s and then left in a water bath at 80 °C for 20 h. To release the hot water extractable organic compounds completely, centrifuge tubes were shaken for 60 s on a vortex shaker, followed by re-centrifuging at 3500 rpm at 20 °C for 20 min. The supernatant was filtered through a 0.45 µm cellulose nitrate membrane filters. The filtrate was mixed with the supernatant, and the filter residue was combined with the corresponding centrifugal residue. All residue was dried at 60 °C and ground manually with an agate mortar. The hot water-resistant residue was weighted and kept in a vacuum desiccator for analysis or additional treatments.

Acid hydrolysis with HCl

Three replicates (2 g each) of the hot water-resistant residue were placed in three 50 mL centrifuge tubes for hydrolysis with 6 M HCl (25 mL, Fig. 2). All tubes with suspension were placed on a shaker (TitroWiCo Orbital Shaker, Bochum, Germany), shaken at 200 rpm for 2 h, and then placed in a water bath at 80 °C for

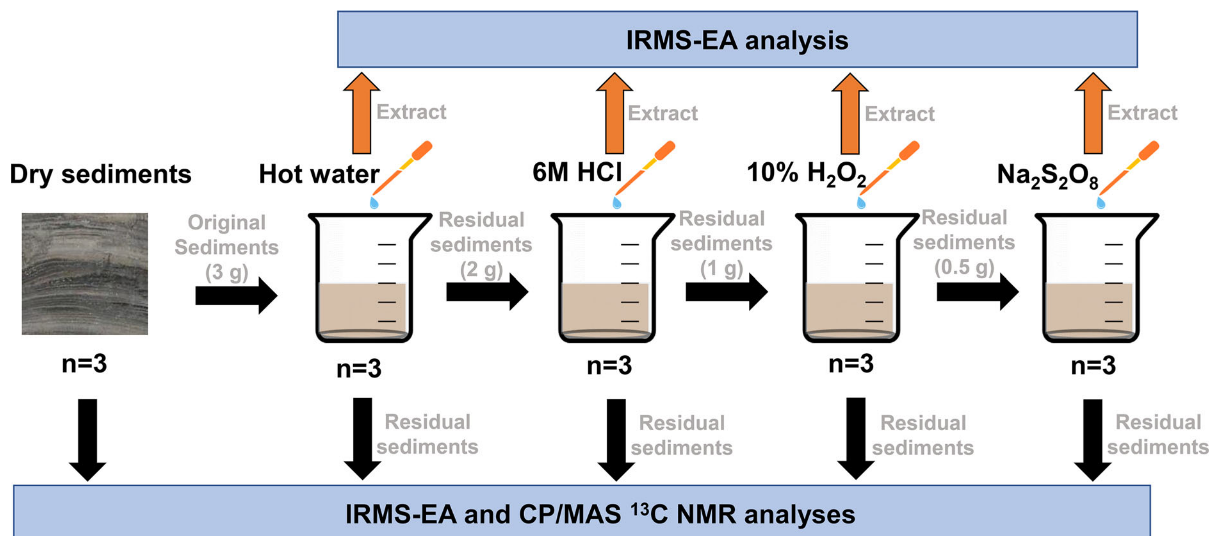


Fig. 2 Diagram of the sequential fractionation procedure. Three replicates were fractionated with successive extraction with hot water, hydrolysis with 6 M HCl, and oxidization with 10% H_2O_2 and $Na_2S_2O_8$. After each fractionation step, extracts and a portion of residual sediments were collected for analysis, and the other portion was maintained for additional fractionation until the end of the procedure. In the diagram, IRMS-EA and CP/MAS ^{13}C NMR are abbreviations for isotope ratio mass spectrometer coupled with elemental analyzer and cross polarization/magic angle spinning ^{13}C nuclear magnetic resonance, respectively

20 h (Silveira et al. 2008). Next, 6 M HCl resistant residue was separated from the supernatant by centrifuging at 3500 rpm at 20 °C for 25 min. The residue was repeatedly rinsed with 10 mL of distilled water and centrifuged (3000 rpm at 20 °C for 15 min). The last step was repeated three times. Centrifugation supernatant and rinses were combined in brown glass bottles and dried at 60 °C. The residue was ground into homogeneous powder and weighed before the next treatment. A portion of the residue (ca. 1 g) was collected for H_2O_2 oxidation, while the remaining material was kept in a vacuum desiccator for subsequent analysis.

Oxidation with H_2O_2

The H_2O_2 oxidation procedure was modified from the methods of Helfrich et al. (2007) and Jagadamma et al. (2010) to consider differences between soils and sediments. One gram of 6 M HCl resistant residue was suspended in 10 mL of distilled water in a 50 mL centrifuge tube. The first two doses of 10 mL 10% H_2O_2 were added to the suspension at an interval of 3 h. The mixed solution was kept in an ultrasonic bath to ensure that the reactants responded adequately. After thoroughly mixing the H_2O_2- and HCl-resistant residue, and the centrifuge tubes were placed in a water bath at 50 °C overnight. The last dose of H_2O_2 (10%, 10 mL) was added, and the tubes were left in the water bath (50 °C) until the suspensions stopped effervescing. The suspensions were shaken on a vortex shaker for 60 s to separate the oxidized OM completely from the residue. The residue was isolated by centrifuging (3500 rpm at 20 °C, 20 min), washed with 10 mL of 10% H_2O_2 and placed in a water bath at 50 °C for 2 h to react. After re-centrifugation of the suspension, the residue was washed three times with 10 mL of distilled water. The centrifugation supernatant, and the H_2O_2 and distilled water rinses were collected for the analysis of stable C isotope ratio. The H_2O_2-resistant residue was dried in an oven at 45 °C and ground for analysis or was retained for the fourth step of the fractionation procedure.

Oxidation with $Na_2S_2O_8$

The residue that had been treated with hot water, HCl and H_2O_2 was then oxidized by $Na_2S_2O_8$. Specifically, 0.5 g of H_2O_2-resistant residue was suspended in 40 mL of Milli-Q water in an ultrasonic bath for 20 min. The suspension was mixed with 4 g of $Na_2S_2O_8$, and the reaction was simultaneously buffered with 4.4 g

$NaHCO_3$ (Helfrich et al. 2007; Lorenz et al. 2008). H_2O_2-resistant residue and chemical reagents were mixed completely on a vortex shaker before being placed in a water bath at 80 °C for 18–24 h, until the effervescence stopped. The centrifuge tubes were again placed on a vortex shaker and then centrifuged at 3500 rpm at 20 °C for 20 min. The supernatant was collected, and the residue was rinsed three times with 10 mL of MilliQ water. Subsequently, rinses were centrifuged (3500 rpm at 20 °C for 10 min) to further separate and purify the residue. To avoid retaining carbonate in the moist residue, the residue was dried and homogenized and then acidized with 20 mL of 1 M HCl for 18 h while being agitated at 180 rpm at 25 °C. The suspension was re-centrifuged at 3500 rpm at 20 °C for 20 min, and the residue was rinsed with Milli-Q water until the pH exceed 6. All supernatants and rinses were collected and combined. Residue was dried at 40 °C and then homogenized for isotope ratio and ^{13}C-NMR analyses.

Analysis of sedimentary organic matter in residue and extracts

Total OC (TOC), total N (TN) and stable C and N isotope ratios of chemically resistant residue were measured. In addition, extracts from the fractionation procedure were analyzed for TOC and stable C isotope composition.

Analysis of elemental and stable isotope ratios

Extracts were analyzed with an isotope ratio mass spectrometer (IRMS, Thermo Fisher Delta V Plus, Bremen, Germany) coupled in continuous-flow mode with an OI Analytical Aurora 1030W TOC analyzer (College Station, Texas, USA). Elemental (C, N) and stable isotope $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of the original sediments and chemically resistant residue were analyzed using a Flash 2000 elemental analyzer coupled with a Delta V Advantage IRMS (Thermo Fisher Scientific, Bremen, Germany). The original mixed sediments ("Field site and sediment sampling" section) with known elemental and isotope compositions were used as reference materials to test the stability of the instruments. Reference materials run after the measurements in this study were interspersed with analyses of samples from other studies. For this procedure, 4 mg of residual sediments were packed into a tin capsule, which was then closed and crimped. Contents of C and N were measured as percentages (by weight), with a standard deviation of ± 0.08% and 0.01% for C (n = 6) and N (n = 6) in standards, respectively. These percentages were converted into absolute C and N mass per gram of dry sediments (mg g^{-1}), and the corresponding atomic C:N was calculated.

Stable isotope composition was converted into $\delta^{13}C$ and $\delta^{15}N$ using the following equation:

$$\delta(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$and δ is the isotope ratio of ^{13}C or ^{15}N relative to the international reference materials: Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Standard deviations of isotope measurements of sediment standards were less than ± 0.1‰ for C (n = 6) and ± 0.2‰ for N (n = 6).

Analysis of biochemical compositions

The compositions of original and residual sediments were analyzed using solid-state ^{13}C-NMR spectroscopy (Bruker Avance 300, Bruker Biospin, Bremen, Germany). The cross polarization/magic angle spinning (CP/MAS) ^{13}C-NMR spectra were obtained at a ^{13}C frequency of 75.47 MHz. The spectroscope was equipped with a 4 mm MAS probe head (product number: Bruker BL4). The 90° proton pulse was set at 3.3 μs, and the decoupling strength during acquisition was 69 kHz. The following conditions were applied: spinning rate of 15 kHz, recycle delay of 3 s and contact time of 2 ms. The samples were then introduced into 4 mm zirconium oxide rotors.

A line-broadening function (LB = 1 Hz) was used for Fourier transformation. Chemical shifts were externally referenced to the glycine resonance at 176.03 ppm. Chemical shift regions of − 10 to 45, 45 to 60, 60 to 95, 95 to 110, 110 to 145, 145 to 160 and 160 to 210 ppm were assigned to alkyl compounds; N-alkyl-methoxy compounds; O-alkyl compounds; O_2-alkyl compounds; aromatic compounds; O-aromatic structures with phenols; and carboxyl or carbonyl compounds, respectively (Pane et al. 2013; Rodríguez-Murillo et al. 2011). The ^{13}C-NMR spectral areas were integrated according to the chemical shift regions to assess the relative contributions of

these functional groups. The main resonance peaks were referred to their typical spectroscopic signals of chemical compounds (Hatcher 1987; KoÈgel-Knabner 2002).

Using a molecular mixing model to estimate biochemical composition

The mixing model of Nelson and Baldock (2005) was used to infer the biochemical composition of sedimentary OM. It categorized OM into six components: carbohydrates, proteins, lignins, lipids, carbonyls and char. The model assumed that these six components were linearly combined and then tested for the best fit to the area-integration results of ^{13}C-NMR spectra. For example, the acquired percentages of several functional groups (Table 2) and the C:N of residual sediments (Fig. 3) were used to constrain the model. This model was used to estimate the percentages of these six organic components in the residual sediments at each step of the sequential extraction procedure. The biochemical compositions of two consecutive extraction steps derived from the ^{13}C-NMR spectra were compared to estimate the removal of organic components by the latter step.

Statistical analysis

The effectiveness of each extraction step was estimated as the percentage of TOC and TN contents extracted by each extraction step relative to the TOC and TN contents in the original sediments. The effectiveness of this procedure was also estimated by changes in δ^{13}C and δ^{15}N values and the percentages of various components between two consecutive extraction steps. A Student's *t*-test (unpaired) was used to compare the extraction effectiveness between two extraction steps using R software (RCore Team 2013). Differences were considered significant at $p < 0.05$. The correlation between C:N and δ^{13}C or δ^{15}N values was tested by a Pearson correlation analysis using the correlation test package of R Studio. Correlations were considered significant at $p < 0.05$. All results were calculated as a mean ± standard deviation (1σ) of three replicate samples.

Results

Variations in stable isotopic compositions of sedimentary organic matter

As the fractionation procedure progressed, OC and N contents of the residual sediments decreased gradually (Fig. 4). The OC content in hot water-resistant residue decreased by 10.8 mg g^{-1} after 6 M HCl hydrolysis. During H_2O_2 oxidation OC was oxidized to CO_2 rather than dissolved in solvents, thus C concentration in the H_2O_2 extracts was low (Table 1). The oxidation with H_2O_2 decreased OC content by an additional 16.3 mg g^{-1}. Similarly, $Na_2S_2O_8$ further oxidized the OC in the H_2O_2-resistant residue, which resulted in an extremely low C concentration in the $Na_2S_2O_8$ extracts (Table 1). Overall, hot water, HCl, H_2O_2 and $Na_2S_2O_8$ extraction removed ca. 5%, 18%, 27% and 44% of TOC, respectively.

Reaction with 6 M HCl removed organic N effectively, decreasing the 5.4 mg N g^{-1} in hot water-resistant to 2.8 mg N g^{-1}, which accounted for about half of the TN in original sediments. In further steps, H_2O_2 and $Na_2S_2O_8$ oxidation decreased N content by 1.0 and 1.2 mg g^{-1}, respectively. Hot water, HCl, H_2O_2 and $Na_2S_2O_8$ extraction removed 8%, 45%, 16% and 17% of TN from original bulk sediments, thus indicating that hot water extraction and 6 M HCl hydrolysis removed larger proportions of N than OC. Consequently, the C:N of residual sediments increased with each extraction step until OC was extensively oxidized by $Na_2S_2O_8$ (Fig. 3a).

Compared to the original bulk sediments, the δ^{13}C value of residual sediments that were treated by hot water decreased by 0.2‰ while the δ^{15}N value increased by 0.5‰ (Fig. 3b and c). Both changes associated with hot water extraction in δ^{13}C and δ^{15}N values were significant ($p < 0.05$), as indicated by a Student's *t*-test. The changes in residue δ^{13}C values coincided with the changes in δ^{13}C values between the original bulk sediments and the hot water extracts (Table 1). Hydrolysis with 6 M HCl decreased δ^{13}C and δ^{15}N values significantly (by 1.0 and 0.7‰, respectively, both $p < 0.005$, Student's *t*-test, Fig. 3b and c), which was consistent with the higher δ^{13}C values (− 27.2‰) of HCl extracts (Table 1). The oxidation with H_2O_2 oxidation decreased δ^{13}C values in residue slightly (Fig. 3b) but decreased δ^{15}N in

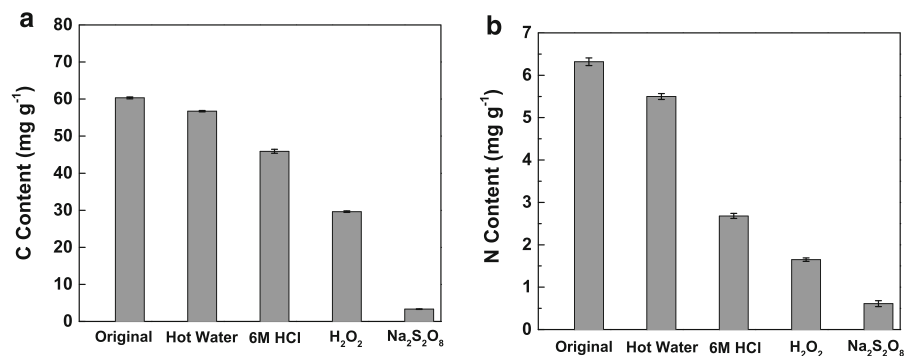


Fig. 3 **a** Carbon (C) and **b** nitrogen (N) contents in residual sediments during the sequential fractionation procedure. Error bars represent one standard deviation of three replicates samples

residue significantly (by 1.3‰, $p < 0.05$, Student's *t*-test, Fig. 3c), which suggests that the $\delta^{13}C$ value of H_2O_2-oxidizable OM was similar to that of 6 M HCl non-hydrolysable OM. Since $Na_2S_2O_8$ oxidized nearly all OC, the $\delta^{13}C$ value of its residue increased from − 29.4‰ to − 27.2‰ ($p < 0.001$, Student's *t* test, Fig. 3b), and its $\delta^{15}N$ value also decreased by a similar degree (5.2‰, Fig. 3c).

Solid-state ^{13}C-NMR analysis of sedimentary organic matter

With solid-state CP/MAS ^{13}C-NMR analysis, representative organic compounds in the original and residual sediments were identified by typical signal peaks of chemical shifts (Fig. 5). The signal peak at 22 ppm may have corresponded to long-chain methyl and methylene OC in fatty acids, waxes and resins (Jagadamma et al. 2010; Vane et al. 2005). Based on previous findings, the peak at 65 ppm was in the overlapping regions of chemical shifts, which represented for proteinaceous C or carbohydrates (Baldock et al. 1992).

The O/O_2-alkyl C that peaked at 87 ppm and 97 ppm were the most prominent polysaccharides or carbohydrates with anomeric C (Baldock et al. 2004; Vane et al. 2005). Resonance peaks at 121 ppm with shoulders were interpreted as a signal of lignins (Hatcher 1987), while a peak at 166 ppm was interpreted as to carboxyl, ester and amide compounds (Monteil-Rivera et al. 2000).

After the 6 M HCl hydrolysis and H_2O_2 oxidation, the peak at 22 ppm in the ^{13}C-NMR spectra of residual sediments remained and became sequentially sharper (Fig. 5), due to a change in biochemical composition or the removal of paramagnetic impurities. Although the peak at 121 ppm strengthened after the 6 M HCl hydrolysis, it decreased after H_2O_2 oxidation (Fig. 5). Sedimentary OM was transformed during the fractionation, since three peaks that occurred after 6 M HCl hydrolysis (in the vicinities of 48, 65 and 79 ppm) were changed to a broad peak at 65 ppm after the H_2O_2 oxidation (Fig. 5). The oxidation of H_2O_2-resistant residue with $Na_2S_2O_8$ resulted in ineffective cross-polarization of the residual sediments, which led to a non-identifiable signal in the ^{13}C-NMR spectrum (Fig. 5).

The chemical shift regions in the ^{13}C-NMR spectra were integrated to quantify functional groups in the original and residual sediments (Table 2). About 40%, 30% and 21% of OC in the original bulk sediments was alkyl C; substituted alkyl C; and aromatic and O-aromatic C, respectively. Hot water extraction changed the chemical structure of sedimentary OM slightly (Fig. 5), decreasing the proportions of N-alkyl, O-alkyl and O-aromatic C by 1.4, 1.8 and 1.5 percentage points, respectively. In the ^{13}C-NMR spectra of 6 M HCl-resistant residue, the percentage of alkyl C increased by ca. 10 percentage points, while the total percentage of O-alkyl and carboxyl C decreased by the same amount. The alkyl C percentage in the 6 M HCl resistant residue increased considerably (ca. 10 percentage points) after H_2O_2 oxidation. The percentage of aromatic C steadily increased with the hot water and HCl extraction, but decreased after H_2O_2 oxidation. Since the noise in the spectrum of $Na_2S_2O_8$ resistant residue was high, the integrated results were not shown in Table 2.

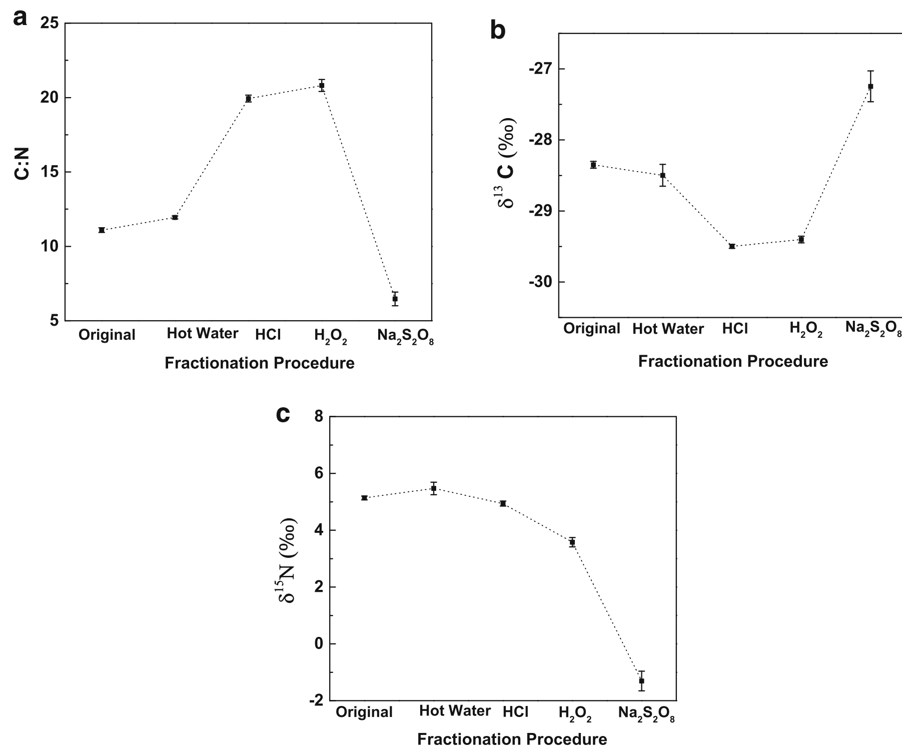


Fig. 4 **a** The atomic carbon-to-nitrogen ratio (C:N), **b** stable C isotope ratio and **c** stable N isotope ratio of residual sediments at each step of the sequential fractionation procedure. Error bars represent one standard deviations of three replicate samples

Table 1 Mean (± standard deviation) carbon concentration and stable C isotope composition of replicate samples (n = 3) of extraction solutions

Organic matter	C (mmol L^{-1})	$\delta^{13}C$ (‰)
Hot water	33.7 ± 3 .1	− 28.1 ± 0.1
6 M HCl	86.6 ± 5.2	− 27.2 ± 0.1
10% H_2O_2	15.3 ± 5.4	− 26.2 ± 0.5
$Na_2S_2O_8$	1.2 ± 0.0	− 24.3 ± 0.4

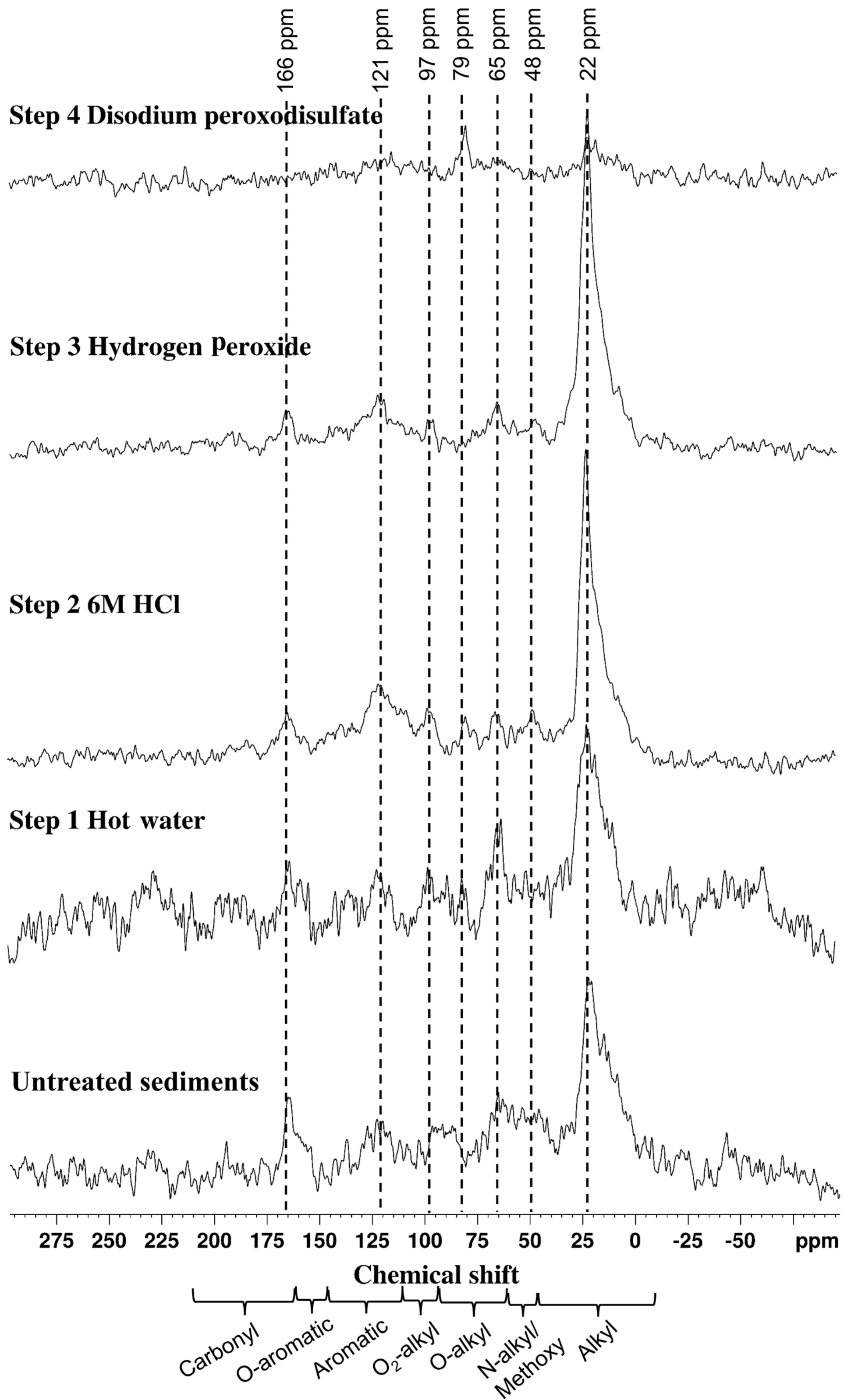
Changes in the biochemical composition of sedimentary organic matter

Although quantitative characterization of sedimentary OM could not be obtained by solid-state CP/MAS ^{13}C-NMR analysis, the mixing model helped to convert signals in the spectrum of each extraction residue into the biochemical composition of sedimentary OM. The model predicted percentages of six major components of OM in the original and residual sediments (Fig. 6), suggesting that chars and carbonyls were absent in the original sediments. Sedimentary OM consisted mainly of lipids, proteins and lignins, and less than 15% of carbohydrates. Since hot water extracted a small fraction of proteins and carbohydrates, the percentage of lipids in the residual sediments increased to nearly 40%. The biochemical composition of hot water non-extractable OM differed little from the original sedimentary OM. A substantial reduction of proteins and carbohydrates and proteins decreased substantially (from 37 to 16%) after 6 M HCl hydrolysis. After oxidation of lignins and complex proteins using H_2O_2, the percentage of lipids in residual sediments was twice that in the original bulk sediments.

Discussion

Using sequential chemical fractionation to remove sedimentary organic matter

Sequential treatment of sediments with hot water, HCl, H_2O_2 and $Na_2S_2O_8$ changed the chemical


166 ppm
121 ppm
97 ppm
79 ppm
65 ppm
48 ppm
22 ppm
Step 4 Disodium peroxodisulfate
Step 3 Hydrogen Peroxide
Step 2 6M HCl
Step 1 Hot water
Untreated sediments
275 250 225 200 175 150 125 100 75 50 25 0 -25 -50 ppm
Chemical shift
Carbonyl
O-aromatic
Aromatic
O2-alkyl
O-alkyl
N-alkyl/
Methoxy
Alkyl

◀ **Fig. 5** Solid-state cross-polarization/magic-angle-spinning ^{13}C nuclear magnetic resonance spectra of original bulk sediments and residual sediments obtained from the sequential fractionation procedure. Spectra were analyzed and stacked using Bruker TopSpin4.0.6

characteristics of residual sediments. After the final step of the chemical fractionation procedure, 95% of TOC and 90% of TN were removed from the original bulk sediments. Labile proteins and carbohydrates in sediments were extracted first using hot water and HCl, while lipids and lignins were sequentially removed using H_2O_2 and $Na_2S_2O_8$.

Although we did not analyze hot water-extracted proteins and carbohydrates, previous studies (Heller and Weiss 2015; Haynes and Francis 1993; Leinweber et al. 1995) consistently demonstrated the microbial origin of this OM fraction. Inherent structural complexity may be one explanation for the existence of proteins and carbohydrates in HCl-resistant residue that were hot water-insoluble but HCl-hydrolysable (Fig. 6). This structural complexity makes these molecules more stable than water soluble ones. Silveira et al. (2008) observed a similar difference when comparing hot water-extractable C and HCl-hydrolysable C. However, it is more likely that some of proteins and carbohydrates extracted using HCl have a chemistry similar to that of the OM extracted by hot water. If so, they may have been physically protected by encapsulation within biogenic minerals or attachment to a mineral matrix or surfaces of organic macromolecules (Ingalls et al. 2004; Ingalls et al. 2003). This hypothesis is supported by the nearly unchanged ^{13}C-NMR spectra (Fig. 5) and stable isotope ratios after hot water extraction (Fig. 3).

Lipids extracted using H_2O_2 and $Na_2S_2O_8$ are considered to have different structures, as indicated by the shifts of resonance peaks in the ^{13}C-NMR spectra after H_2O_2 and $Na_2S_2O_8$ residue oxidations. Moreover, the chemical resistances of H_2O_2 and $Na_2S_2O_8$-oxidized lipids can be differentiated, because they counteract H_2O_2 oxidation differently. These findings are supported by the similar study of Jagadamma et al. (2010) who revealed distinct structures of H_2O_2- and $Na_2S_2O_8$-oxidized lipids using ^{13}C-NMR analyses of extracted soil OM.

In the early diagenesis of sedimentary OM, proteins and carbohydrates are susceptible to microbial reformation and decomposition due to their weak chemical bonds and high bioavailability (Arndt et al. 2013; de Leeuw and Largeau 1993). The stability of lipids varies greatly in sediments and is determined by the degree of saturation and stereochemical and structural properties (Middelburg 2019). Nonetheless, most lipids are less degradable in comparison with proteins and carbohydrates. For lignins, primary studies have reported their limited degradability in lacustrine sediments, which is due to their refractory nature in anoxic environments after deposition (Ishsiwatari and Uzaki 1987; Louchouarn et al. 1997; Szklarz and Leonowicz 1986). In general, the sequential removal of sedimentary OM by our fractionation procedure is consistent with the preferential degradation of organic components in sediments as revealed by the stable isotope composition and ^{13}C-NMR analyses. Thus, the fractionation procedure can be interpreted as simplified early in-situ diagenetic processes.

Table 2 Relative distribution of organic carbon (OC) functional groups in residual sediments using the fractionation procedure. Data are the integrated results of the ^{13}C-NMR spectra. Results for the $Na_2S_2O_8$-resistant residue are not shown due to the low signal-to-noise ratio of the ^{13}C-NMR spectrum.

Functional group	Untreated (%)	Hot water extraction (%)	6 M HCl hydrolysis (%)	H_2O_2 oxidation (%)
Alkyl C	40.2	40.9	50.4	60.9
N-alkyl/methoxy C	9.2	7.9	5.8	4.5
O-alkyl C	17.2	15.3	8.5	8.4
O_2-alkyl C	4.5	4.9	7.9	4.5
Aromatic C	10.5	13.9	15.5	11.8
O-aromatic C	10.6	9.1	7.4	4.9
Carboxyl C	7.9	8.0	4.4	5.0

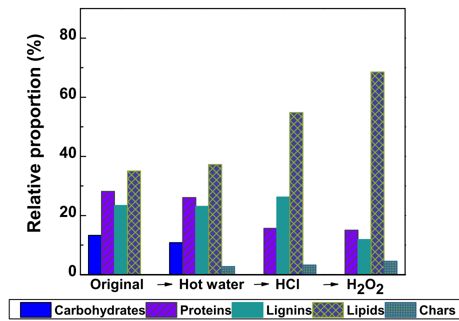


Fig. 6 Estimated percentages of carbon assigned to the main organic components in residual sediments. Data were derived from results of ^{13}C nuclear magnetic resonance analyses, which were calculated using a mixing model of Nelson and Baldock (2005)

Influence of biochemical composition on stable isotope ratios

Early diagenesis of sedimentary OM involves selective preservation of a succession of compounds as well as a vast range of enzymatic reactions, including microbial degradation of labile components and growth of bacteria (Harvey et al. 1995; Hedges and Keil 1995).

Thus, preferential removal of N- or C-enriched organic compounds will change the biochemical composition and the C:N of OM in residual sediments. Moreover, labile organic components (proteins and carbohydrates) are generally enriched in ^{15}N and ^{13}C, while more stable components (lipids and lignins) are relatively depleted in these isotopes (Böttcher et al. 1998; Hobbie and Werner 2004; Ogrinc et al. 2005; Rieley et al. 1991). Preferential degradation of proteins and carbohydrates during early diagenesis would enrich the lighter isotopes and thus decreased the isotope composition of ^{15}N and ^{13}C in the residual OM.

Pearson correlation test revealed that δ^{13}C values of the original bulk sediments and residual sediments are negatively and linearly correlated to C:N ($r^2 = 0.95$, $p < 0.005$, Fig. 7a). Theoretically, the gradual depletion of ^{13}C during early in-situ diagenesis of sediments can be attributed to (i) preferential degradation of isotopically heavy OM components, (ii) formation of isotopically light OM components (e.g. for bacterial growth) and (iii) isotopic fractionation related to hydrolysis of reactive OM components (Freudenthal et al. 2001; Meyers and Ishiwatari 1993). Although the influence of each mechanism was difficult to distinguish in our study, the ^{13}C-NMR and isotope ratio analyses of residue indicate the influence of preferential removal of N-enriched proteins and OC-enriched carbohydrates on the stable isotope composition of bulk sediments. For example, during the oxidation of lignins and lipids by H_2O_2 and $Na_2S_2O_8$, the δ^{13}C value increased greatly (Fig. 3b) and the biochemical composition of the residual sediments changed (Fig. 6). Therefore, it is reasonable to assume that changes in biochemical composition are closely related to the stable C isotope composition of sedimentary OM.

The correlation between δ^{15}N and C:N was not significant ($p > 0.05$, Fig. 7b). The lack of correlation between δ^{15}N and C:N is consistent with previous studies of other lacustrine and marine sediment samples (Hassan et al. 1997; Ogrinc et al. 2005; Woodward et al. 2011). No evident shifts in the δ^{15}N value (less than 0.2‰) were observed after hot water extraction and 6 M HCl hydrolysis, despite the substantial change in the C:N of residual sediments (Fig. 7b). The nearly invariable δ^{15}N value in the present study indicates that changes in biochemical composition do not fundamentally alter the stable N isotope composition of bulk sediments. It also agrees well with Lehmann et al. (2002), who attributed the decline of sedimentary δ^{15}N value primarily to the addition of a ^{15}N-depleted substrate caused by bacterial growth rather than the preferential degradation of proteins. Alternatively, the results of the present study help to interpret the increase in δ^{15}N value as being a consequence of isotopic fractionation processes during deamination (Freudenthal et al. 2001). Our findings supplement laboratory studies that indicate alteration of isotope signals associated with microbial growth and metabolism (Freudenthal et al. 2001; Lehmann et al. 2002).

Implications for the application of stable carbon and nitrogen isotope ratios in research

Stable C and N isotope compositions in lacustrine sediments may provide information about past nutrient limitations (Brenner et al. 1999), primary production (Teranes and Bernasconi 2000) and early diagenesis in aquatic ecosystems (Macko et al. 1994). They may also record extreme weather and

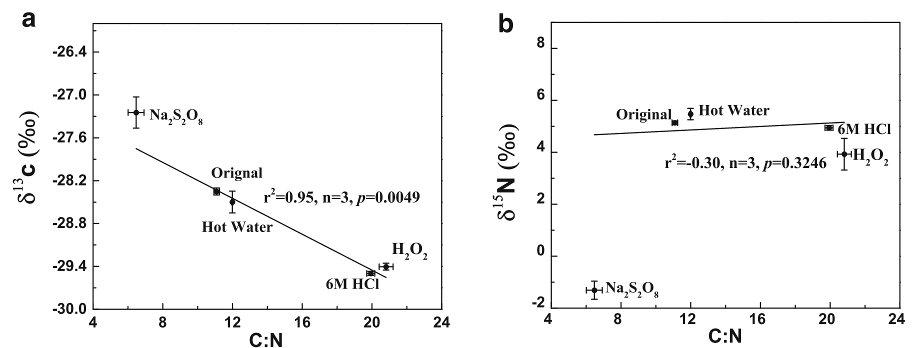


Fig. 7 Correlation between stable carbon (**a**) or nitrogen (**b**) isotope ratio and atomic carbon-to-nitrogen ratio (C:N) in original and residual sediments from the fractionation procedure (hot water, HCl, H_2O_2 and $Na_2S_2O_8$). Error bars represent one standard deviations of three replicate samples

vegetation successions in watersheds (Leng and Marshall 2004; Meyers 2003). Nonetheless, since the 1980s, organic geochemists have observed that diagenetic processes can modify stable isotope signals in lacustrine and marine sediments (Hatcher et al. 1983; Spiker and Hatcher 1984). The present study demonstrates that changes in biochemical composition (Fig. 6) have a strong influence on bulk C:N and stable C isotope compositions, causing a shift up to 1 per mil point (Fig. 3b). The magnitude of shifts in stable isotope ratios induced by preferential degradation is similar to those of isotope alterations, which are interpreted as changes in paleo-environments. For example, a decrease of 1.3‰ in the $\delta^{13}C$ value was observed in a sediment core from the Rochester Basin of Lake Ontario for the period 1980 to 1990. This decrease has been explained exclusively as a decline in productivity in this region (Hodell and Schelske 1998). The present study, however, demonstrates that preferential degradation of isotopically light OM components during early diagenesis can also explain the decrease in the $\delta^{13}C$ value in the core.

The $\delta^{15}N$ value decreased by only 0.2‰ (Fig. 3c) after more than half of the N-containing organic compounds had been extracted using hot water and HCl (Fig. 4b). The decrease in $\delta^{15}N$ increased to 1.6‰ after another 16% of TN from recalcitrant lignins and lipids had been removed using H_2O_2 under oxic conditions (Figs. 3c, 4b). This degree of removal of this recalcitrant TN fraction during in-situ diagenesis is unusual due to the absence of oxygen in the sediment in nature. Consequently, in-situ diagenesis may not shift the stable N isotope composition appreciably. In principle, our results suggest that preferential degradation of proteins and carbohydrates has a limited influence on the stable N isotope composition of bulk sediments. However, as previous studies demonstrated, microbial metabolism involved in early diagenesis can shift $\delta^{15}N$ values by up to 4‰ (Macko and Estep 1984; Meyers and Ishiwatari 1993; Sigman et al. 1999). Thus, it is possible that diagenetic alterations in stable N isotope ratios mask the initial isotope information of sediments and thus complicate application of $\delta^{15}N$ in paleo-environmental research.

Conclusions

Diagenetic processes in sediments may alter paleo-environmental signals from stable isotope ratios and thus hinder accurate interpretations. In particular, preferential degradation of isotopically heavy proteins and carbohydrates results in a post-depositional alteration of stable C isotope ratios. This effect might become large enough to interfere with isotope records used for paleo-environmental reconstruction. The strong correlation between $\delta^{13}C$ and C:N values of residual sedimentary OM during sequential fractionation processes implies that stable C isotope ratios depend greatly on the biochemical composition of OM in sediments. In contrast, degradation during early diagenesis seemed an unlikely primary process for mediating alterations in stable N isotope ratios after deposition. As alternative explanations, we suggest microbial growth and metabolism.

Our simulation of preferential degradation of sedimentary OM identified the complex mechanisms that alter stable C and N isotope ratios during early diagenesis. Stable isotope measurements combined with chemical structure analyses provide insights into mechanisms that govern diagenetic alteration of sedimentary OM. Overall, we suggest future studies that focus more on paleo-signal formation and its diagenetic alteration.

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