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The potential distortion of sedimentary $\delta^{15}N$ and C_{org}/N ratios by NH_4^+ and the effects of pre-analysis sample treatment

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Abstract

We examined the susceptibility of $\delta^{15}N$-signals and C_{org}/N ratios in organic-rich sediments to pre-analysis sample treatment. Each sample was subjected to three different kinds of processing. For comparative purposes, the first measurement series (MS-1) was carried out on untreated sediment. In MS-2, the sediment was rinsed with distilled water. In MS-3, analyses were carried out on decalcified and rinsed material, in MS-4 the samples were decalcified without being subsequently washed. The sediment yielded conspicuously different results depending on the type of processing it was subjected to. Rinsing, irrespective of whether acidification was included or not, induced substantial modifications in $\delta^{15}N$ accompanied by a pronounced loss of NH_4^+ (up to 14 wt% of the initial N-content). Molar C_{org}/N ratios, on the other hand, were only affected by a combination of acidification and rinsing. The discrepancies are ascribed to the influence of decomposition-derived ammonium (NH_4^+). In untreated sediment (MS-1), NH_4^+ seems to produce misleading shifts in both $\delta^{15}N$-signals and C_{org}/N ratios. Presumed mechanisms involved are as follows: Firstly, nitrogen isotopes fractionate during NH_4^+-volatilisation in the heating oven, where the sediment is put to desiccate. Secondly, NH_4^+-ions are able to escape that fractionation when adsorbed to negatively-charged SiO_2-surfaces. The adsorption capacity of SiO_2 increases with increasing pH of the pore water and hence with increasing carbonate content. Our findings raise serious doubts about whether untreated sediment (MS-1) can provide reliable C_{org}/N and $\delta^{15}N$-records. Pre-analysis acidification plus rinsing (MS-3) seems to eliminate the deceptive influence of NH_4^+-adsorption and -outgassing.

Key Words

Introduction

Nitrogen isotope abundance ratios ($\delta^{15}N$) and C/N ratios are both important instruments in the study of marine food webs. Their value – as with every proxy indicator – depends on the reliable recording of the original phenomenon in the absence of the direct measure. In this regard, the deceptive influence of sample preparation on the original C/N ratios and $\delta^{15}N$-signals is of great interest and has already been addressed by several authors (Nieuwenhuize et al. 1994; Bunn et al. 1995; Holmes et al. 1999; Jacob et al. 2005; Kennedy et al. 2005; Carabel et al. 2006; Ng et al. 2007).

Various studies have shown, for instance, that the removal of carbonate via acidification not only alters $\delta^{13}C$ but may likewise bias $\delta^{15}N$ (Bunn et al. 1995; Jacob et al. 2005; Carabel et al. 2006; Ng et al. 2007). As a consequence, many authors now refrain from pre-analysis decalcification when measuring $\delta^{15}N$ and prefer to conduct independent measurements using untreated subsamples (Goering et al. 1990; Thornton & McManus 1994; Bunn et al. 1995; Bouillon et al. 2002; Carabel et al. 2006).

Our findings clearly indicate that this approach is not applicable to all kinds of material. The goal of this study is to investigate the effects of common processing methods on the organic-rich sediments from the central Namibian shelf and to establish which can be most trusted with respect to the $\delta^{15}N$- and C/N record.

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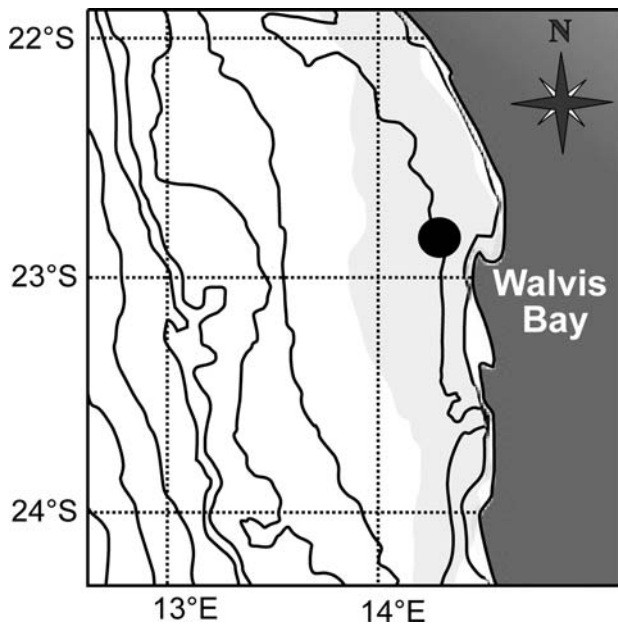
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Figure 1. The provenance of core 180 (22.88°S/14.41°E; water depth 48 m). The organic-rich diatomaceous ooze coating the inner shelf is shaded grey.

Material and methods

Core 180

Core 180 was retrieved from the northern Benguela Upwelling area during METEOR cruise M57-3 (15/03/2003 to 08/04/2003) (Fig. 1). The 5 m long core consists of organic-rich diatomaceous ooze (average opal 54.3 %, average organic matter 9.8 %, average carbonate 5.9 %; Bremner & Willis 1993), which coats the inner shelf as a belt running parallel to the coast at a depth of up to 150 m (Shannon & O'Toole 1998; Struck et al. 2002; Brüchert et al. 2004). Its presence testifies to the high productivity of the region. The deposit has an average thickness of 5.1 m, an estimated age of 5000 years with a mean sedimentation rate amounting to 1 mm/a (Bremner & Willis 1993).

The total organic carbon content of core 180 averages 5.5 wt%. The sediment is primarily made up of phytoplankton remains, mainly diatom frustules (Vavilova 1990; Graml 2001). This explains the high percentage of biogenic silica (opal) and why unaltered δ¹⁵N should more or less correspond to the isotopic signature of the first trophic level. A dilution by higher trophic levels as well as terrestrial detritus (i.e. clay minerals) is considered negligible (Holmes et al. 1998; Pichevin et al. 2005).

Sample processing and isotopic measurements

A total of 100 syringe samples were taken at 5 cm-intervals. The sediments were desiccated in a heating oven (40 °C) and homogenised using an agate mortar. Each sample was subsequently split into four parts with each subsample being subjected to an individual pre-analysis sample treatment (Fig. 2).

(1) The first measurement series (MS-1) was carried out on ± 8 mg of untreated sediment wrapped in tin-foil cups. [N] and [TC] (total carbon) measured relate to the bulk sediment volume.
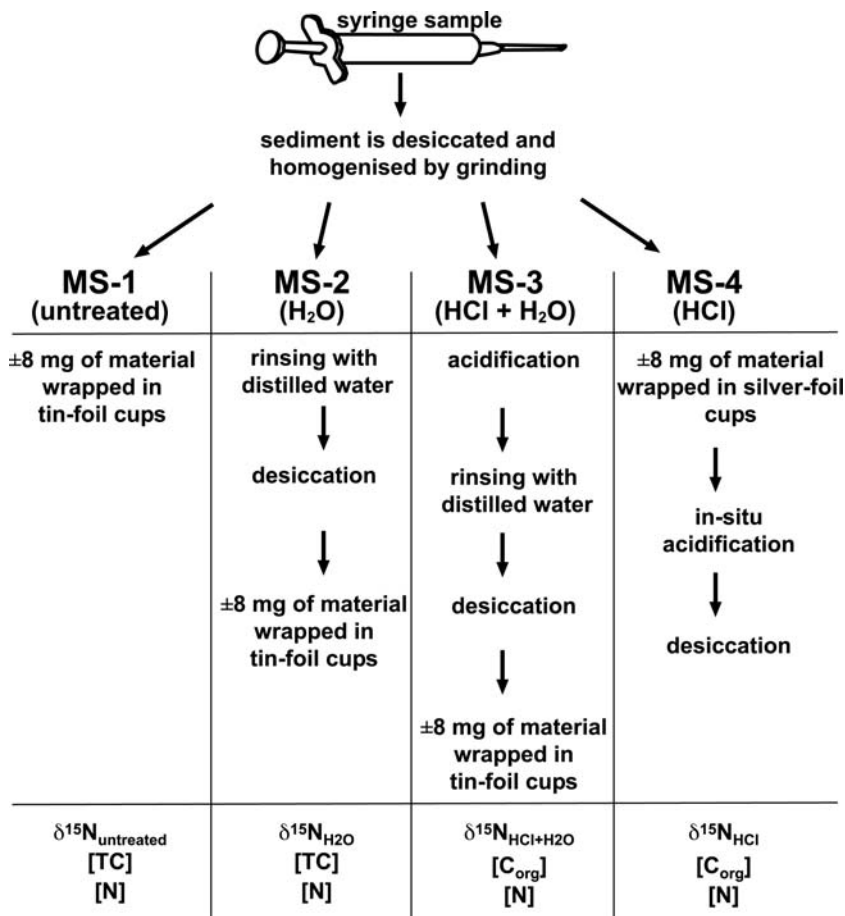

Figure 2. The single measurement series (MS) in brief. MS-1 and MS-2 yield the total carbon content (TC), whereas, due to the acidification process, MS-3 and MS-4 yield the amount of organic carbon (C_{org}).

(2) In MS-2, the material was rinsed, shaken, and centrifuged a total of 3 times at 3000 rpm before being desiccated a second time (40 °C). The measurement was carried out on ± 8 mg of sediment wrapped in tin-foil cups. Note that only about a quarter of the samples was subjected to this type of treatment ($n = 22$). As some compounds may be lost during rinsing, [N] and [TC] measured in MS-2 relate to a smaller sediment volume than [N] and [TC] in MS-1. This makes [N] and [TC] appear proportionally larger compared to MS-1.

(3) In order to determine the amount of organic carbon (C_{org}), carbonate needs to be eliminated via acid treatment prior to measurement. Acidification was conducted in two different ways: In the first case (MS-3), the sediment was put into a centrifuge tube and treated with 2N hydrochloric acid. It was then thoroughly rinsed with distilled water and centrifuged a total of 3 times at 3000 rpm. Measurements were made on ± 8 mg of dried (40 °C) sample material wrapped in tin-foil cups. As some compounds (including carbonate) are lost during rinsing, [N] and [C_{org}] in MS-3 again relate to a smaller sediment volume than [N] and [TC] in MS-1.

(4) In MS-4 the samples were directly acidified in tarred silver cups by adding 2N hydrochloric acid until no more CO_2 was released. Prior to analysis, the acid was allowed to evaporate in the heating oven (40 °C). Different from MS-3, [N] and [C_{org}] relate to the bulk sediment volume since no subsequent washing was applied (NB: Note that the sample's weight was taken before adding the acid.).

The isotopic composition of nitrogen ($\delta^{15}N$) and total weight percent (wt%) of carbon and nitrogen were determined with a Thermo Flash EA 1112 Elemental Analyser connected to an isotope-ratio mass-spectrometer (Finnigan, Delta V). The reference gas was pure N_2 nitrogen from a cylinder calibrated against IAEA-standards N-1 and N-2. The external reproducibility of the isotope measurements was tested with an internal standard (peptone) after every 5th measurement. As regards the concentrations of C and N, reproducibility is $\pm 3\%$ of the original amount present. For $\delta^{15}N$, the analytical precision of the lab standard was $\pm 0.2\,‰$ (1σ standard deviation). The isotope abundance ratios are reported in the conventional δ-notation in per mil (‰) with respect to atmospheric N_2 (AIR).

Ammonia concentration in the rinsing water

Ammonium (NH_4^+) is a by-product of organic matter decomposition. In organic-rich and anoxic sediments NH_4^+ is abundantly produced and easily preserved.

As outlined below, our findings gave strong reason to believe that NH_4^+ exerts a deceptive influence on the original $\delta^{15}N$- and C_{org}/N record. In order to substantiate that assumption, we decided to test the rinsing water in MS-2 and MS-3 for NH_4^+. The measurements were made on the same selection of samples subjected to MS-2 ($n = 22$). The concentration of NH_4^+ was measured by means of the photometrical test 'LCK 303 Ammonium Cuvette Test' of Hach-Lange (http://www.hach-lange.co.uk). For MS-3, the pH was adjusted to 7 by the addition of 1 molar NaOH-solution. The amount of NH_4^+ always lay within the accepted methodological range (2–47 mg/l).

Results

Delta^{15}N vs. depth

The down-core distributions of $\delta^{15}N_{HCl}$ and $\delta^{15}N_{untreated}$ are similar in both quality (pattern) and quantity (isotope composition) (Fig. 3A). Both records show large but comparable variations spanning roughly 8 to 15 ‰ ($\delta^{15}N_{untreated}$) and 9 to 15 ‰ ($\delta^{15}N_{HCl}$). Median $\delta^{15}N_{HCl}$ (11.4 ‰) is slightly higher than median $\delta^{15}N_{untreated}$ (10.2 ‰) (Fig. 3B; Table 1).

Washing with distilled water, irrespective of whether prior acid treatment is included (MS-3) or not (MS-2), entails strong modifications in the down-core development of $\delta^{15}N$ (Fig. 3A; Table 1). In both measurement series, the range of $\delta^{15}N$-variation is reduced to within the standard error inherent in the method. Both data sets appear much smoother than $\delta^{15}N_{untreated}$.

The lowering from mean 10.2 ‰ (or 10.0 ‰; see Table 1) in MS-1, to 8.5 ‰ in MS-3 and 8.4 ‰ in MS-2 suggests a mobile phase with comparably high $\delta^{15}N$-values (Fig. 3B). The offsets distinctly exceed the measurement precision and are almost equivalent to the isotopic difference between two trophic levels (Fry 1988). Given the magnitude of this discrepancy, the records would lead to completely different conclusions regarding nutrient supply and consumption.

C_{org}/N ratios vs. depth

With MS-3 and MS-4 it is possible to directly calculate C_{org}/N. In contrast, MS-1 and MS-2, not having been acidified, provide no information about [C_{org}]. This means that we must derive this information from elsewhere. As regards MS-1, we can easily use [C_{org}] of MS-4, as both [N] from MS-1 and [C_{org}] from MS-4 refer to the bulk sediment volume (the $\delta^{13}C$-values in MS-4 oscillate minimally around -19.5 ‰ (not shown), which indicates that the removal of carbonate was complete.).

This is not possible with MS-2 because [N] refers to an amount smaller than the bulk sediment volume. For the sake of legibility, the calculation of [C_{org}] in MS-2 is shown elsewhere (see caption next to Fig. 4).

Table 1. Standard deviation, median, minimum and maximum values of C_{org}/N and $\delta^{15}N$. MS-2 is limited to a selection of samples ($n = 22$). Values in brackets are based on this same selection of samples. This is in order to ensure a better comparability of MS-2 with the other measurement series.

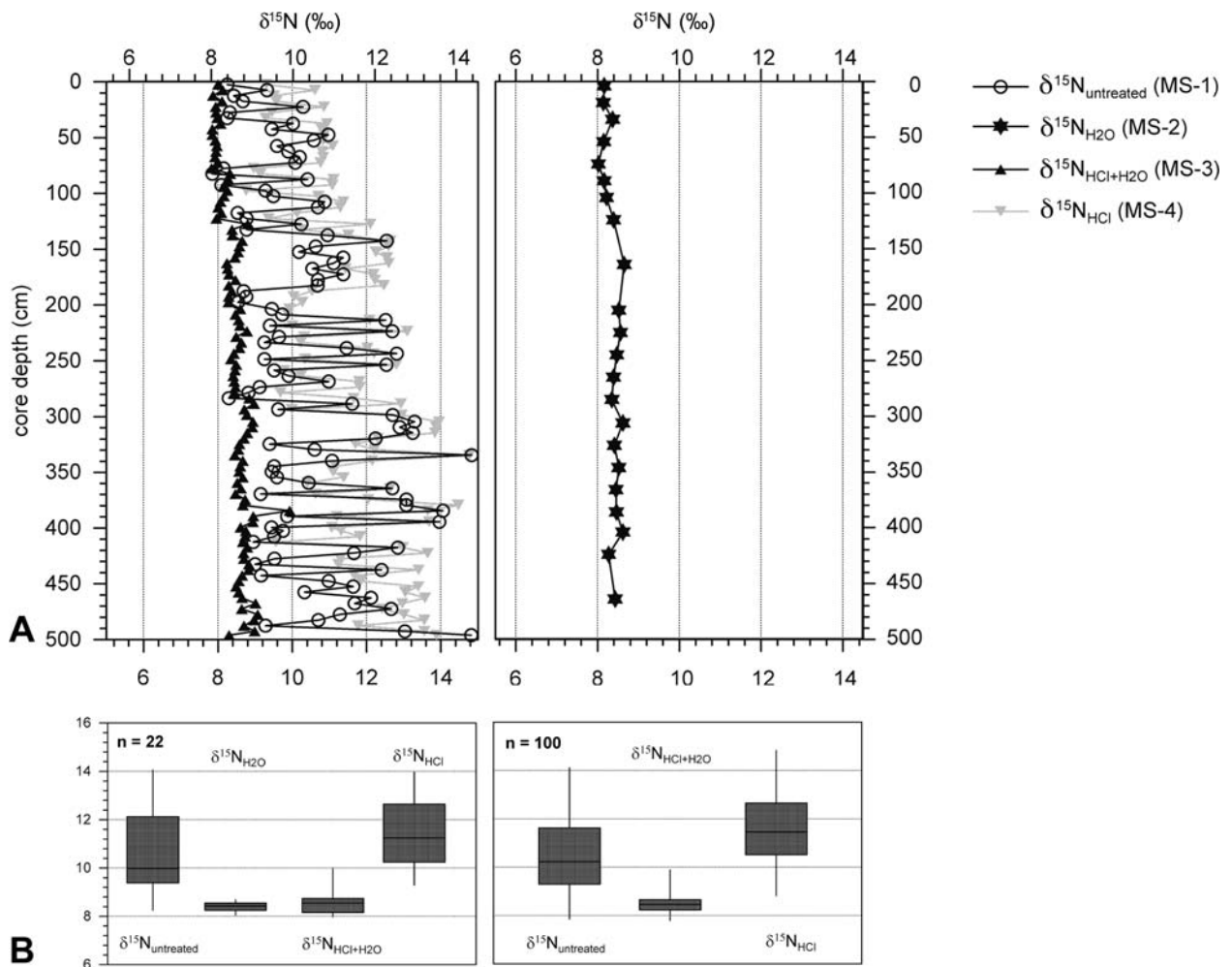|	MS-1 untreated	MS-2 H_2O	MS-3 HCl + H_2O	MS-4 HCl		MS-1 untreated	MS-2 H_2O	MS-3 HCl + H_2O	MS-4 HCl
C_{org}/N					$\delta^{15}N$ (‰)				
standard deviation	0.6 (0.5)	0.7	0.2 (0.2)	0.4 (0.4)	standard deviation	1.7 (1.8)	0.2	0.4 (0.5)	1.4 (1.5)
median	7.2 (7.3)	7.4	7.5 (7.5)	7.3 (7.3)	median	10.2 (10.0)	8.4	8.5 (8.5)	11.4 (11.2)
minimum	5.4 (5.9)	5.5	7.1 (7.2)	5.7 (6.9)	minimum	7.9 (8.3)	8.0	7.8 (7.9)	8.8 (9.3)
maximum	8.9 (8.2)	8.6	8.5 (8.4)	8.7 (7.7)	maximum	14.8 (14.1)	8.7	9.9 (9.9)	14.8 (14.0)

Figure 3. A. Downcore development of $\delta^{15}N$. For reasons of clarity, $\delta^{15}N_{H2O}$ (MS-2) is shown separately; **B.** Delta^{15}N-data pooled in boxplots. For better comparison of MS-2 with the other measurement series, the boxplots to the left are based on the same selection of samples processed in MS-2.

C_{org}/N ratios are neither affected by washing nor by in-situ acidification. $[C_{org}/N]_{H2O}$ and $[C_{org}/N]_{untreated}$ show a markedly similar development (Fig. 4, right chart) and $[C_{org}/N]_{HCl}$ deviates only sporadically from $[C_{org}/N]_{untreated}$. C_{org}/N strongly reacts, however, to acidification plus rinsing (MS-3). The combination of acidification and rinsing leads to pronounced downcore data-smoothing, accompanied by a slight increase of median C_{org}/N (from 7.2 in untreated material to 7.5 in acidified plus rinsed material; Table 1).

Ammonium in rinsing water

The rinsing water from MS-2 and MS-3 contains – as expected – significant amounts of ammonium nitrogen. In MS-2, the loss averages 3.5 wt%, ranging from 0.4 to 10.8 wt% (Fig. 5). In MS-3, the loss averages 6.3 wt%, spanning 1.1 to 14 wt%. The offset between the losses in MS-2 and MS-3 averages 2.1 wt%. Obviously, prior acidification (MS-3) somehow mobilises an even greater quantity of NH_4^+.

Discussion

A study of the available literature shows that the effects of sample preparation on $\delta^{15}N$ and C/N are far from unanimous. Bunn et al. (1995), for instance, found that acidification and subsequent rinsing increased the mean $\delta^{15}N$-signatures of shrimp tissue and decreased those of seagrass to a degree that may confuse the interpretation of food webs. Rinsing is a common practice applied in order to remove acid remains (Stoner & Zimmerman 1988; Kang et al. 2003) but the fact of the matter is that several authors strongly advise against it in order to minimise the loss of dissolved organic matter (Nieuwenhuize et al. 1994; Bunn et al. 1995; Jacob et al. 2005; Carabel et al. 2006). Still, acidification alone does not guarantee the stability of organic compounds, either: Ng et al. (2007) and Jacob et al. (2005) reported elevated C/N ratios in decalcified sample volumes, suggesting a proportionally greater loss of N relative to C. Moreover, the $\delta^{15}N$-signature of sedimentary matter (Kennedy et al. 2005; Carabel et al. 2006), marine algae
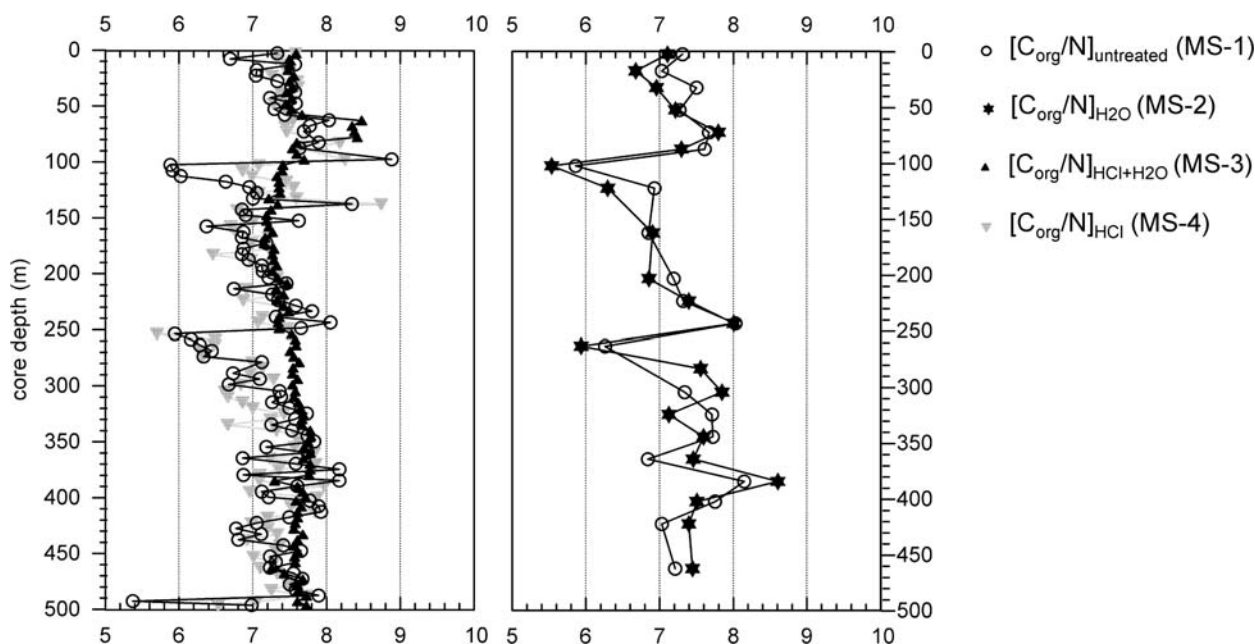
Figure 4. Downcore development of C_{org}/N. The right chart shows $[C_{org}/N]_{H2O}$ (MS-2) along with the corresponding $[C_{org}/N]_{untreated}$ data (MS-1), revealing their striking similarity. Since rinsing eliminates no carbonate, the amount of organic carbon in MS-2, i.e. $[C_{org}]_{MS-2}$, had to be calculated in an indirect manner. $[C_{inorg}] = [TC]_{MS-1} - [C_{org}]_{MS-4}$; $[TC]_{MS-1}$ and $[C_{org}]_{MS-4}$ both refer to the bulk sediment volume and are thus comparable; $[C_{org}]_{MS-2} = [TC]_{MS-2} - [C_{inorg}]$. Note that $[TC]_{MS-2}$ refers to a smaller sediment volume (due to the loss of various compounds in the course of rinsing) than $[C_{inorg}]$ (which refers to the bulk sediment volume). For this reason, $[C_{inorg}]$ turns out to be slightly underestimated when applied in the equation. The underestimation of $[C_{inorg}]$ results in an overestimation of $[C_{org}]_{MS-2}$ and consequently $[C_{org}/N]_{H2O}$. Overall, however, the error is expected to be small. Besides that, our conclusions are based on down-core patterns rather than absolute values.
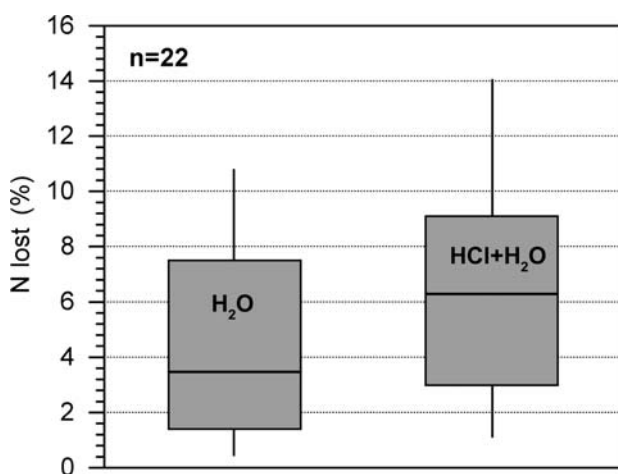

Figure 5. The amount of NH_4^+-N washed away by the rinsing water in MS-2 (H_2O) and MS-3 ($HCl + H_2O$). The losses relate to the amount of nitrogen present in untreated sediment (MS-1). The measurements were made on the same selection of samples subjected to MS-2 ($n = 22$). On average, acidification plus rinsing mobilises 2.1 % more NH_4^+-N than rinsing alone.

and cyanobacteria (Ng et al. 2007), invertebrates and fish (Jacob et al. 2005) decreased significantly in response to in-situ acidification. In contrast, mollusc tissue (Ng et al. 2007) and algal material examined by Kennedy et al. (2005) proved rather resistant.

In tests conducted by Holmes et al. (1999) neither the application of weak hydrochloric acid nor subsequent rinsing significantly affected sedimentary $\delta^{15}N$.

It clearly shows that the influence of sample preparation is as variable and manifold as the types of material investigated. According to our own results, washing may leave pronounced marks on the $\delta^{15}N$-record while C_{org}/N is only affected when samples are subjected to both acidification and rinsing. The questions are: first, which record can be trusted to provide the prime isotopic signal required for palaeoceanographic reconstructions? Second, can the loss of NH_4^+ be held responsible for the changes of C_{org}/N and $\delta^{15}N$ occurring in the course of sample processing? We pre-empt the answer – yes, there is a variety of evidence that substantiates the influence of ammonium on both C_{org}/N and $\delta^{15}N$.

The potential impact of NH_4^+ on C_{org}/N and the role of carbonate

C_{org}/N ratios are a widely used instrument in distinguishing between marine and terrestrial organic matter (Schubert & Calvert 2001). The observation that NH_4^+ is capable of biasing the original C/N ratio and causing misinterpretation is not new. Several studies have already reported fixation and adsorption of NH_4^+ in and onto clay minerals and accordingly low C/N ratios (e.g. Müller 1977 and references therein; Rosenfeld 1979;

von Breymann & Suess 1988; Schubert & Calvert 2001; Morse & Morin 2005). In core 180, clay minerals are negligible and so is their significance in this context. There must be another reason for the observed deviations of C_{org}/N. On the evidence of the excellent correlation between $[CaCO_3]$ and C_{org}/N in MS-1 (Fig. 6A), carbonate appears to be the key to the answer.

Due to the alkaline nature of the carbonate ion (CO_3^{2-}), the pH of the interstitial water is slightly raised while carbonate dissolves (Faure 1992). An increasing pH promotes the adsorption of dissolved ammonium ions to silicate surfaces (Brunelle 1978). Biogenic silica is fairly abundant in the form of diatom frustules. The isoelectronic point of silica is around pH 1. For further explanation: The isoelectric point (IEP) is the pH at which a particular molecule carries no net electrical charge. The surfaces of mineral oxides that are surrounded by a solution with a pH higher than its IEP result negatively charged which is compensated for by adsorbed cations. The reverse is true if the particle is dipped in a medium with a pH lower than its IEP (Brunelle 1978). However, the negative polarisation of silicate surfaces only becomes significant at a pH above 7. This is the threshold above which the amount of NH_4^+ adsorbed to silica surfaces increases rapidly (Brunelle 1978). The pH in marine sediments oscillates around 8, held more or less stable due to the buffering qualities of ammonium and carbonate (Ben-Yaakov 1973). At these pH-ranges (pH > 7) even slight changes in the H^+/OH^--ratio have pronounced effects on NH_4^+-adsorption (Brunelle 1978).

The negative correlation between C_{org}/N and $[CaCO_3]$ in MS-1 (Fig. 6A) is ascribed to the combination of NH_4^+-adsorption and -outgassing: The amount of adsorbed ammonium rises along with the carbonate content as the adsorption capacity of SiO_2 is enhanced at higher pH-values. At the same time, it is predominantly adsorbed (as opposed to dissolved) ammonium

▶

Figure 6. C_{org}/N vs. $[CaCO_3]$: $[CaCO_3]$ was calculated by subtracting $[C_{org}]$ (MS-4) from the total amount of carbon (MS-1). The resulting amount of inorganic carbon was multiplied by 8.33, according to the molar relationships in $CaCO_3$. $CaCO_3 = ([TC]_{MS-1} - [C_{org}]_{MS-4}) \times 8.33$. The negative correlation in (**A**) testifies to the increasing number of NH_4^+-ions adsorbed to negatively charged SiO_2-surfaces, the higher the amount of carbonate. The dissolution of carbonate lifts the pH in the interstitial water, which increases the surface polarisation and adsorption capacity of SiO_2. The higher the percentage of adsorbed NH_4^+-ions the lower the N-loss due to outgassing. Washing with distilled water (**B**) does not change anything about the negative correlation. Instead, acidification lowers the adsorption capacity of SiO_2, causing the release of adsorbed NH_4^+-ions (**C** and **D**). During subsequent rinsing they are washed away with the rinsing water. This is why the negative correlation is completely erased in MS-3 (**C**). Acidification without rinsing seems to release previously adsorbed NH_4^+ during later drying as NH_3 (**D**).

that is kept from outgassing in the form of NH_3 in the heating oven (more on the ammonium loss due to outgassing in the following chapter).

Note that our reasoning requires the pore water to be undersaturated with respect to carbonate (if the solution was already supersaturated, rising $[CaCO_3]$ would not lead to enhanced dissociation of $CaCO_3$), a prerequisite
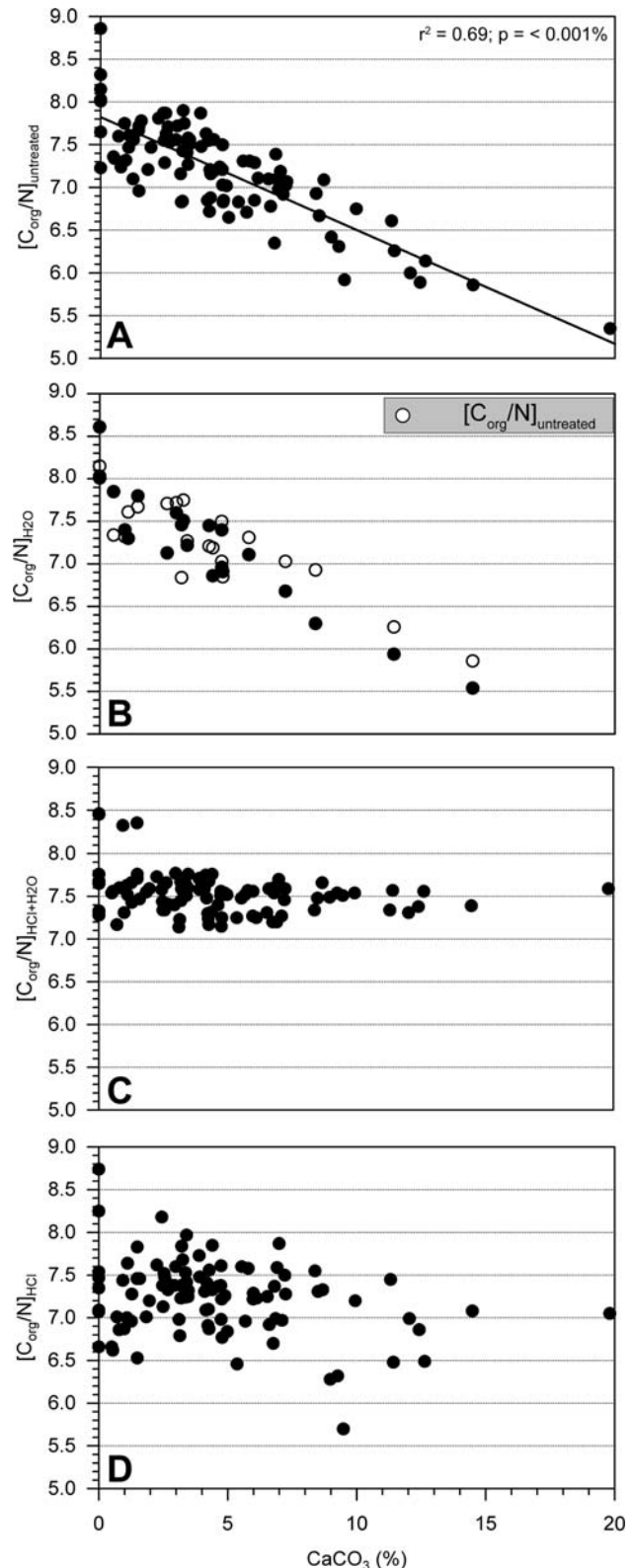
that is met by the high partial pressures of CO_2 (produced by OM matter decay) and the comparably low temperatures (Faure 1992).

Also note that NH_4^+ has NH_3 as a conjugate base. This implies that the amount of NH_4^+ decreases in favour of NH_3 as the pH rises. However, this effect is seemingly outweighed by the concomitantly increasing adsorption capacity of SiO_2 as the pH increases. The fact that the solubility of SiO_2 increases as the pore waters get more basic (Brunelle 1978; Faure 1992) does not disturb the pattern either.

Acidification plus rinsing has a considerable impact on the C_{org}/N ratios. In MS-3, the originally inverse correlation between $[CaCO_3]$ and C_{org}/N is completely erased (Fig. 6C). Lower C_{org}/N ratios of carbonate-rich sample splits are adapted to the C_{org}/N ratios in carbonate-poor sample splits and mean C_{org}/N is slightly raised (see also Table 1).

What happens in the course of acidification plus rinsing? As the pH is lowered, the SiO_2-adsorption capacity diminishes and the SiO_2-surfaces result oversaturated with respect to ammonium. Ammonium is released into the pore water and carried away in the course of rinsing. C_{org}/N consequently rises.

In-situ acidification (MS-4) brings about the same release of formerly adsorbed NH_4^+. However, in contrast to MS-3, the liberated NH_4^+-molecules are not washed away. In the heating oven some of them outgas to form gaseous NH_3 (see below). This outgassing is considered the reason for the disturbance of the originally inverse correlation between $[CaCO_3]$ and C_{org}/N (Fig. 6D).

Note that the amount of ammonium lost in MS-2 still averages more than half of the amount lost in MS-3 (Fig. 5). Assuming that rinsing alone releases hardly any adsorbed NH_4^+, the bulk of the ammonium lost in MS-2 represents dissolved NH_4^+. If this hypothesis is right, how is it that the leaching of dissolved NH_4^+ does not result in rising C_{org}/N ratios? $[C_{org}/N]_{untreated}$ and $[C_{org}/N]_{H2O}$ are virtually identical (Figs 4, 6B). The most likely explanation is the simultaneous loss of dissolvable organic matter with high C_{org}/N ratios: Organic matter that has already been subjected to some microbial activity usually exhibits higher C_{org}/N ratios than fresh organic matter. This is because nitrogen is more susceptible to decay than carbon and, therefore, preferentially lost (Rosenfeld 1981; Lehmann et al. 2002). Apparently these compounds are sufficiently unstable to be leached away together with dissolved NH_4^+, thus inhibiting the increase in C_{org}/N. In contrast, acidification plus rinsing is capable of additionally mobilising originally adsorbed NH_4^+; this additional loss leads to the observed increase of C_{org}/N ratios in MS-3.

The weakness of the correlations notwithstanding, Figure 7 substantiates our hitherto held ideas and confirms the control of carbonate, or rather pH, on the mobility of NH_4^+ in MS-2 and MS-3. The less $CaCO_3$, the less adsorbed NH_4^+ and the greater the loss during rinsing (Fig. 7A). The positive correlation in Figure 7b further corroborates the assumption that acidification
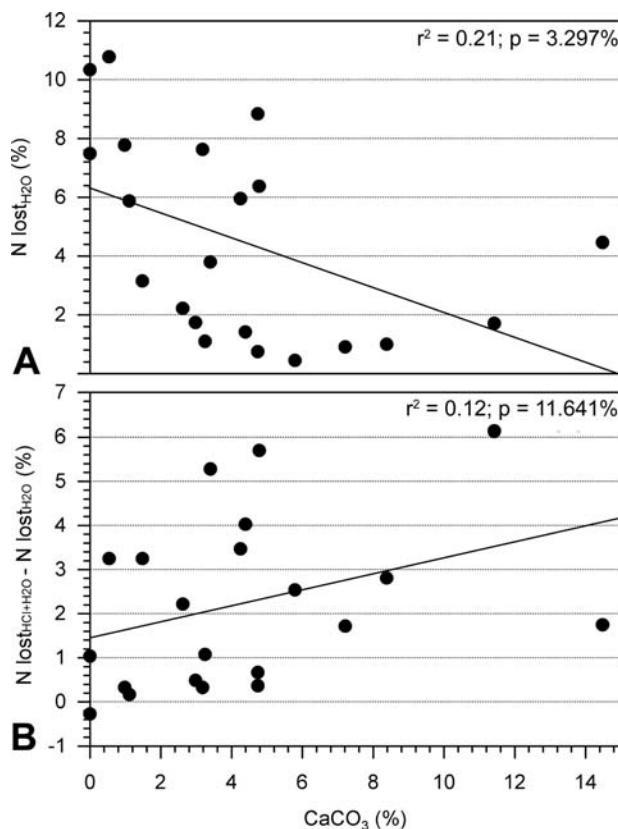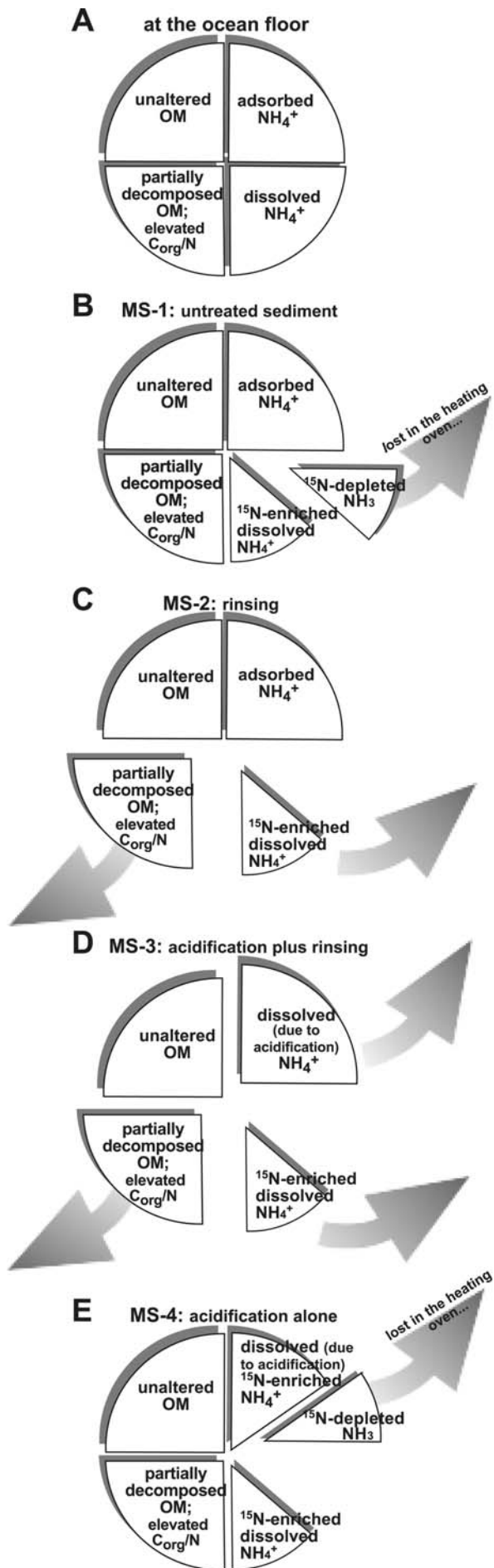

Figure 7. The control of $CaCO_3$ on the fate of NH_4^+ during sample treatment. Both charts substantiate the ability of $CaCO_3$ to promote NH_4^+-adsorption; **A.** Samples containing lower carbonate tend to loose more NH_4^+ during rinsing (MS-2). This is attributed to the slightly lower pH of the pore waters, the consequently less negative surface charge of the SiO_2-particles and the higher percentage of NH_4^+ dissolved in interstitial waters; **B.** Chart (B) testifies to the rising proportion of adsorbed NH_4^+ as carbonate and the pH increases. Acidification plus rinsing mobilises both adsorbed and dissolved NH_4^+ while rinsing alone mobilises only dissolved NH_4^+. At low $CaCO_3$, where only few NH_4^+ is adsorbed, the offset between N lost$_{HCl + H2O}$ and N lost$_{H2O}$ tends to be low. The increasing offset with higher $[CaCO_3]$ is due to the rising proportion of adsorbed NH_4^+ and its loss in the course of acidification plus rinsing.

plus rinsing (MS-3) mobilises both adsorbed and dissolved NH_4^+, while rinsing alone (MS-2) mobilises only the latter.

The potential impact of NH_4^+ on $\delta^{15}N$

Rinsing caused average $\delta^{15}N$ to drop by 1.6 ‰ (Fig. 3B; Table 1). According to our above line of reasoning, rinsing does predominantly (if not exclusively) mobilise dissolved NH_4^+. This implies a $\delta^{15}N$-enrichment in dissolved NH_4^+ compared to the residual sediment. The ^{15}N-enrichment in dissolved NH_4^+ may be due to nitrogen isotope fractionation during the drying process. When NH_4^+ transforms to gaseous NH_3, the light isotope (^{14}N) may accumulate in the gaseous phase, thus leaving ^{15}N-enriched NH_4^+ behind (Fig. 8B). The process is comparable to kinetic isotope fractionation

A at the ocean floor

B MS-1: untreated sediment

C MS-2: rinsing

D MS-3: acidification plus rinsing

E MS-4: acidification alone

of oxygen isotopes during the vaporisation of water and can reach pronounced proportions. In a study conducted on simulated urine patches on grassland soils, the isotopic composition of the NH_3 volatilised was depleted by up to almost 30 ‰ compared to the original urea-N added (Frank et al. 2004).

The inverse relation between $\delta^{15}N$ and [N] in untreated material (Fig. 9A) substantiates that ammonia volatilisation – accompanied by the discrimination against ^{15}N – is taking place. The outgassing of ^{15}N-depleted NH_3 results both in the reduction of [N] and an increase of the $\delta^{15}N$-signatures. After rinsing, the negative correlation is completely erased (Fig. 9B); this agrees with the leaching of ^{15}N-enriched ammonium ions as illustrated in Figure 8C.

Acidification plus rinsing (MS-3) and rinsing alone (MS-2) yield extremely similar $\delta^{15}N$-patterns (Figs 3, 9B–C). Apparently, the mobilisation of originally adsorbed NH_4^+ in MS-3 has no additional effect on the isotopic signature of the sediment. This suggests that adsorbed NH_4^+ is resistant to both the outgassing and fractionation during the drying process (Figs 8B, D).

On the basis of the available evidence, $\delta^{15}N$ is strongly controlled by dissolved NH_4^+. As outlined in the above section, the amount of dissolved NH_4^+ depends on the adsorption capacity of SiO_2, which co-varies with the amount of carbonate, or rather, the pH of the interstitial waters. The lower the carbonate content, the lower the pH and the higher the amount of dissolved NH_4^+, which is the fraction liable to fractionation in the heating oven. Figure 10 reflects the anticipated relationship between carbonate and $\delta^{15}N$ reasonably well. The lower the carbonate content, the more pronounced the shift towards higher $\delta^{15}N_{untreated}$-values (Fig. 10A). Due to the leaching of isotopically "heavy" NH_4^+-molecules in both MS-2 and MS-3, the negative correlation is lost and average $\delta^{15}N$ is again lowered (Figs 10B–C).

In-situ acidification (MS-4) increases the amount of dissolved NH_4^+-molecules (Fig. 8E) compared to the amount already present in the original sediment (Fig. 8B). This enhances the loss of ^{15}N-depleted NH_3 during desiccation and might explain why average

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Figure 8. Schematic summary of the processes occurring during various methods of sample preparation. Arrows mark the fractions lost during processing; **A.** Chart (A) identifies the fractions that are relevant as regards the modification of C_{org}/N and $\delta^{15}N$ during sample treatment. NH_4^+ is excreted in the course of organic matter (OM) decayal, leaving partially decomposed OM with elevated C_{org}/N behind. If not stated otherwise, C_{org}/N and $\delta^{15}N$-values reflect unaltered OM; **B.** In the heating oven, some of the dissolved NH_4^+ is lost in the form of ^{15}N-depleted NH_3, thus leaving a ^{15}N-enriched residual behind; **C.** Rinsing mobilises intermediate and by-products resulting from deanimation reactions, exept for adsorbed NH_4^+. Adsorbed NH_4^+-ions are released during acidification; **D.** The subsequent rinsing in MS-3 induces their loss; **E.** In MS-4, unfixed NH_4^+ are not washed away but fractionate during desiccation, thereby increasing the ^{15}N-enrichment in the residual.

$\delta^{15}N_{HCl}$ is slightly higher than average $\delta^{15}N_{untreated}$ (Fig. 3B). Figure 10D furthermore reveals that the increase of $\delta^{15}N$ is more pronounced where $CaCO_3$ is abundant, i.e. where we expect a higher proportion of NH_4^+ to be released.

The presence of NH_4^+ seems equally problematic with $\delta^{15}N$ as with C_{org}/N. NH_4^+ is generated during OM decayal. As our data indicates, the decayal itself does not seem to produce the observed ^{15}N-enrichment in the residual sediment. This is worth noting because

it disagrees with the widely held belief in nitrogen isotope fractionation during protein hydrolysis (e.g. Melander 1960; Gaebler et al. 1966; Saino & Hattori 1980; Altabet & McCarthy 1985; Schäfer & Ittekkot 1993; Altabet & Francois 1994; Montoya 1994; Ostrom et al. 1997; Sachs & Repeta 1999).

What makes us so certain that deanimation occurred without fractionation is that MS-2 and MS-3 yield so markedly similar $\delta^{15}N$-signatures (Fig. 3B). The acid-induced mobilisation of adsorbed NH_4^+ in MS-3 does not additionally influence the isotopic signature of the sediment. Apparently, adsorbed NH_4^+ is unfractionated per se. Its $\delta^{15}N$-signature seemingly corresponds to that of fresh organic matter, otherwise its loss during acidification plus rinsing should have induced additional isotopic shifts. Our data clearly demonstrates that the observed shift towards higher $\delta^{15}N$-signals in MS-1 truly results from kinetic isotope fractionation during the drying process, i.e. during the transformation of NH_4^+ to gaseous NH_3.

Which record to trust

It appears that the use of untreated material for $\delta^{15}N$-analyses is not, contrary to popular belief (see above), advisable a priori. Our findings have shown that the down-core scattering of $[C_{org}/N]_{untreated}$ and $\delta^{15}N_{untreated}$ is primarily to be ascribed to the misleading influence of NH_4^+, rather than being indicative of environmental fluctuations.

If interested in the original signal, it is recommended that NH_4^+ be removed prior to measuring $\delta^{15}N$ and C_{org}/N. This appears to be done best by a combination of acidification and subsequent rinsing (MS-3). Acidification plus rinsing eliminates the effects of NH_4^+-adsorption and -outgassing and restores the original signal of both C_{org}/N and $\delta^{15}N$. If $\delta^{15}N$ is the sole parameter of interest, it is enough to remove dissolved NH_4^+ as it is only dissolved NH_4^+ that undergoes fractionation during desiccation; then rinsing alone suffices (MS-2) in order to eliminate the decomposition-, or more precisely, outgassing-derived shift towards higher $\delta^{15}N$-signals.

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Figure 9. $\delta^{15}N$ vs. [N]. Dissolved NH_4^+ is lost in the form of gaseous, ^{15}N-depleted NH_3; A. Its outgassing results in the simultaneous reduction of [N] and accumulation of ^{15}N-enriched NH_4^+ in the residual, hence the inverse relation; B–C. The negative correlation is erased in both MS-2 and MS-3, implying that rinsing alone provokes the leaching of ^{15}N-enriched NH_4^+. The acid-induced loss of adsorbed NH_4^+ in MS-3 has no additional influence on $\delta^{15}N$, because adsorbed NH_4^+ has not undergone any fractionation; D. As ^{15}N-enriched NH_4^+ is not washed away in MS-4, the negative correlation remains. NB: In MS-1 and MS-4, [N] refers to the bulk sediment volume. In MS-2 and MS-3, the sample splits have already undergone processing and consequently lost a certain amount of components when being weighed. This is why the percentage of [N] appears somewhat higher in MS-2 and MS-3 (B, C).
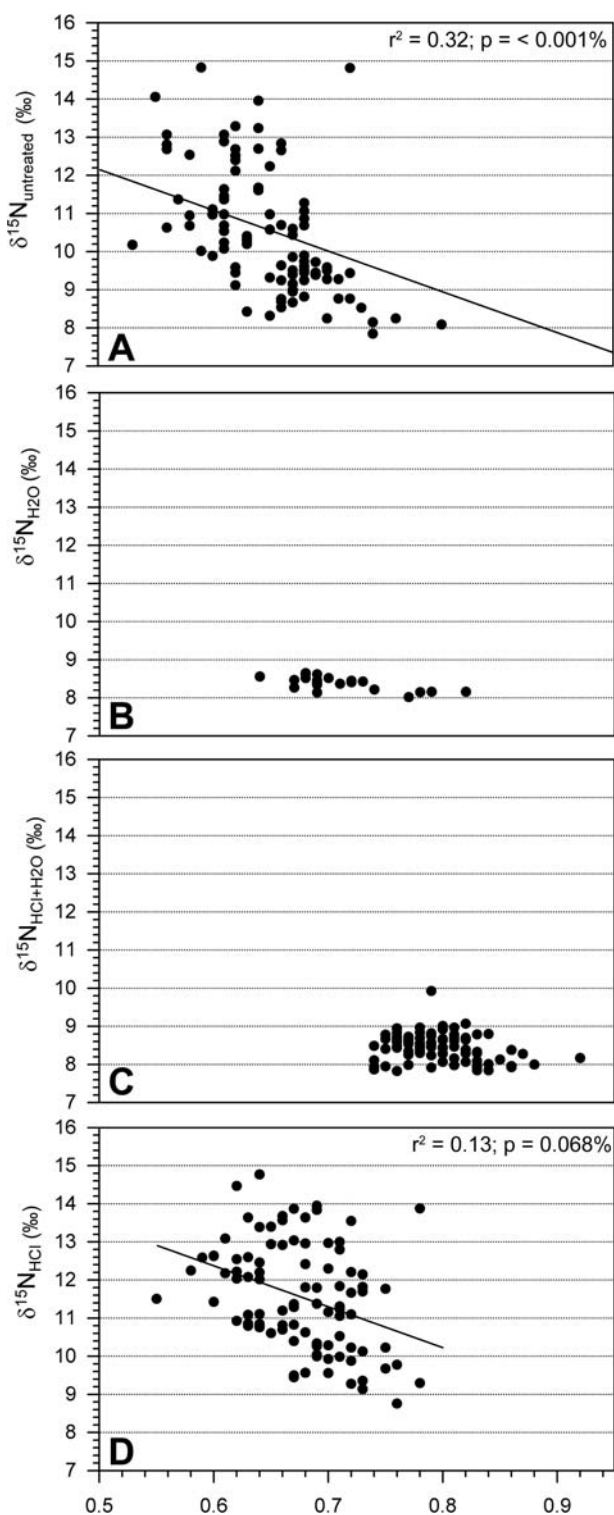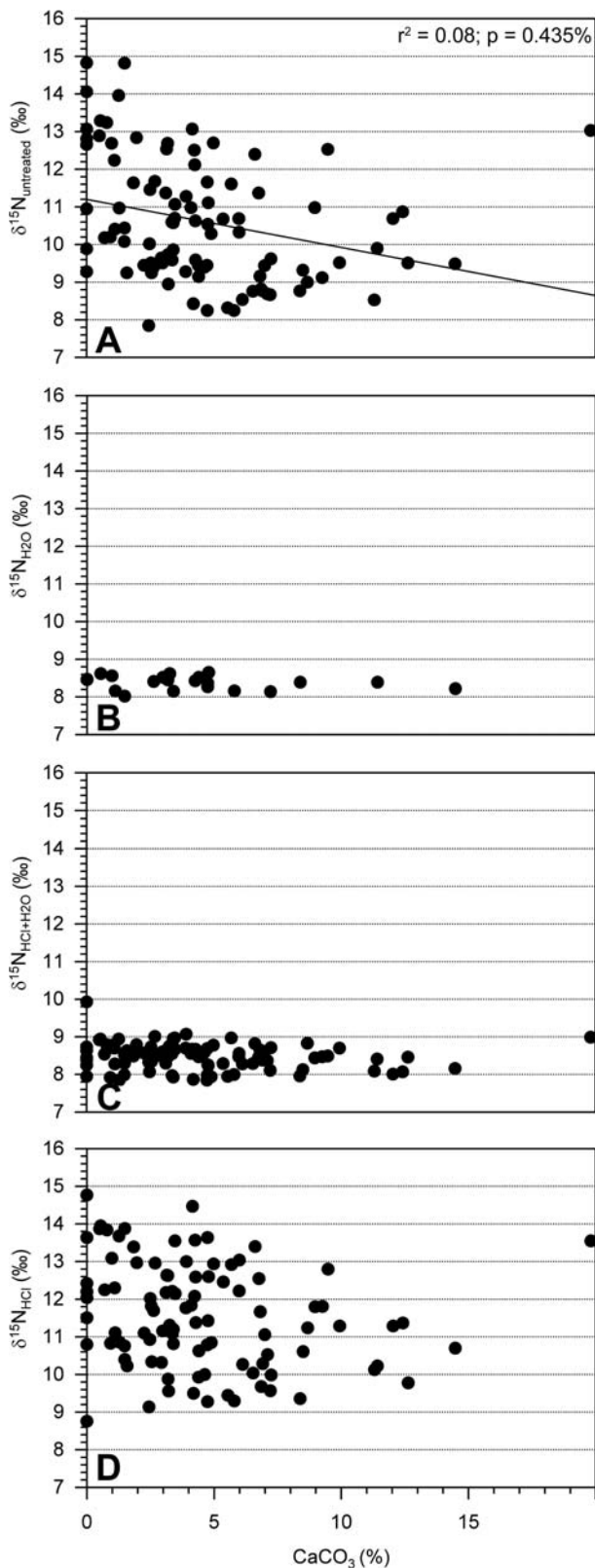
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Figure 10. $\delta^{15}N$ vs. [$CaCO_3$]. The negative correlation in MS-1 (**A**) reflects the enhanced outgassing of ^{15}N-depleted NH_3 the lower the amount of carbonate, i.e. the lower the negative surface polarisation of SiO_2. Only dissolved NH_4^+ is able to outgas and fractionate. For further details see text. Washing alone (**B**) or washing and acidification (**C**) reduces the $\delta^{15}N$-variability completely. Here the remaining fraction of porewater-NH_4^+ after its incomplete degassing is washed away. The treatment with HCl alone (**D**) does not remove this fraction completely.

[2] The outgassing and fractionation only concerns NH_4^+-ions dissolved in the pore waters. In contrast, NH_4^+-molecules adsorbed to negatively-charged SiO_2-surfaces remain unaffected. The adsorption capacity of SiO_2 increases with increasing pH, i.e. rising carbonate content.

[3] We conclude that excessive confidence in untreated material is inappropriate when NH_4^+-molecules are involved: Ammonia volatilisation is capable of producing misleading fluctuations in $\delta^{15}N_{untreated}$ without any environmentally-triggered background. Our results clearly indicate that the higher the amount of dissolved NH_4^+, the more we must suspect unwanted shifts towards higher $\delta^{15}N$ in untreated sediment. At the same time, it remains unclear how many of the dissolved NH_4^+-ions turn into gaseous NH_3. Due to this uncontrollable and variable outgassing, [C_{org}/N]$_{untreated}$ is likewise considered unreliable.

[4] Acidification plus rinsing seems capable of wiping out the effects of NH_4^+-adsorption and -outgassing and associated distortions of $\delta^{15}N$ and C_{org}/N: Acidification reduces the negative surface polarisation of SiO_2 and liberates adsorbed ammonium ions. Subsequent rinsing ideally mobilises all decomposition-derived intermediate products, including both dissolved and formerly bound ammonium. Certainly we cannot exclude the possibility of parts of "fresh" organic matter being concomitantly washed away.

[5] In organic-rich and anoxic sediments, where the conditions are favourable for the presence of NH_4^+, we assume acidified and rinsed material to deliver the trustworthiest record with decayal-derived influences being eliminated.

Several authors have called for a standardisation of pre-analysis sample treatment, fearing that the different methodology may hamper the comparability of data (e.g. Jacob et al. 2005; Ng et al. 2007). We do only support this view as long as it is the same type of material analysed.

Due to compositional differences, however, sediments may require completely different approaches in order to overcome possible misinterpretations of C_{org}/N ratios and $\delta^{15}N$-values (e.g. Schubert & Calvert 2001). The chemistry of the interstitial waters, the porosity and the solid/water ratio are further aspects to consider.

Our results certainly do not warrant any generalisation. Instead, they highlight the importance of carefully investigating the varying effects of different processing

Conclusions and recommendations

[1] Our data provide strong evidence of nitrogen isotope fractionation during desiccation when NH_4^+ transforms into gaseous NH_3. Thereby, ^{14}N accumulates in the gaseous phase, thus leaving ^{15}N-enriched NH_4^+ behind.

methods, rather than following a simple recipe. It is recommended that random measurements be conducted on acidified plus rinsed sample splits in addition to untreated material, in order to better evaluate the potential influence of ammonium on $\delta^{15}N$ and C_{org}/N.

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