Concentration dependent nitrogen isotope fractionation during ammonium uptake by phytoplankton under an algal bloom condition in the Danshuei estuary, northern Taiwan

Kon-Kee Liu a,⁎, Shuh-Ji Kao b, Kuo-Ping Chiang c, Gwo-Ching Gong d, Jeng Chang e, Jun-Shiang Cheng a, Cheng-You Lan a

a Institute of Hydrological and Oceanic Sciences, National Central University, Jungli 32001, Taiwan, ROC
b Research Center for Environmental Changes, Academia Sinica, Nankang, Taipei 115, Taiwan, ROC
c Institute of Marine Environmental Chemistry and Ecology, National Taiwan Ocean University, Keelung 20224, Taiwan, ROC
d Institute of Marine Environmental Chemistry and Ecology and Center of Excellence for Marine Bioenvironment and Biotechnology, National Taiwan Ocean University, Keelung 20224, Taiwan, ROC
e Institute of Marine Biology, National Taiwan Ocean University, Keelung 20224, Taiwan, ROC

A R T I C L E   I N F O

Article history:
Received 18 November 2012
Received in revised form 2 October 2013
Accepted 6 October 2013
Available online 25 October 2013

Keywords:
Particulate nitrogen
River estuary
Diatoms
Isotope effect
Ammonium assimilation
Variable isolate fractionation

A B S T R A C T

In July 2009 an intense algal bloom with maximum Chl-a concentration reaching 166 μg L−1 occurred in the highly eutrophic Danshuei River estuary, which receives waste discharges from the densely populated Taipei metropolitan area in northern Taiwan. The estuary is often burdened with very high concentration of ammonium (up to ~550 μM), which dominates the dissolved inorganic nitrogen species in the estuary. The observed ε15N values of particulate nitrogen ranged from −8.6% to 0.2%, and the ε15N values of coexisting ammonium ranged from 4.6 to 11.9%. Notably the offset between δ15NAm and δ15NH4 (Δδ15N) showed significant correlation with ammonium concentration. The ε-values were calculated to be between −4.7 and −16.4‰. The range overlaps with that of previous estimates (−6.5 to −18.1‰) based on field observations. We plotted all field observed ε-values vs. corresponding ammonium concentrations and found a trend similar to that previously observed for marine bacterium, Vibrio harveyi, in laboratory cultures. Thus, we constructed a concentration dependent curve of the ε-value for ammonium uptake by phytoplankton in natural waters. The curve shows the maximum magnitude of ε-value (−20‰) at ammonium concentration around 100 μM with decreasing isotope effect on both sides; at lower concentrations, the ε-value diminishes to zero; at higher concentrations, it slopes gradually towards an asymptotic value around −2‰. More than half of the ε-values derived from laboratory cultures of diatoms also fall on this curve. However, a few culture-based ε-values fall on another curve with similar pattern but considerably larger maximum magnitude. The maximum isotope effect is probably attributed to the cumulative isotope effects from ammonium deprotonation and the subsequent membrane diffusion of ammonia. This study provides the first field observed evidence of concentration dependent nitrogen isotope fractionation during ammonium uptake by phytoplankton and reconciles partially the disparity between estimates from field observations and from laboratory cultures.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The Danshuei River (Fig. 1), also known as the Tamsui River, is the largest river in northern Taiwan. It flows through the densely populated metropolitan area of the Taipei City and New Taipei City, where the total population exceeds six million. The high population brings a heavy load of anthropogenic nutrients to the river due to inadequate waste treatment (Lo, 2001; Liu et al., 2007). Consequently, the Danshuei River estuary is highly eutrophic with observed ammonium concentrations up to 500 μM (Sun and Peng, 2001; Wen et al., 2008).

The dominant phytoplankton in the Danshuei estuary are diatoms (Wu and Chou, 2003). In July 2009 there was an intense algal bloom with the chlorophyll-a (Chl-a) concentration reaching as high as 166 μg L−1, during which we obtained water samples for various chemical analyses and isotopic analyses of ammonium and particulate nitrogen (PN). Since ammonium was the dominant species of dissolved inorganic nitrogen in the estuary, the occasion provided an excellent opportunity to study nitrogen isotope fractionation during ammonium assimilation in the field.

Nitrogen isotope fractionation during ammonium uptake may be represented by the ε-value, which was first introduced by Mariotti et al.
The definition is as follows (see Supplement A for discussion on terminology and conventions):

\[ \varepsilon = (\alpha - 1) \times 1000\%o, \]

where \( \alpha \) is the isotope effect (Sigman et al., 2009), or the isotope separation factor (Hoefs, 2009). Please note that all \( \varepsilon \)-values referred to in the text have been converted to the convention described here, if they were defined differently.

An \( \varepsilon \)-value of \(-9.1\%o\) was estimated from changes in the nitrogen isotopic composition of ammonium during its consumption in the Delaware estuary in spring (Cifuentes et al., 1989). A pair of \( \varepsilon \)-values, slightly smaller in magnitude, \(-7.9\%o\) and \(-6.5\%o\), was reported for ammonium supported phytoplankton growth after a storm in the Chesapeake Bay (Montoya et al., 1991). An \( \varepsilon \)-value of \(-18.1\%o\) was estimated for ammonium utilization in the Scheldt estuary (De Brabandere et al., 2007).

The \( \varepsilon \)-values obtained from laboratory cultures showed even larger variation. Wada and Hattori (1978) grew Phaeodactylum tricornutum with an initial ammonium concentration of 3.5 mM and found \( \varepsilon \)-value of \(-2\%o\), when 40% of ammonium was consumed. Hoch et al. (1992) performed cultures of the marine heterotrophic bacterium, Vibrio harveyi, and obtained \( \varepsilon \)-values ranging from \(-4\%o\) to \(-27\%o\), while using initial ammonium concentrations from 23 to 182 \( \mu \)M, and \(-27\%o\) to \(-14\%o\), while using initial ammonium concentrations from 182 \( \mu \)M to 23.3 mM. Basing on these results, they suggested nitrogen isotope fractionation dependent on ammonium concentration due to changes in the pathways of ammonium transport and assimilation. Pennock et al. (1996) conducted culture experiments of diatoms in media with initial ammonium concentrations from 50 to 100 \( \mu \)M, and obtained \( \varepsilon \)-values ranging from \(-5\%o\) to \(-29\%o\); they also suggested concentration dependency of isotope fractionation. Waser et al. (1998) carried out culture experiments of diatoms with initial concentration of 166 \( \mu \)M and reported \( \varepsilon \)-values of \(-19\%o\) and \(-20\%o\). Vo et al. (2013) reported \( \varepsilon \)-values in the wide range of \(-5.1\%o\) to \(-30.2\%o\) for different strains of Escherichia coli.

While large differences exist between field observations and laboratory culture studies of nitrogen isotope fractionation during ammonium uptake by phytoplankton, which have been recognized by some (e.g., Fogel and Cifuentes, 1993; Sigman et al., 2009), none have attempted to reconcile the differences or considered the validity of concentration dependent nitrogen isotope fractionation under natural conditions. Our observations in the highly eutrophic Danshuei estuary allowed us to investigate isotope fractionation during ammonium uptake at rather high level of ammonium concentrations in the field in the hope of better understanding the phenomenon and resolving the apparent disparity. This investigation has given us new insight into the concentration dependency of isotope effects and brought a new perspective to the issue so that its applicability under natural conditions will be validated and the concentration dependent function expressed in a more quantitative manner.

Since ammonium-rich water bodies are widespread in coastal environments adjacent to highly urbanized or industrialized regions, such as the Pearl River Delta (e.g., Dai et al., 2008) or the Scheldt estuary (e.g., De Brabandere et al., 2007), this study will shed light on the nitrogen isotopic characteristics of ammonium based algal production of organic matter. During the Mesozoic Oceanic Anoxic Events, when deposition of organic rich sediments occurred, the deep ocean was probably dominated by the reduced nitrogen species. It has been suggested that

![Fig. 1. Map of the Danshuei River system in northern Taiwan. The sampling location was at the Chunyang Bridge.](image-url)
upwelling of anoxic deep waters would have supplied ammonium as an important nitrogen source to support growth of eukaryotic primary producers in the upper water column (Higgins et al., 2012). If this was the case, our new findings can contribute to better interpretation of the nitrogen isotopic records in the geologic history.

2. Materials and methods

2.1. Sample collection

Water samples were collected from the Danshuei estuary at the Chunyang Bridge (Fig. 1) during a strong algal bloom in July 2009. A bucket, pre-washed with distilled water prior to deployment, was lowered from the bridge to collect the river water within ca. 50 cm from the surface. Before collecting the sample, the bucket was rinsed with the river water twice. Such sampling was done every 3 h over about four semidiurnal tidal cycles, from 7 to 9 July 2009. The water depth varied between 2.5 m and 5.0 m following the tide. A similar sampling was carried out from 9 to 11 February 2009, when the algal biomass was relatively low.

For collection of suspended particulate matter (SPM), water samples of 200 mL to 500 mL in volume were measured with a calibrated cylinder, which was prewashed with distilled water and rinsed with sample water, and filtered through quartz fiber filters with a hand-operated pump in the open on site. The filtration flask was prewashed with distilled water. The quartz filters, 47 mm in diameter, were preheated at 500 °C in an oven for the removal of organic matter and wrapped in aluminum foil before use; after filtration, the filters were folded and wrapped again in aluminum foil and stored at 450 °C in an ice chest. For salinity determination, water samples were stored at 4 °C in an ice chest. For Chl-a, the frozen samples were transferred to a freezer for storage at about −20 °C. The stored samples were usually analyzed within 3 months after collection.

In order to establish the seasonal variation of Chl-a over an annual cycle in the Danshuei River estuary, we conducted weekly sampling from May 2010 to August 2011. For each sampling occasion, water samples were collected at both the high tide (slack before ebb) and the low tide (slack before flood). The samples were filtered for Chl-a analysis. Every month, additional water samples, 500 mL in volume, were collected, preserved with 25 mL of 37% formalin and kept at room temperature. Within 2 months, selected samples were examined for identification and counting of phytoplankton. Phytoplankton cells in a 100 mL water sample were concentrated by the Utermöhl method (Hasle, 1978). They were identified and counted using an inverted epifluorescence microscope (Nikon-TMD 300) at 200 × 400 × or 400 × (Hasle and Syvertsen, 1996; Marumo et al., 1966). Cells were counted in two rows in the middle of the settling chamber and the counting area occupied about 5.2% of the entire chamber. The total number of cells observed in the two rows was used to estimate the phytoplankton density.

2.2. Chemical analyses

The chemical analyses performed on the frozen filtered water samples were conducted shortly after collection. The procedures are described briefly below. The frozen samples were thawed under tap water prior to analysis. Nitrate was analyzed by the standard pink azo dye method adapted for flow injection analyzer (Morris and Riley, 1963; Strickland and Parsons, 1972; Pai et al., 1990). Nitrite was also analyzed by the pink azo dye method using a batch procedure (Strickland and Parsons, 1972). Ammonium was analyzed by the modified indo-phenol method by reacting with hypochlorite and phenol under alkaline conditions (Solórzano, 1969; Pai et al., 2001). The algal pigments retained on GF/F filters were extracted in 90% acetone and the extract was measured with a fluorometer, Turner 10-AU-005 (Parsons et al., 1984; Welschmeyer, 1994; Gong et al., 2000). The precision of the measurement represented by the standard deviation of repeated analyses was better than ±0.3 μM for nitrate and nitrite, ±0.5 μM for ammonium, and ±0.1 μg L−1 for Chl-a.

2.3. Isotopic analyses

Determination of δ15N-NH4 was based on the procedure of Holmes et al. (1998). For ammonia extraction, 250 mL of filtered sample water (often diluted with deionized doubly distilled water to desired concentration of ammonium, 50 μM or less) was transferred to 500–M HDPE incubation bottles, to which pre-ashed NaCl (12.5 g) and MgO (0 · 75 g) and the ammonium trap were added. The ammonium trap consisted of a Whatman GF/D filter acidified with 25 μL of 2 M H2SO4 sandwiched between two Teflon filter membranes. Samples, blanks and working standards of (NH4)2SO4, which all had volumes of 250 mL and ammonium concentrations between 24 and 96 μM, were incubated for 2 weeks so that all ammonia could diffuse out of the solution and be trapped. During incubation samples were shaken thoroughly in an orbital shaker and maintained at room temperature. After incubation, the floating ammonium traps were retrieved from the incubation bottles and the filters with trapped ammonium were removed from the package, dried and stored in a desiccator for no more than 1 month before analysis. Prior to analysis they were wrapped in tin boats. The recovery of the ammonium retrieval procedure was nearly 100% within the error of nitrogen quantification by the beam intensity of the mass spectrometer. The blank was 0.2 ± 0.1 μmol on average, which was corrected by dilution series of samples (Cheng, 2010).

The filters with SPM were dried at 70 °C in an oven and then acidified with 1 mL of 1 N HCl solution in a fume hood to remove carbonate (Gordon, 1969). Then, the filters were dried at 60 °C in an oven for 48 h. The filters with trapped ammonium and the decarbonated SPM samples were analyzed for nitrogen and carbon contents and their isotopic compositions in a continuous flow elemental analyzer (Flash EA-1100 NC, Thermo-Finnigan)–isotope ratio mass spectrometer (Thermo Finnigan DELTAplus Advantage) system.

The nitrogen isotopic composition is presented by the delta notation:

$$\delta^{15}N = \left\{\frac{\text{[15N]/[14N]}_{\text{sample}}}{\text{[15N]/[14N]}_{\text{standard}}} - 1\right\} \times 1000 \text{‰}$$

The nitrogen isotopic measurements were calibrated with working standard and then converted to the δ-values with respect to atmospheric nitrogen. The accuracy was estimated to be better than ±0.2‰ based on periodic analyses of an international standard (USGS 40, l-glutamic acid: −4.5 ± 0.2‰). A laboratory standard (KNO3) with δ15N of 13.8‰ was used to check the stability of the system for isotopic analysis every month. The long-term mean of the measurements was 13.8 ± 0.3‰ in four years. For particulate nitrogen samples, the precision was estimated to be better than ±0.2‰ by a working standard (Merck Acetanilide: −1.5 ± 0.2‰) and field samples. The reliability of the diffusion method was checked by running solutions of a working standard of ammonium sulfate (24–96 μM) and the procedure yielded a mean value of −0.23 ± 0.2‰, which was very close to the result (−0.30 ± 0.2‰) obtained by direct combustion of the working standard in the EA-IRMS system.
3. Results

We first report Chl-α results from the weekly sampling to reveal the variation pattern over an annual cycle, which allows us to qualify the observations in July 2009 as an exceptionally strong algal bloom condition. Then we report the chemical hydrography and nitrogen isotopic data obtained during the bloom.

3.1. Seasonal variation of Chl-a

The data of Chl-a concentration in surface water of the Danshuei estuary obtained in February and July 2009 and from the weekly sampling from mid-2010 to mid-2011 are all plotted within an annual cycle (Fig. 2). It is clear that the low-tide samples had higher Chl-a concentrations than the high-tide samples, indicating that the freshwater end-member had higher concentration of phytoplankton, while the seawater end-member had lower concentration.

During the weekly sampling period (May 2010–Aug. 2011), the observed Chl-a concentrations during low tides varied from a minimal level of 8–16 μg L⁻¹ in winter to a maximal level of 70–80 μg L⁻¹ in summer, while those observed during high tides ranged from 1–5 μg L⁻¹ in winter to 12–20 μg L⁻¹ in summer. Compared to the same month in other years, July 2009 showed an extraordinarily strong Chl-a peak, which reached as high as 166 μg L⁻¹, indicating it an exceptionally strong algal bloom.

The phytoplankton composition in the estuary in summer showed significant variation along the salinity gradient. In the lower salinity water (S < 5), diatoms accounted for >96% of phytoplankton cells with the rest being dinoflagellates and chlorophytes in comparable abundances. In higher salinity waters (S > 10), diatoms were less but still dominant, accounting for 94% or less, while chlorophytes were the distant second. More detailed information on the phytoplankton composition is provided in Supplement B. In all cases, the diatoms were dominated by Fragilaria spp. The observed relative abundances of diatoms and trend agreed with previous findings (Wu and Chou, 2003).

3.2. Observations during the algal bloom

Four cycles of semidiurnal tides elapsed during the sampling period in July 2009 (Fig. 3). The temperature remained high throughout the tidal cycles with a narrow range from 29 °C to 31.5 °C (Fig. 3a). Even so, the tidal signals were visible with higher temperatures at high tide, while the diel cycle also showed noticeable influences on temperature with elevated temperature at noon. The tidal signals were more evident in the salinity data (Fig. 3a). The salinity minimum reached 2 at low tide, indicating these samples very close to the freshwater end-member, while the salinity maximum reached only 15, which was considerably lower than the salinity outside the river mouth around 31 (Wen et al., 2008). This implies the contribution of seawater end-member less than 50% in all the water samples collected at the Chunyang Bridge and the dominance of the riverine system in the observed conditions.

Most chemical constituents, including ammonium, Chl-a, particulate organic carbon (POC) and particulate nitrogen (PN), varied inversely with respect to the salinity variation, implying higher concentration in the freshwater end-member and lower in the seawater end-member (Fig. 3b–d). The ammonium concentrations were very high, varying between 200 μM and 520 μM with the peak values corresponding to salinity minima, indicating low tides when the river water end-member dominated the water body and lowered the salinity. By comparison, the nitrate and nitrite concentrations were rather low, varying in the ranges of 0–30 μM and 3–7 μM, respectively. The variation of nitrate roughly followed that of salinity, indicating that the seawater end-member was more enriched in nitrate, but that of nitrite was rather irregular. It is noted that the concentration of dissolved reactive phosphate ranged from 1.5 to 12.4 μM with a mean of 7.2 ± 2.8 μM (Cheng, 2010). The available phosphate was never depleted to such a degree to pose as a limiting factor for phytoplankton growth in the water body that we studied.

The δ¹⁵N values of ammonium ranged from 4.6 to 11.9‰, and those of particulate nitrogen were considerably lower, ranging from −8.6‰ to 0.2‰. Although the variation of ammonium and PN concentrations followed the tidal movement, their δ¹⁵N did not show any evident tidal influences, suggesting that some other factor, such as algal growth, was probably the main control over the isotopic compositions.

As noted by Holmes et al. (1998) dissolved organic nitrogen (DON) could interfere with nitrogen isotopic analysis of ammonium in the diffusion method; hence, it is desirable to assess possible interferences from DON in our analysis. However, since DON was not analyzed in this study, we did the assessment by assuming the DON levels similar to those (2–20 μM) that we previously observed in the lower reaches of the Danshuei River (Huang, 2003). According to Holmes et al. (1998), the blanks attributable to DON degradation represent 0.1–2.5% of total DON present in coastal or river waters. If the same degree of DON degradation occurred in our case, it would have produced a blank of 0.002–0.5 μM. The estimated maximum blank would have been only about 0.1–0.25% of the ammonium, present in the diffusion bottle. Therefore, the interferences from DON should be insignificant, if any.

4. Discussion

We first discuss the dominance of autochthonous organic matter from phytoplankton bloom and show the lack of evidence for the allochthonous organic matter in the POM pool. Then we explore the potential sources of nitrogen for the algal growth during our sampling period and illustrate that ammonium was the major nitrogen source. Thus we derive the nitrogen isotope fractionation factor during ammonium assimilation from co-existing particulate nitrogen and ammonium. Finally we demonstrate the consistent trend revealed from our results and previous findings so that we derive a concentration dependent isotope fractionation factor for ammonium uptake by phytoplankton.

4.1. Dominance of autochthonous organic matter

Following the example of Cifuentes et al. (1988), we used the POC/Chl ratio to explore the origin of particulate organic matter (POM) in our samples. These authors suggest that the POC/Chl ratio (wt/wt) ratio less than 200 represents freshly formed biomass, whereas the ratio above 200 suggests dominance of degraded POM. In our case, the POC/Chl ratio varied between 33 and 62 with a mean of 47.9 ± 8.5, suggesting
the POM primarily as freshly formed algal biomass. This also agreed reasonably well with the range, 50–75, observed for coastal diatom blooms in summer (Schaefer and Lewin, 1984). In addition, the linear regression between POC (μM) and Chl-a (μgL⁻¹) gave the following relationship:

\[
[\text{POC}] = 3.40[\text{Chl-a}] + 44.3 \quad R^2 = 0.82. \quad p<0.001.
\]  

The slope corresponds to a C/Chl-a ratio of 41. Both the good correlation and the slope indicate the increase of POM in our samples mainly attributed to phytoplankton growth. By contrast, the C/Chl-a ratios observed in February 2009 ranged from 219 to 619 with an average of 370 ± 108, indicating the predominance of detrital material in the POM pool under non-blooming conditions. Therefore, the POM collected in July 2009 comprised mainly of freshly formed phytoplankton biomass.

Moreover we wish to show that allochthonous organic matter originating from the open sea or from land was not a significant contributor in the suspended POM pool. Shah et al. (2000) reported that the typical sea surface Chl-a concentration in the northern Taiwan Strait off the Danshuei River mouth in summer was about 0.3 μgL⁻¹ or less. As mentioned earlier the fraction of the open sea water in the estuarine water we sampled was less than 50%, which implies the contribution of Chl-a from the open shelf less than 0.15 μgL⁻¹. While the Chl-a concentration found in our samples was all greater than 30 μgL⁻¹, the Chl-a from the open shelf accounted for less than 0.5% in all samples.

Concerning terrigenous organic matter in the estuary, Mariotti et al. (1984) demonstrated a positive correlation between δ¹⁵NPN and salinity in the Scheldt estuary; low δ¹⁵NPN (1.3–1.6‰), implying land derived organic material, corresponded to the low end of salinity, whereas the high δ¹⁵NPN (7‰), implying marine organic matter, corresponded to

![Fig. 3: Time-series of observations at the sampling station in the Danshuei estuary in July 2009. The shaded period indicates ebb tides. In each panel, the left axis is for the solid circles with dashed lines, and the right axis for the crosses with solid lines. (a) Temperature and salinity; (b) [NH₄⁺] and [NO₃⁻] (μM); (c) [NO₂⁻] (μM) and [Chl-a] (μgL); (d) [POC] and [PN] (μM); and (e) δ¹⁵N values of ammonium and PN.](image-url)
the high end of salinity. This indicates that the nitrogen isotopic composition of suspended particulate matter (SPM) may serve as a good indicator of the relative contributions of terrigenous and marine organic matter. In our case, the $\delta^{15}$N$_{PM}$-salinity relationship for the observations in July 2009 (Fig. 4) showed no correlation, implying no isotopic evidence for a two-end-member mixing involving terrigenous organic. In other words, essentially all PN in our samples originated from the algal growth process without showing a discernible second-end-member from land.

The C/N ratio is another indicator of the origin of organic matter. Canuel et al. (1995) observed a negative correlation of the C/N ratio of POM and salinity in the San Francisco Bay with the highest value (~16) at the riverine end and the lowest value (~7) at the seaward end. In our case, we found good correlation between POC and PN as follows:

$$[\text{POC}] = 7.37[\text{PN}] - 1.2 \quad R^2 = 0.96, p<0.001.$$  

The slope, 7.37, was close to the Redfield ratio, suggesting an origin of algal biomass. It is noted that the POC:PN ratio showed a correlation with salinity (Fig. 4), but the weak trend was opposite to what was observed in the San Francisco Bay. This indicates that the variation of C:N ratio was served in the San Francisco Bay. This indicates that the variation of C:N ratio with salinity (Fig. 4), but the weak trend was opposite to what was expected from mixing of terrigenous (high C/N) and marine (low C/N) end-members.

The C/N ratio is another indicator of the origin of organic matter. Canuel et al. (1995) observed a negative correlation of the C/N ratio of POM and salinity in the San Francisco Bay with the highest value (~16) at the riverine end and the lowest value (~7) at the seaward end. In our case, we found good correlation between POC and PN as follows:

$$[\text{POC}] = 7.37[\text{PN}] - 1.2 \quad R^2 = 0.96, p<0.001.$$  

The slope, 7.37, was close to the Redfield ratio, suggesting an origin of algal biomass. It is noted that the POC:PN ratio showed a correlation with salinity (Fig. 4), but the weak trend was opposite to what was observed in the San Francisco Bay. This indicates that the variation of C:N ratio was served in the San Francisco Bay. This indicates that the variation of C:N ratio with salinity (Fig. 4), but the weak trend was opposite to what was expected from mixing of terrigenous (high C/N) and marine (low C/N) end-members.

**4.2 Ammonium as predominant nitrogen source for algal growth**

As shown earlier, ammonium was the predominant species of dissolved inorganic nitrogen (DIN) in the Danshuei estuary, while the combined oxidized form ($\text{NO}_x$), namely, nitrate plus nitrite, accounted for 1.7% to 14.6% with a mean of 6.9 ± 3.3%. Besides, it is well known that ammonium is preferred to nitrate during nitrogen assimilation by phytoplankton because ammonium is reduced nitrogen whereas $\text{NO}_x$ is the oxidized form that has to be reduced before assimilation (McCarthy, 1980). It is clear that ammonium should be the predominant nitrogen source for the observed algal bloom. Even so, it is worth investigating whether $\text{NO}_x$ uptake could be of any significance. Contrary to the trend shown by ammonium, $\text{NO}_x$ concentration increased with increasing salinity (Fig. 5). The highest percentage of $\text{NO}_x$ in DIN occurred in the high end of the salinity range. If uptake of $\text{NO}_x$ had been important during the algal bloom, the most important contribution should have occurred in the high salinity waters. It was observed in the same samples analyzed for this study that $\text{NO}_x$ was more enriched in $^{15}$N with a mean $\delta^{15}$N value of 20.0 ± 3.1‰ (Cheng, 2010). Hence, $\delta^{15}$N$_{PN}$ would have revealed an increasing trend with increasing salinity, should algal $\text{NO}_x$ uptake have occurred. However, no such trend was found (Fig. 4), indicating that uptake of $\text{NO}_x$ was not evident during the algal bloom. It is worth mentioning that the high $\delta^{15}$N value of $\text{NO}_x$ was probably attributed to denitrification under low oxygen conditions in the bottom water of the estuary (Lan, 2013; Wen et al., 2008).

In spite of the presumed ammonium uptake, the ammonium concentration showed a quasi-conservative property manifested as a strong negative correlation with salinity (Fig. 5) as the following:

$$[\text{NH}_4^+] = -16.94S + 451, \quad R^2 = 0.683.$$  

where S is salinity. This suggests mixing as the main control of ammonium concentration in the estuary, while the uptake was limited. If the PN concentration represented the amount of uptake, the removed ammonium accounted for 6.9% to 18.9% of the original ammonium concentration. It is noted that the sum of ammonium and PN concentrations showed a better correlation with salinity, as shown below:

$$[\text{NH}_4^+] + [\text{PN}] = -20.81S + 527, \quad R^2 = 0.761$$  

indicating that a more conservative behavior characterized the sum.

Since ammonium may be oxidized to nitrite and nitrate during nitrification, we examined whether inclusion of $\text{NO}_x$ (NO$_3^-$ + NO$_2^-$) in the sum would make the property even closer to perfectly conservative behavior. The regression gave the following result:

$$[\text{NH}_4^+] + [\text{PN}] + [\text{NO}_3^-] + [\text{NO}_2^-] = -19.62S + 541, \quad R^2 = 0.738.$$  

The lower R$^2$ indicates that inclusion of $\text{NO}_x$ does not improve the conservative behavior. Because some of the $\text{NO}_x$ could have originated from the open shelf, it deserves investigating whether such $\text{NO}_x$ could...
alter the linear relationship. The typical nitrate concentration in the surface water just outside the Danshuei River mouth in summer was less than 1 μM (Shiah et al., 2000). Therefore, such NO3 would have accounted for less than 0.5 μM in the estuarine water that we sampled and can hardly interfere with the linear relationship in question (Fig. 5). Since the residence time of the estuarine water is only 1–2 days (Wang et al., 2004), it is reasonable to assume that the fraction of ammonium removed by nitrifiers was very little.

4.3. Nitrogen isotope fractionation during ammonium uptake

Because ammonium was the predominant nitrogen source for the algal bloom and algal biomass was the major component of suspended PN, the nitrogen isotopic offset ($\Delta^{15}$N) between the coexisting ammonium and PN should reflect isotopic fractionation during ammonium uptake by phytoplankton. The calculated isotopic offsets range from −17.8 to −5.1‰, with a mean of $-13.0 \pm 3.2$‰.

Although Hoch et al. (1992) and Pennock et al. (1996) have long suggested dependency of nitrogen isotopic fractionation during ammonium uptake by phytoplankton. The calculated isotopic offsets range from −17.8 to −5.1‰, with a mean of $-13.0 \pm 3.2$‰.

It is noted that this regression result is not intended to represent the concentration dependency of isotope fractionation, which requires rigorous derivation of the $\varepsilon$-values and thorough consideration of all available data.

For rigorously derived $\varepsilon$-values, we need to consider detailed conditions of ammonium uptake during phytoplankton growth, such as whether the process took place in a closed or open system. Because of the very short residence time of the estuarine water and the fast ammonium uptake during algal bloom, it is reasonable to assume a closed system, in which the Rayleigh type isotopic fractionation occurred. Then we may use the following equation to calculate the $\varepsilon$-value:

$$
\varepsilon = \left\{ \ln(1 - (1 - f)R_p/R_i) / \ln(f) - 1 \right\} \times 1000 \%
$$

where $f$ is the fraction of the residual ammonium in the system and $R_i$ and $R_p$ denote, respectively, the initial isotopic ratio of the substrate, namely, ammonium, and the observed isotopic ratio of the product, namely, the algal biomass or PN. Eq. (9) may be derived from the definition of $\varepsilon$-value, i.e., Eq. (1), and the following well known equation for Rayleigh type isotopic fractionation (e.g., Zeebe and Wolf-Gladrow, 2001):

$$
R_p/R_i = (1 - f^\alpha)/(1 - f).
$$

The ratio of the isotopic ratios may be expressed as:

$$
\frac{R_p}{R_i} = \left(1000 + \delta^{15}N_{PN}\right) / \left(1000 + \delta^{15}N_{NH_4}\right).
$$

Because the sum of ammonium and PN concentrations was nearly conservative, the initial values of ammonium concentration and its isotopic composition may be derived by combining the observed ammonium and PN properties:

$$
\left[\text{NH}_4^+\right] = \left[\text{NH}_4^+\right] + [\text{PN}]
$$

and

$$
\delta^{15}N_{NH_4} = \left(\delta^{15}N_{NH_4} - \left[\text{NH}_4^+\right] + \delta^{15}N_{PN}[\text{PN}]/\left(\left[\text{NH}_4^+\right] + [\text{PN}].
$$

Thus $f$ may be evaluated as:

$$
f = \left(\frac{\left[\text{NH}_4^+\right]}{\left[\text{NH}_4^+\right]}\right)^\alpha.
$$

The data for calculation and the results are listed in Table 1. The estimated $\varepsilon$-value ranges from −4.7 to −16.4‰ with a mean of $-12.0 \pm 2.9$‰. It is noted that the $\varepsilon$-values calculated under an open system assumption are slightly larger in magnitude. The equation used for the calculation is the following:

$$
\varepsilon_{op} = \left\{ R_p/R_i - 1 \right\} \times 1000 \%
$$

where $R_i$ denotes the isotopic ratio of the substrate, i.e., ammonium. The results, listed in Table 1, show that the mean difference is only $0.9 \pm 0.4$‰. For the following discussion, we refer to only the $\varepsilon$-values calculated under the closed system assumption.

4.4. Comparisons with previous estimates

All estimates of $\varepsilon$-value during ammonium uptake by phytoplankton from previous studies are summarized in Table 2 and plotted along side with estimates from this study in a diagram of $\varepsilon$-value vs. ammonium concentration (Fig. 7a). It is noted that each $\varepsilon$-value was plotted at the midpoint between the initial and final ammonium concentrations, over which the $\varepsilon$-value was estimated, while the horizontal error bar covers the concentration range. Before discussing the figure, we have

![Fig. 6. The $\delta^{15}$N shift between particulate nitrogen (PN) and ammonium vs. ammonium concentration. There exists a significant correlation that implies concentration dependency of isotope fractionation.](image)
to qualify the plotted values that are listed in Table 2. Some of the original estimates that were calculated using a different convention for the isotope fractionation coefficient, namely, $\delta^{15}N$, were converted to the convention of this study. Moreover, some published data allowed us to estimate the $\varepsilon$-value for ammonium uptake. Details are explained below.

For the study by Waser et al. (1998), we have listed not only their final estimates but also the initial estimate based on the first stage of the culture. For the $\varepsilon$-value reported by Cifuentes et al. (1989) the uncertainty was estimated using the data presented in their Fig. 7. De Brabandere et al. (2007) reported the overall $\varepsilon$-value for ammonium consumption by both phytoplankton and nitrifying bacteria. Hence, we used their reported data to calculate the $\varepsilon$-value solely for ammonium uptake by phytoplankton using the following approach: (1) estimating the increase of algal biomass in terms of nitrogen and its isotopic composition during the spring bloom in April 2002 reported in their study, (2) estimating the $\delta^{15}N$ value of coexisting ammonium, and (3) calculating the $\varepsilon$-value from the aforementioned quantities. The calculation is detailed below.

The increase in biomass is estimated from the increase of Chl-$a$ from 25 $\mu$g L$^{-1}$ in late March to 138 $\mu$g L$^{-1}$ in mid-April when the bloom occurred. The Chl-$a$ to PON conversion was done with the assumption of the Chl-$a$:PON ratio of 1.59 g/mol, which corresponds to a Chl-$a$: POC ratio of 1:50. Thus it is calculated that the biomass concentration

### Table 2

<table>
<thead>
<tr>
<th>$[\text{NH}_4]^+$ (mM)</th>
<th>$\varepsilon$-Value (‰)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab culture results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.00 ± 0.700</td>
<td>$-6 \pm 4$</td>
<td>Lab culture of Phaeodactylum tricornutum</td>
</tr>
<tr>
<td>23 ± 12</td>
<td>$-4 \text{ to } -27$</td>
<td>Lab cultures of the marine heterotrophic bacteria, Vibrio harveyi</td>
</tr>
<tr>
<td>182 ± 23,300</td>
<td>$-27 \text{ to } -14$</td>
<td>Lab culture of Skeletonema costatum</td>
</tr>
<tr>
<td>12.5 ± 12.5</td>
<td>$-4.8$</td>
<td></td>
</tr>
<tr>
<td>12.5 ± 12.5</td>
<td>$-7.8$</td>
<td></td>
</tr>
<tr>
<td>12.5 ± 12.5</td>
<td>$-11.0$</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>$-28.9$</td>
<td></td>
</tr>
<tr>
<td>30.0 ± 15.0</td>
<td>$-27.1$</td>
<td></td>
</tr>
<tr>
<td>30.0 ± 15.0</td>
<td>$-23.7$</td>
<td></td>
</tr>
<tr>
<td>30.0 ± 15.0</td>
<td>$-28.3$</td>
<td></td>
</tr>
<tr>
<td>75.1 ± 22.9</td>
<td>$-20.7$</td>
<td></td>
</tr>
<tr>
<td>17.00 ± 5.0</td>
<td>$-19.6$</td>
<td>Lab culture of Thalassiosira pseudonana</td>
</tr>
<tr>
<td>90 ± 80</td>
<td>$-19.0$</td>
<td></td>
</tr>
<tr>
<td>90 ± 80</td>
<td>$-20.0$</td>
<td></td>
</tr>
<tr>
<td>Field observations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 ± 35.0</td>
<td>$-9.1 \pm 1.0$</td>
<td>Delaware Bay, Feb–March 1987</td>
</tr>
<tr>
<td>4.8 ± 0.8</td>
<td>$-7.9$</td>
<td>Storm induced algal growth in Chesapeake Bay, Sept. 27-Oct. 5, 1985</td>
</tr>
<tr>
<td>13.6 ± 3.6</td>
<td>$-8.6$</td>
<td></td>
</tr>
<tr>
<td>9.64 ± 5.3</td>
<td>$-18.1 \pm 2.0$</td>
<td>Overall isotope effect of ammonium removal in the Scheldt estuary</td>
</tr>
<tr>
<td>81.6 ± 12.5</td>
<td>$-16.3$</td>
<td>Algal bloom in Scheldt estuary, April 2002 (see text)</td>
</tr>
</tbody>
</table>
(in terms of nitrogen) grew from 15.8 μM to 86.6 μM with corresponding δ15N values of 1.8‰ and −2.6‰, respectively (De Brabandere et al., 2007). The increase was calculated to be 70.8 μM. The nitrogen isotopic composition of the biomass increment was calculated to be −3.6‰ from isotope mass balance. The δ15N NH4+ increased only slightly from 12.4 to 13.3‰ in the first half of April 2002 (De Brabandere et al., 2007). The mean of the two δ15N NH4+ values (12.9‰) was used for the estimation of the ε-value, which was calculated using Eq. (15). The resultant ε-value was −16.6‰, which is close to the overall ε-value of −18.4 ± 2.0‰ for ammonium consumption reported by the authors.

As revealed in the expanded list of ε-values (Table 2), the range from field observations spans from −6.5 to −18.1‰, which overlaps to a large extent with our estimates. More importantly all data points based on field observations revealed a consistent pattern of ε-value varying with [NH4+]) (Fig. 7a). The trend shows a maximum isotope effect of about −20‰ around the ammonium concentration of 100 μM, while the isotope effect drops rapidly at lower [NH4+] but decreases more gradually towards an asymptotic value at higher [NH4+]. Aside from four data points all estimates based on laboratory cultures of diatoms fall near the aforementioned trend.

It is intriguing that the trend resembles that observed for laboratory cultures of the marine heterotrophic bacterium, V. harveyi (Hoch et al., 1992), suggesting that the processes responsible for the nitrogen isotope fractionation during ammonium uptake by the diatom dominated phytoplankton assemblage in the Danshuei estuary are probably similar to those for the aforementioned bacterium (Fig. 7b). In the next section we attempt to derive empirical functions to express the concentration dependent ε-value based on the processes proposed by Hoch et al. (1992).

4.5. Ammonium uptake and concentration dependent ε-value

The kinetic isotope effect, unlike the thermodynamic isotope effect, which tends to approach a certain constant equilibrium value at constant temperature, is kinetic in nature and may vary considerably as the rate determining steps of biochemical pathways change with the ambient condition as well as the species of organisms and their cellular conditions. Assimilation of ammonia by aquatic eukaryotic photosynotrophs (Falkowski and Raven, 2007) is primarily brought about by the sequential action of glutamine synthetase (GS) and glutamine 2-oxoglutarate amino transferase (GOGAT). At low levels of ammonium, membrane transport is by an active ammonium transport system (Kleiner, 1986). At higher levels of ammonia, passive diffusion of NH4+ becomes more important for membrane transport (Hoch et al., 1992).

According to Hoch et al. (1992), at very low level of ammonium (<1 μM), the rate-limiting step during ammonium uptake is the active membrane transport of ammonium. While Hoch et al. (1992) suggested no isotope effect associated with this transport, a moderate ε-value of (−14.1 ± 0.8‰) was reported for E. coli (Vo et al., 2013). However, if the ambient ammonium is quickly exhausted at very low concentration, little isotope effect would be manifested. Consequently, the ε-value may approach zero at very low [NH4+].

At high level of ammonium, transport may become so fast that it does not pose significant limitation on assimilation, and the rate-limiting step may rest with assimilation by the aforementioned enzyme systems. While the GS/GOGAT system is in operation under normal conditions, assimilation by GDH could be important at very high ammonium concentration (Helling, 1994). Under such a condition, the efflux of NH3 out of the cell by molecular diffusion should carry the isotope signal out of the cell as suggested by a similar process during nitrate uptake (Granger et al., 2011). Hoch et al. (1992) suggested an ε-value of −14% for the GDH-mediated ammonium assimilation by V. harveyi, but the notion was put into doubt as the genome sequence of V. harveyi lacks such enzyme (Vo et al., 2013). On the other hand, Vo et al. (2013) reported a reverse ε-value of +8.8 ± 0.4‰ for GDH-mediated ammonium assimilation by E. coli. In light of the conflicting evidence, we adopt the ε-value of −2‰ reported by Wada and Hattori (1978) as the asymptotic value for the high end of ammonium concentration. Apparently more study is needed to clarify the conflicting reports.

For the intermediate ammonium concentration, both active membrane transport and passive diffusion of NH3 are in action. It has been suggested that the overall isotope effect for ammonia uptake by passive diffusion could be as large as −39‰ (O’Leary, 1978). This rather large ε-value was attributed to the cumulative isotope effects from NH4+ deprotonation and membrane diffusion of NH3 (Hermes et al., 1985); the former was reported to be −19.2‰ (Hoch et al., 1992), and the latter was found to be −10.9‰ for E. coli (Vo et al., 2013). The combined effect, though not as large as that reported by O’Leary (1978), is indeed rather large. This uptake process becomes more important with increasing ammonia concentration, and could account for the maximum isotope effect observed during ammonium uptake. By contrast, the ε-value of active transport of ammonium was found to be −14.1‰ for E. coli (Vo et al., 2013). When the active transport becomes more important, it tends to diminish the isotope effect and probably limits the manifestation of the maximum isotope effect due to assimilation via membrane diffusion of ammonia.

Within the cell, NH3 assimilation by GS is usually the dominant process at median to low ammonium concentration (Taiz and Zeiger, 2002). Its ε-value was reported to be −12.5 to −8‰ by Hoch et al. (1992), which is in contrast to the insignificant isotope effect observed for E. coli by Vo et al. (2013). In any case, the isotope effect is small.

The trend of the concentration dependent ε-value may be represented by the following empirical formula:

\[ ε = A \exp\left(-bC/(1 + \kappa/C) + \varepsilon_0 [1 - \exp(-bC)]\right) \]  

where C is the ammonium concentration, A is a scaling coefficient, \( \kappa \) resembles a half saturation ammonium concentration for the isotope effect, and \( b \) is a decay coefficient representing the diminishing control of ammonium transport as the rate-limiting step with increasing concentration of ammonium. The term, \( \varepsilon_0 \), is the asymptotic value associated with the isotope effect at very high ammonium level, when the contribution of transport to the isotope effect is diminishing. As suggested above, when \( C \) approaches zero, so does the function; and when \( C \) becomes very large, the function approaches the asymptotic value. At intermediate level, relatively large isotope effect may arise from various processes, which are expressed in the fashion of Michaelis–Menten kinetics.

The Curve-1 (solid curve in Fig. 7) was proposed to fit all estimates of ε-values based on field observations. The parameters used for Curve-1 are listed in Table 3. To fit the estimates from our observations, we intentionally let the curve pass through those data points at or near the upper limit of the distribution. This is to take into consideration the potential dilution effect and the background SPM as explained in the following. Even though we compensated for the ammonium consumed by algal uptake, the estimation of the initial ammonium concentration by including the amount transformed to PN was truly the initial value, only if no mixing occurred during the algal bloom. If mixing did occur, it would have most likely decreased the concentration through dilution by mixing with the seawater end-member, which was depleted in ammonium. Therefore, the presumed initial ammonium concentration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Curve-1 (fitting all field estimates)</th>
<th>Curve-2 (fitting the extreme values from lab cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (‰)</td>
<td>−39</td>
<td>−55</td>
</tr>
<tr>
<td>b (μM⁻¹)</td>
<td>0.0025</td>
<td>0.0030</td>
</tr>
<tr>
<td>( \kappa ) (μM)</td>
<td>55</td>
<td>38</td>
</tr>
<tr>
<td>( \varepsilon_0 ) (‰)</td>
<td>−2</td>
<td>−2</td>
</tr>
</tbody>
</table>
probably represents the lower limit of the possible initial value. In other words, most data points would tend to fall to the left of their true positions in Fig. 7 due to dilution. The background SPM probably comprised mainly of re-suspended sediment and might have a δ15N value in the range of 1–4‰ (Liu et al., 2007). The heavier isotopic composition would have made the δ15N(NH4)2O closer to δ15N(NH4)NO3 and cause the data points to fall below the curve. On the other hand, it cannot be ruled out that local point sources of pollutants that contained ammonium or very intensive remineralization of organic matter that produced ammonium might raise the ammonium concentration after the algal bloom occurred. These possible processes might explain the single point above the curve.

More than half of the ε-values estimated from culture experiments also fall on Curve-1 (Fig. 7), but four ε-values reported by Pennock et al. (1996) fall far from Curve-1 due to their rather large magnitudes that resemble the maximum isotope effect for bacterial ammonium uptake (Fig. 7b) observed by Hoch et al. (1992). We proposed another curve (Curve-2, the dashed curve in Fig. 7) to fit these data points by using the same function for Curve-1, namely, Eq. (16), but a different set of parameters (Table 3). It is noteworthy that the maximum isotope effects manifested by both curves are within the combined isotope effects from the coupled processes of ammonium deprotonation followed by membrane diffusion of ammonia, suggesting that these processes could be responsible for the maximum isotope effect during ammonium uptake. However, the alternative assimilation pathway by active ammonium transport tends to reduce the manifestation of the maximum isotope effect. Because the culture conditions often differ considerably from the natural conditions, we consider Curve-1 to be better representation for natural conditions.

While both Curve-1 and Curve-2 resemble the concentration-dependent trend of the ε-values observed for V. harveyi (Hoch et al., 1992), neither is identical to the latter. Because most phytoplankton in the Danshuei estuary were autotrophic eukaryotes, their enzymes for ammonium assimilation are surely different in some amino acid sequences from those of heterotrophic bacteria. Besides, the related enzymes are mostly located within organelles, such as, cytosol, chloroplast, and mitochondrion (Taiz and Zeiger, 2002; Masclaux-Daubresse et al., 2010), so that more transport processes through membranes are involved. It is conceivable that nitrogen isotope fractionation of autotrophic eukaryotes differs from that of heterotrophic bacteria.

As we have demonstrated the variable isotope fractionation depending on ammonium concentration, we must face the paradox that the ε-values reported from this and other studies were derived basing on equations for constant isotope fractionation. Looking back on the processes involved, we recognize that phytoplankton must have experienced changes in ammonium concentration during growth, especially under bloom conditions, and, therefore, the changing isotope fractionation. For the phytoplankton in our samples, the changes in ε-value corresponding to changes in ammonium concentration should be relatively small, 0.3 to 1.4‰ as estimated according to Fig. 7a. Therefore, the equation based on constant isotope fractionation, i.e., Eq. (9), may serve as a reasonable approximation to the actual situation. The concentration range, over which the isotope effect is most sensitive to the change in ammonium concentration, should be less than 60 μM (Fig. 7a). However, if isotope fractionation diminishes to zero at very low [NH4+ ] as suggested by Hoch et al. (1992), the actual trend should be close to what we have proposed (Fig. 7).

5. Summary and conclusions

In July 2009 we observed an intense algal bloom in the highly eutrophic Danshuei estuary, where the Chi-a concentration reached as high as 1666 μg L⁻¹, almost double the maximum value observed under ordinary summer conditions. The dominant algal species were the pennate diatoms, Fragilaria spp. The observed δ15N values of particulate nitrogen ranged from −8.8‰ to 0.2‰, while the coexisting ammonium had δ15N values ranging from 4.6 to 11.9‰. The ε-values during ammonium uptake were calculated to be between −4.7‰ and −16.4‰, with the range overlapping considerably with the previously reported range of −6.5‰ to −18.1‰ observed in the field. Fitting all field observed ε-values, we constructed a curve of concentration dependent isotope fractionation curve for ammonium uptake by phytoplankton with the following expression:

\[
e = -39 \exp(-0.0025C)/(1 + 55/C) - 2[1 - \exp(-0.0025C)],
\]

where C is the ammonium concentration (μM). The curve shows a maximum isotope effects of about −20‰ around the ammonium concentration of 100 μM, whereas the isotope effect diminishes to zero as ammonium becomes null, and decreases towards an asymptotic value of −2‰ at higher ammonium concentrations. More than half of the ε-values derived from laboratory cultures also fall on this curve, but a few estimates fall on another curve, which shows similar pattern but stronger maximum isotope effect. The curves show resemblance to the trend of the concentration dependent ε-values observed for the marine heterotrophic bacterium, V. harveyi, by Hoch et al. (1992), implying that similar but not identical biochemical pathways are responsible for nitrogen isotope fractionation during ammonium uptake by autotrophic eukaryotes or heterotrophic bacteria.

This study provides the first field observation attesting the notion of concentration dependent nitrogen isotope fractionation during ammonium assimilation in the aquatic environment, which was observed only in laboratory cultures before. It also reconciles partially the disparity between field and laboratory studies. The maximum isotope effects around ammonium level of 100 μM probably represent partial manifestation of the combined effects from NH2 deprotonation and NH3 membrane diffusion. It warrants further investigation what controls the degree of manifestation of the maximum isotope effect. Besides the ε-value for millimolar level of ammonium is not well constrained and warrants further investigation. Since the culture conditions often differ considerably from the natural conditions, we recommend the curve based on field observations (namely, Curve-1 in Fig. 7 or Eq. (17)) as more applicable for natural conditions.

Acknowledgments

We are grateful for the four anonymous reviewers who provided critical but constructive comments that helped improve the manuscript. We acknowledge the technical assistance in isotopic analysis at Academia Sinica, Taipei. This study was supported by the National Central University and the grant NSC 98-2611-M-008-003 from the National Science Council (Taiwan). This is NCU-IHS Contribution #211.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marchem.2013.10.005.

References


