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Analyzing biases of nitrogen contents and δ^{15}N values arising from acidified marine sediments with different $CaCO_3$ concentrations

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Abstract

In some studies, the researchers pretreated and measured organic carbon, nitrogen and their isotopes (δ^{13}C and δ^{15}N) of marine sediment together, to save costs and resources of analysis. However, the procedure of acidification to remove inorganic carbon for analysing δ^{13}C can affect the values of nitrogen and δ^{15}N, and the biases vary a lot depending on the $CaCO_3$ contents of sediments. In this study, the biases of total nitrogen (TN) and δ^{15}N values arising from acidified sediments were compared between the $CaCO_3$-poor (1%–16%) and $CaCO_3$-rich (20%–40%) samples. TN and δ^{15}N values were altered during acid treatment (without centrifugation) that possibly led to N-containing compounds volatilization. For $CaCO_3$-poor samples, acidification led to a range of 0%–40% TN losses and 0‰–2‰ shift in δ^{15}N values; and 10%–60% TN losses and 1‰–14‰ shift in δ^{15}N values for $CaCO_3$-rich samples. The biases from most samples exceeded the precision of the instrument (0.002% for TN and 0.08‰ for δ^{15}N), and high biases could mislead our judgment for the environmental implication of the data. Thus, avoiding co-analysis of organic carbon, nitrogen and their isotopes (δ^{13}C and δ^{15}N) in sediments, even for $CaCO_3$-poor marine sediments, is necessary.

Key words: stable isotope nitrogen, nitrogen, $CaCO_3$, acidification, marine sediment

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1 Introduction

Organic matter contents (e.g., total carbon and nitrogen) and their isotope fingerprints (δ^{13}C and δ^{15}N) in the sediments are important environmental proxies to evaluate the sediment quality and trace the material sources in coastal and estuarine ecosystems (Tucker et al., 1999; Voss et al., 2000; Savage, 2005). For example, anthropogenically enriched organic nitrogen (e.g., human sewage, livestock runoff) can significantly elevate the values of δ^{15}N to 10‰–25‰ (Sweeney and Kaplan, 1980; Savage, 2005). In some studies, the researchers pretreated and measured organic carbon, nitrogen and their isotopes (δ^{13}C and δ^{15}N) in marine sediment together, to save costs and resources of analysis (e.g., Middelburg and Nieuwenhuize, 1998; Kennedy et al., 2005; Hu et al., 2006; Wang et al., 2013). Acidification is a necessary procedure to remove inorganic carbon for meeting the requirement of total organic carbon (TOC) and δ^{13}C measurements. However, when samples were acidified, the loss of total nitrogen (TN) and even the shift of δ^{15}N values occurred (Bunn et al., 1995; Jacob et al., 2005; Jaschinski et al., 2008; Serrano et al., 2008; Schlacher and Connolly, 2014). This biases caused by acidification varied greatly depending on the species of acid reagent (e.g., HCl, H_2SO_3, H_3PO_4 or HF) and carbonate contents in the sediments (Schubert and Nielsen, 2000; Lorrain et al., 2003; Kennedy et al., 2005; Schmidt and Gleixner, 2005; Fernandes and Krull, 2008). For examples, Harris et al. (2001) only detected a slight increase in δ^{15}N values (0.04‰–0.14‰) after acidification using HCl fumigation; Fernandes and Krull (2008) also found 1‰–2‰ deviation of δ^{15}N values between the acidified (using HCl washing) and non-acidified samples. For the biases of nitrogen content, Lohse et al. (2000) reported there were approximately 28%–47% losses in nitrogen compounds for $CaCO_3$-rich (55%–61%) samples after acidification using H_2SO_3; Kennedy et al. (2005) however only found about 0.3% nitrogen losses in the acidified samples (30% $CaCO_3$) using HCl. Therefore, there is uncertainty

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on the biases in TN and δ^{15}N values after acidification, and it is necessary to evaluate the biases according to the $CaCO_3$ contents of the sediments.

Global mean $CaCO_3$ content in marine surface sediments is approximately 34.8%, with a wide range of approximately 0%–80% (Ridgwell and Hargreaves, 2007). For examples, the samples from north and west Pacific Ocean contain approximately 0%–20% $CaCO_3$, but $CaCO_3$ contents in the samples from Atlantic Ocean can reach to 80% (Ridgwell and Hargreaves, 2007). In this study, TN and δ^{15}N values in 236 marine sediment samples with higher $CaCO_3$ contents (20%–40%) from the coastal waters in the western Australia and 273 samples with lower $CaCO_3$ contents (0%–16%) from the coastal waters in China's seas were measured, respectively, under acidified and non-acidified conditions. The biases of TN and δ^{15}N values were evaluated and the correlation between the biases and $CaCO_3$ contents in the sediments were examined to understand the impact of $CaCO_3$ contents.

2 Materials and methods

2.1 *Sample collection*

Two sediment cores were collected from the coast of King George River (KGR) in the northwestern Australia, and 236 sediment samples were obtained by slicing at 1-cm interval. Two and one sediment cores were collected from the Bohai Sea and Yellow Sea in China, respectively, and 221 sediment samples were obtained by slicing at 1-cm interval. In addition, 52 surface marine sediment samples were collected from the South Yellow Sea, China (Table 1). Totally, 509 marine sediment samples were stored in a freezer at –20°C preparing for the analysis of TOC, TN, δ^{15}N values and $CaCO_3$ contents.

Table 1. Description of sample property

Samples identifier	Location	Number of samples	Description	TOC1)/%	TN2)%	C/N2)	δ^{15}N2)/‰	$CaCO_3$/%
KGR6	13°55′13.44″S, 127°19′13.8″E	114	Down-core marine sediment from King George River, northwestern Australia (11.8 m depth)	0.40–0.75	0.03–0.11	8–19	3.6–5.7	20–31
KGR10	13°53′08.88″S, 127°08′22.56″E	122	Down-core marine sediment from King George River, northwestern Australia (11.2 m depth)	0.38–0.83	0.04–0.12	7–22	4.0–7.8	23–40
B62-2	38°52′12″N, 119°40′12″E	76	Down-core marine sediment from Bohai Sea, China (24 m depth)	0.46–0.92	0.06–0.12	6–12	4.9–6.4	1–7
B24-1	38°09′27.6″N, 122°29′0.24″E	63	Down-core marine sediment from North Yellow Sea, China (45 m depth)	0.68–1.14	0.09–0.18	7–12	4.5–5.6	1–5
H10-1	34°59′24″N, 123°29′56.04″E	82	Down-core marine sediment from South Yellow Sea, China (78 m depth)	0.20–1.09	0.03–0.14	7–14	3.8–6.2	1–7
SMS	–	52	Surface marine sediment from South Yellow Sea, China	0.14–1.18	0.02–0.19	6–21	3.7–6.0	1–16

Note: 1) Measurement on acidified sample; 2) measurement on unacidified whole sample.

2.2 *Analytical procedures*

TOC, TN and δ^{15}N values were measured using a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to an elemental analyzer (Flash EA 1112, Thermo Scientific, Italy) in Littoral Environment et Sociétés (LIENSs)-UMR7266, France. Firstly, all samples were freeze-dried and homogenized by grinding. Each sample was divided into two shares, using for the pretreatment with and without acidification, respectively. One share was acidified by adding 1 mL of 0.5 mol/L HCl (or 1 mol/L HCl) to every 100 mg of samples. The acidified samples were dried at <60°C, then mixed with 1 mL Milli-Q waters and freeze-dried again. The freeze-dried samples were weighed into 5 mm×8 mm tin capsules, and the prepared capsules were used for the measurement of total organic carbon (TOC), total nitrogen ($TN_{acidified}$) and stable nitrogen isotope ($\delta^{15}N_{acidified}$). The corrections for TOC and $TN_{acidified}$ values were performed because the weight of samples had increased after decarbonation (formation of $CaCl_2$). The other sample share, using for non-acidification treatment, was weighed directly and placed into 5 mm×8 mm tin capsules for the measurement of total carbon (TC), total nitrogen ($TN_{unacidified}$) and stable nitrogen isotope ($\delta^{15}N_{unacidified}$). Based on the results of TOC and TC, the $CaCO_3$ contents were estimated using $CaCO_3$=(TC–TOC)×8.33 (Stein et al., 1994; Schubert and Calvert, 2001).

The isotopic composition results are expressed relative to atmospheric N_2 for δ^{15}N. Replicate measurements of an acetanilide standard (Thermo Scientific) indicated that the δ^{15}N analytical errors were less than 0.1‰. The total organic matter and total nitrogen weight data obtained as part of the stable isotope analyses. Replicate measurements of a certified reference material (Low Organic Content Soil, Elemental MicroAnalysis, UK) indicated relative errors of 0.025% and 0.002% for total organic carbon and total nitrogen, respectively.

2.3 *Statistical analysis*

We tested the differences of TN and δ^{15}N values between non-acidified and acidified samples, using software SPSS 16.0 (*t*-test). The correlation between these differences and $CaCO_3$ contents were analyzed respectively using SigmaPlot 12.0. The biases in TN and δ^{15}N values after acidification were calculated, respectively, using the following equations:

$$\Delta \mathrm{N}\ (\text{of the TN}) = \frac{\mathrm{TN}_{\text{unacidified}} - \mathrm{TN}_{\text{acidified}}}{\mathrm{TN}_{\text{unacidifed}}} \times 100, \tag{1}$$

$$\Delta^{15}\mathrm{N} = \delta^{15}\mathrm{N}_{\text{unacidified}} - \delta^{15}\mathrm{N}_{\text{acidified}}. \tag{2}$$

3 Results and discussion

3.1 *$CaCO_3$ contents of all samples*

The $CaCO_3$ contents were approximately 20%–40% and 1%–16% for the sediments collected from the northwestern Australia and China's seas, respectively (Table 1). Based on the $CaCO_3$ contents of global marine sediments (Ridgwell and Hargreaves, 2007), the samples were divided into $CaCO_3$-poor samples ($CaCO_3$ contents <20%) and $CaCO_3$-rich samples ($CaCO_3$ contents >20%), respectively, for a comparison.

3.2 *Effects of acid treatment on TN*

The results of paired-sample *t*-test indicated a significant difference in TN contents (P=0.000<0.01, 2-tailed) between the samples measured with and without acid treatment. For the non-acidified samples, TN contents ranged from 0.02%–0.19%, while this range reduced to 0.01%–0.16% for the acidified samples. About 99% non-acidified vs. acidified TN values are located below the ideal 1:1 relation, indicating that acidification resulted in the losses of nitrogen contents from the samples (Fig. 1a). In addition, losses of nitrogen contents were on average higher in $CaCO_3$-rich samples compared to $CaCO_3$-poor samples after acidification. About 0%–40% and 10%–60% of nitrogen contents were lost from non-acidified $CaCO_3$-poor samples and $CaCO_3$-rich samples, respectively (Fig. 2a). Moreover, our data suggest a statistically significant positive correlation between the losses of nitrogen contents and the $CaCO_3$ contents (Fig. 2a). These findings partially agree with Lohse et al. (2000) and Fernandes and Krull (2008), who found a particularly high losses of nitrogen

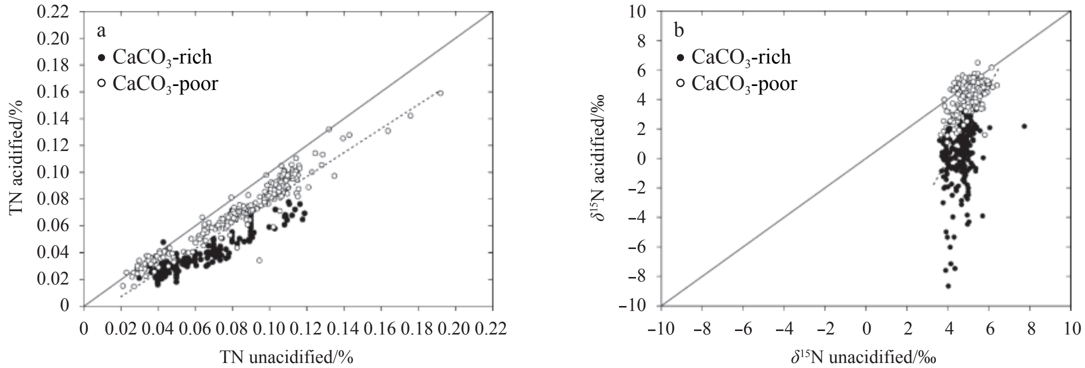

Fig. 1. Scatter plots of unacidified vs. acidified values for TN and $\delta^{15}N$. The ideal relation (1:1) is described by the solid line. The dashed lines indicate the statistic relation by regression analysis.

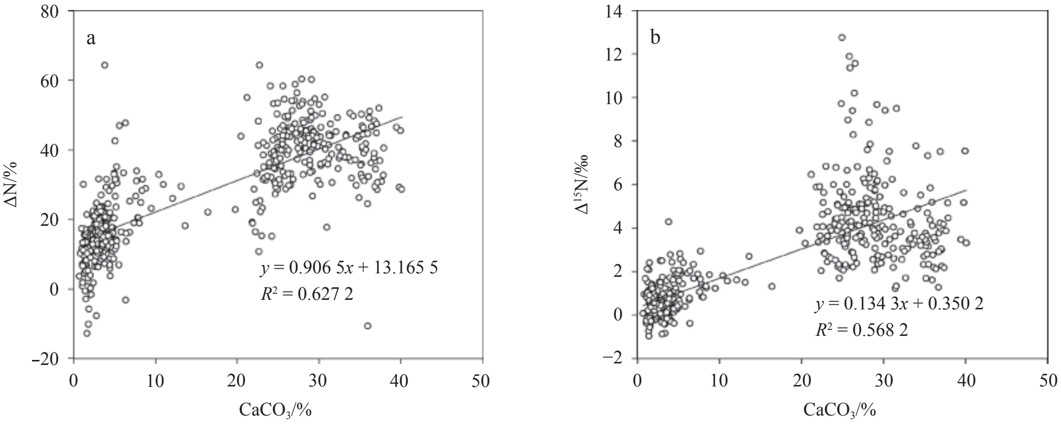

Fig. 2. Correlation between $CaCO_3$ contents and the biases in TN and $\delta^{15}N$ values after acidification.

Table 2. Comparison of differences caused by acidification in sedimentary TN and $\delta^{15}N$ values with references

Sample type	$CaCO_3$/%	ΔN/%	$\Delta^{15}N$/‰	Acidification method	Possible derives	Analysis mode	Reference
DSS1)	55–61	28–47	–	*in situ* H_2SO_3	H_2SO_3volatilises nitrogenous organic matter	single6)	Lohse et al. (2000)
	7–17	2–4	–				
SMS2)	–	11–29	–	direct HCl acidification	nitrogen appears to be liberated during direct acidification	single	Ryba and Burgess (2002)
MPOM3)	<5	0	0	HCl fumes for 4 h	HCl fume treatment did not alter $\delta^{15}N$ and N values for low carbonate contents	dual7)	Lorrain et al. (2003)
DSS1)	30	0.3	0.005	*in situ* 2 mol/L HCl	weak HCl (2 mol/L) did not significantly affect $\delta^{15}N$ and TN values	dual	Kennedy et al. (2005)
SMS2)	>30	30–64	1–2	HCl washing	losses through volatilisation or solubilisation (acid-soluble compounds)	single	Fernandes and Krull (2008)
SES4)	<10	19–40	0–1				
SOILB5)	–	15	0.7	*in situ* 5% HCl	loss of soluble organic and/or inorganic N species, e.g., ^{15}N-rich species (e.g., protein, ammonium)	single	Brodie et al. (2011a, b)

Note: Single or dual indicate separate or simultaneous C and N analyses; 1) deep-sea sediment; 2) surface marine sediment; 3) marine suspended particulate organic matter; 4) surface estuarine sediment; 5) international soil standard; 6) measurement on unacidified whole sample for TN and $\delta^{15}N$; 7) measurement on acidified sample for TN and $\delta^{15}N$.

contents in $CaCO_3$-rich (>30%) samples, up to 64% after acid treatment (Table 2), but no significant correlation between the loss of nitrogen contents and the $CaCO_3$ contents.

The loss of nitrogen contents from acidified samples was observed in the present and previous studies, although the acidification methods were different (Table 2). In our study, we used an acid treatment with 0.5 mol/L or 1 mol/L HCl in glass tubes (Peng et al., 2015), which avoided the loss of acid-soluble organic matter and/or fine particles by rinsing process (rinse method) and intensive effervescence (*in situ* acidification). This indicates that volatilisation of nitrogen compounds seems to be the most reasonable explanation for our results, and further investigation is still need for the explanation of the mechanism. Previous studies have demonstrated that in the $CaCO_3$-rich samples the fraction of proteinaceous compounds absorbed on carbonate surface layers became unstable with the increasing of acid addition, and this acid-soluble component was lost during carbonate dissolution (Fernandes and Krull, 2008). Meanwhile, this fraction of acid-soluble organic matter is greater in more calcareous sediments (Froelich, 1980). This may explain the higher losses observed in the $CaCO_3$-rich samples compared to the $CaCO_3$-poor samples.

3.3 *Effects of acid treatment on $\delta^{15}N$*

Nitrogen isotope ($\delta^{15}N$) values of samples measured with and without acidification are shown in Fig. 1b. There have significant shifts in $\delta^{15}N$ values between acidified and non-acidified samples, and these shifts have statistically significant positive correlation with the $CaCO_3$ contents, indicating that losses of acid-soluble nitrogen compounds with different $\delta^{15}N$ values (Fig. 2b). For $CaCO_3$-poor sediments the measured $\delta^{15}N$ values after acid treatment varied in the range of –1‰ to 6‰ (Fig. 1b). The shift of $\delta^{15}N$ values of these samples after acidification (up to 3‰) is similar to the deviation (0‰–2‰) observed by Fernandes and Krull (2008) (Table 2). For $CaCO_3$-rich samples about 40% of the measured $\delta^{15}N$ values were significant negative after acidification (down to –9‰, Fig. 1b), and bias in $\delta^{15}N$ values (up to 14‰) were remarkable compared to non-acidified samples (Fig. 2b). Schmidt and Gleixner (2005) also found some samples with unusual negative $\delta^{15}N$ values (e.g., –4.24‰ for a podzol Sample 6) after acid treatment due to the accumulation of coal-derived products. However, it is really difficult to understand what cause such depletion for part of $CaCO_3$-rich samples, since there is no rinse of the samples, which could trigger a loss of ^{15}N-enriched compounds. Volatilisation of nitrogenous compounds like chloramine should lead to an enrichment of $\delta^{15}N$ values not depletion.

In addition, our data suggest that isotopic shifts were not consistent for all samples (Fig. 2b). The depletion of $\delta^{15}N$ values was observed in 95% samples after acid treatment reflecting the loss of ^{15}N-enriched compounds such as protein and ammonium (Brodie et al., 2011a, b) (Fig. 2b). The loss of heavy isotope during acid treatment had previously been reported by other studies (Table 2). However, about 5% acidified samples (originating in $CaCO_3$-poor sediments collected from the Bohai Sea) detected a 0‰–1‰ increase signature in $\delta^{15}N$ values, suggesting the loss of ^{14}N-enriched compounds. Harris et al. (2001) observed a small increase (0.04‰–0.11‰) in $\delta^{15}N$ values of acidified soil samples following HCl fumigation. This contrasting shifts (depletion or enrichment) in $\delta^{15}N$ values probably influenced by the organic matter composition and clay mineralogy of samples (Baldock and Skjemstad, 2000; Schubert and Calvert, 2001; Fernandes and Krull, 2008), but more work need to do to identify differences in acid-soluble compounds, inorganic nitrogen and mineralogy of samples containing different $CaCO_3$ contents during acid treatment.

4 Conclusions

Our results suggest that the analysis of nitrogen contents and its isotopic composition should be measured on non-acidified whole sample, because acidification to remove carbonates in marine sediments leads to significant nitrogen losses through volatilisation and isotopic shifts, particularly for samples with high $CaCO_3$ contents (>20%). Meanwhile, there was a statistically significant positive correlation between the $CaCO_3$ contents and the biases in nitrogen contents and its isotopic composition after acid treatment (Fig. 2), indicating that the $CaCO_3$ contents in the sample play an important role in determining what extent the nitrogen fraction may have been affected by acid treatment.

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