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δ^{15}N dynamics of ammonium and particulate nitrogen in a temperate eutrophic estuary

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Abstract We monitored the stable nitrogen isotopic composition (δ^{15}N) of suspended matter and ammonium in the freshwater stretch of the Scheldt estuary (Belgium) over a full year to investigate for seasonal evolution and possible co-variation between isotopic signatures. The δ^{15}N value of ammonium remained rather constant during winter (average = +11.4‰) but increased significantly with the spring and summer bloom, reaching values as high as +70‰. This enrichment of the ammonium pool in ^{15}N coincided with significant ammonium depletion during summer period, suggesting a close causal relationship. Based on a semi-closed system approach we deduced an apparent fractionation factor associated with NH_4^+ utilization (i.e. combining effects of uptake and nitrification) of 18.4‰ (SE = 2.0‰), which is similar to values reported in literature. Observed variations of ammonium δ^{15}N could account for about 69% of δ^{15}N variation in suspended matter.

Keywords Ammonium · Fractionation · Scheldt estuary · Stable isotopes

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Introduction

The natural stable isotopic composition of dissolved nitrogen species has been shown to be a powerful tool to understand N cycling in a variety of aquatic systems. Such studies use the variation in the stable isotopic composition of dissolved N, induced by a process-specific level of discrimination against the heavy or light isotope, to reveal the dominant processes acting on the N-pool (Cifuentes et al. 1989; Horrigan et al. 1990; Montoya et al. 1990; 1991; Velinsky et al. 1991; Ostrom et al. 1997; Wu et al. 1997; Sigman et al. 1999; Lehmann et al. 2004). Other studies have used the δ^{15}N of dissolved inorganic N as a proxy for the δ^{15}N of autochthonous organisms, and as such successfully distinguished between autochthonous and allochthonous organic matter sources in mixed suspended matter pools (Mariotti et al. 1984; Caraco et al. 1998).

Previous studies have shown that the spatio-temporal variation of δ^{15}N of dissolved inorganic nitrogen (DIN) in natural aquatic systems

can be large (Mariotti et al. 1984; Cifuentes et al. 1989; Horrigan et al. 1990; Montoya et al. 1990, 1991; Velinsky et al. 1991; Velinsky and Fogel 1999). Variations in $\delta^{15}N_{DIN}$ can be transferred to the microbial community (phytoplankton and bacteria) via the assimilation of ammonium and nitrate. Evidently, this variability has to be taken into account when $\delta^{15}N$ is to be used as a tool to study food webs. The $\delta^{15}N$ signature of NH_4^+ should have a larger impact on the $\delta^{15}N$ of the microbial community than the one of NO_3^-, given the preferential uptake of NH_4^+ over NO_3^- by microbial organisms (Mariotti et al. 1984; Cifuentes et al. 1988; Montoya et al. 1991; Velinsky and Fogel 1999). Since the lifetime of most microbial organisms at the base of the food web is very short, it is important to know the short term variability of $\delta^{15}NH_4^+$. However, high temporal resolution studies of $\delta^{15}NH_4^+$ signatures in estuaries are scarce.

Nitrification and NH_4^+ uptake are processes that discriminate against the heavy isotope and therefore have the potential to enrich the NH_4^+ pool in ^{15}N. Studies investigating the fractionation factors associated with nitrification and NH_4^+ uptake report a broad range of values. Horrigan et al. (1990) report that the fractionation associated with water column nitrification in the Chesapeake Bay (USA) varied between 12.7‰ and 16‰, while Cifuentes et al. (1989) report a fractionation factor of 9.1‰ for algal NH_4^+ uptake in the Delaware Estuary (USA). The fractionation factor associated with biological NH_4^+ uptake estimated by Velinsky et al. (1991) varies from 5–15‰ in the Black Sea to 20–30‰ in Framvaren Fjord (Norway). Fractionation factors for laboratory cultures of algae and nitrifying bacteria range from 3‰ to 27‰ (e.g. Wada 1980; Pennock et al. 1996) and from 14‰ to 38‰ (Mariotti et al. 1981; Casciotti et al. 2003), respectively.

The present study describes the fortnightly to monthly variation of $\delta^{15}NH_4^+$ in the Scheldt estuary. We estimate the apparent fractionation factor associated with NH_4^+ consumption and investigate how the variability in $\delta^{15}NH_4^+$ is transmitted to the $\delta^{15}N$ of suspended matter.

Materials and methods

Study site

The Scheldt River drains the urbanized and industrialized areas of France, Belgium and The Netherlands before discharging into the North Sea (Fig. 1). The Scheldt estuary is the stretch of the Scheldt River subject to tides and extends over about 155 km. The edge of salt intrusion is situated at approximately 100 km from the mouth of the estuary, so that the Scheldt estuary has an extensive freshwater section (55 km) of which part is bordered by freshwater marches. On average 39% of the water transported in the Scheldt estuary originates from the Scheldt River proper; the remaining 61% of the water originates from the tributaries Dender (11%), Durme (6%) and Rupel (44%). In the freshwater section, however, the flow is dominated by the Scheldt River itself (Scheldt: 70%, compared to Dender: 20% and Durme: 10%). Discharge of the Scheldt estuary for the period of study is generally low (187 $m^3 s^{-1}$; measured at Schelle: Fig. 1) with highest values found during winter and lowest during summer (Taverniers 2001, 2002). As a result, water residence times are long, ranging between 2 and 3 months (Soetaert and Herman 1995a; Regnier et al. 1997).

The nitrogen cycle of the Scheldt estuary has been extensively studied over the past 30 years. The three main processes described so far which affect DIN concentrations in the Scheldt are nitrification, denitrification and biological uptake (Wollast 1982), with nitrification being the most important (Regnier et al. 1997). In general, most of the nitrogen is processed in the pelagic rather than benthic compartment (Soetaert and Herman 1995b; de Wilde and de Bie 2000). Furthermore, a recent study has shown that the freshwater tidal marshes bordering the estuary do not represent a significant N-source (Gribsholt et al. 2005). Although NH_4^+ is efficiently recycled within the estuary (Middelburg and Nieuwenhuize 2000) a net consumption of NH_4^+ is observed due to the fact that nitrification exceeds aerobic mineralization and other ammonium generating processes (Soetaert and Herman 1995b).

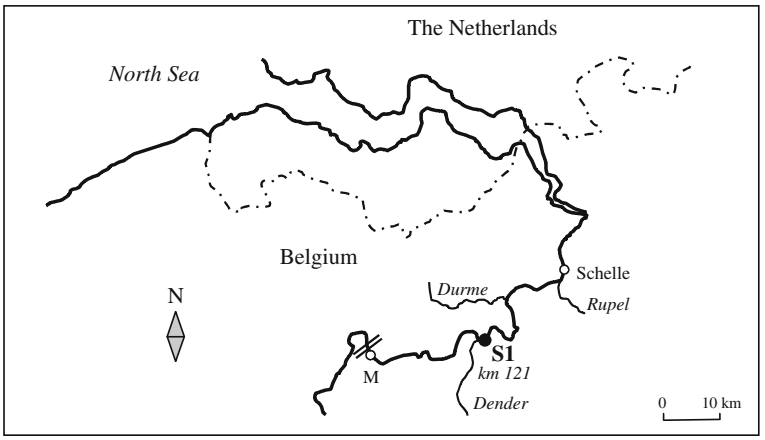

Fig. 1 Map of the Scheldt estuary and its major tributaries showing the sampling station S1 located in the freshwater section at km 121 from the mouth of the estuary

Previous studies report the Scheldt estuary to be characterized by high nutrient loadings (Struyf et al. 2004), extensive phytoplankton blooms (Muylaert et al. 2000) and high bacterial production rates (Goosen et al. 1997). On an annual basis, however, the balance of these processes is in favor of bacterial degradation, making the Scheldt estuary a net heterotrophic system (Frankignoulle et al. 1996, 1998; Hellings et al. 2001).

Sampling and analytical protocols

The study was carried out at a freshwater station (S1), situated below the confluence of the Scheldt River and the Dender tributary, at 121 km from the river mouth (Fig. 1) and lasted from 30 November 2001 till 19 November 2002. Environmental parameters were monitored fortnightly between 30 November 2001 and 29 July 2002 and monthly between 23 August 2002 and 19 November 2002. The physico-chemical and biological parameters monitored included dissolved oxygen concentration ($[O_2]$), temperature, $[NH_4^+]$, $[NO_3^-]$, $[NO_2^-]$, Chlorophyll *a* ([Chl-*a*]) and suspended matter ([SPM]) concentration and $\delta^{15}N$ of suspended particulate organic matter (SPOM) and NH_4^+. Dissolved oxygen concentrations and temperature were measured in situ. Water for determination of SPM load and $\delta^{15}N$ of SPOM was filtered in triplicate on pre-weighted and pre-combusted GF/F filters (Whatman; $\varnothing$ = 47 mm). SPM concentrations were assessed gravimetrically. The filtered water was kept for analyses of $[NH_4^+]$ (indophenol blue method), $[NO_2^-]$ and $[NO_3^-]$ (cadmium reduction method) and $\delta^{15}NH_4^+$. Triplicate samples for the determination of [Chl-*a*] were collected separately by filtering water on similar GF/F filters. [Chl-*a*] was determined using the spectrophotometric method of Lorenzen. Discharge data (Q) were obtained from Taverniers (1999, 2000, 2001, 2002).

$\delta^{15}NH_4^+$ composition was determined using an original simplified diffusion method (Diaconu et al. 2005). The sample was first diluted to reach an approximate concentration of 10 μmol l^{-1}. A glass gas-washing bottle (Schott) was filled with 250 ml of the diluted sample. The bottle holds an external gas inlet tube connected to a large gas diffusion disc located at the bottom. The gas outlet of the bottle is connected to an in-line INOX filter holder (Pall Life Sciences), holding a 25 mm diameter GF/D glass-fiber membrane (Whatman) impregnated with 50 μl 2.5 mol l^{-1} $KHSO_4$. After adding 1.5 ml KOH 80% to the sample, the bottle is immediately closed and air is bubbled under controlled flow through the sample in order to strip the NH_3-gas out of the solution. When passing through the acidified filter, the NH_3 in the air flow is trapped. After extraction, the filter is removed and dried for 12 h at 50°C. Filters resulting from the extraction of Milli-Q water using the same protocol served as blank. Testing out this method showed that after 15 h, more than 80% of the initial ammonium present in the solution was trapped on the filter. The risk of contaminating the sample with NH_4^+ produced from DON breakdown during the

extraction procedure at high pH is assumed to be minimal. Indeed, the extraction is performed over a time span of 15 h, which is shorter than the time span over which DON contamination will occur (Holmes et al. 1998). In addition, tests done with Scheldt water samples of various salinities (and thus likely to carry variable loads of DON) spiked with standard amounts of $^{15}N\text{-}NH_4^+$, showed no significant difference between measured and expected ^{15}N in the trapped NH_4^+ (Diaconu et al. 2005). Extractions made with a $^{15}N\text{–}NH_4^+$ standard solution showed that fractionation occurred, with $\delta^{15}NH^+_{4\,\text{extracted}} = \delta^{15}NH^+_{4\,\text{solution}} - 2‰$. All measurements were subsequently corrected for this fractionation.

The stable N isotopic composition of SPOM and extracted NH_4^+ on filters was determined with a Finnigan Delta^{XL}plus isotope ratio mass-spectrometer coupled on-line to an elemental analyzer (Flash series 1112). Nitrogen isotope ratios are expressed relative to atmospheric nitrogen. The results are reported in the standard δ-notation:

$$\delta^{15}\text{N}(‰) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000$$

$$R = {}^{15}\text{N}/{}^{14}\text{N}$$

From replicate measurements (n = 4 to 6) on reference material IAEA-N1: $\delta^{15}N = +0.43 \pm 0.7‰$ we deduced a reproducibility for combined elemental analyzer—IRMS analysis of 0.02‰, for $\delta^{15}N$ measurements on SPOM we had a reproducibility (n = 10) of 0.6‰ and for ammonium extractions, triplicate analysis of natural samples typically resulted in standard deviations of 2‰.

Results

Water column characteristics

Discharge at station S1 was calculated as the sum of the discharges of the Scheldt River, measured at the head of the estuary (station M; km 155) and the Dender tributary. Q ranged between 9 (July 2002) and 450 $m^3\ s^{-1}$ (February 2002) and was generally higher during winter-early spring (Fig. 2a). Water temperatures ranged between 5°C (late-December 2001) and 23°C (late-July 2002) and correlated inversely with $[O_2]$ (Fig. 2b). Generally, lowest O_2 concentrations were observed during summer-autumn with nearly anoxic conditions in September 2002 ($[O_2]$ as low as 0.9 mg l^{-1}). During late autumn, winter and spring, O_2 concentrations were much higher with a maximum value of 9.1 mg l^{-1} observed in December 2001. [SPM] generally fluctuated between 41 (July 2002) and 133 mg l^{-1} (October 2002); (Fig. 2c). However, during late winter-early spring 2002 and late summer 2002, SPM concentrations were markedly higher with maximum values as high as 230 mg l^{-1} (March 2002) and 201 mg l^{-1} (August 2002). The [Chl-*a*] evolution marked a brief spring phytoplankton bloom and a longer lasting bloom during summer-early autumn (Fig. 3c). A maximum spring [Chl-*a*] value of 137 ± 4 μg l^{-1} was recorded in April 2002, while the summer bloom, lasting from June till October 2002, reached maxima of 101 μg l^{-1} and 192 ± 11 μg l^{-1} in late-June and September 2002, respectively.

$[NO_2^-]$ showed a maximum of 51 μmol l^{-1} in June 2002 (Fig. 3a). NO_3^- concentrations were an order of magnitude higher than the ones of NO_2^- with a maximum of 535 μmol l^{-1} observed in April 2002 and a minimum of 212 μmol l^{-1} observed in August 2002 (Fig. 3a). NH_4^+ concentrations were approximately 3 times lower than NO_3^- concentrations (Fig. 3b). Largest $[NH_4^+]$ depletions were met in July and August 2002 (<1 μmol l^{-1} in August 2002), while a maximum of 185 μmol l^{-1} was observed in May 2002.

$\delta^{15}{}_{\text{N-SPOM}}$

$\delta^{15}N$ signatures of SPOM averaged +3.2 ± 0.8‰ during winter 2001–2002 (November till March), suggesting that SPOM consisted mainly of sewage and terrestrial detritus (range $\delta^{15}N = +1‰$ to +3‰; Mariotti et al. 1984; Middelburg and Nieuwenhuize 1998; Fisseha 2000). $\delta^{15}N_{\text{SPOM}}$ values subsequently decreased to −2.6‰ during the spring bloom (Fig. 3d) where after they increased to a maximum of +12.5‰ in July 2002 when high [Chl-*a*] levels and high $[NO_2^-]$ coincided with

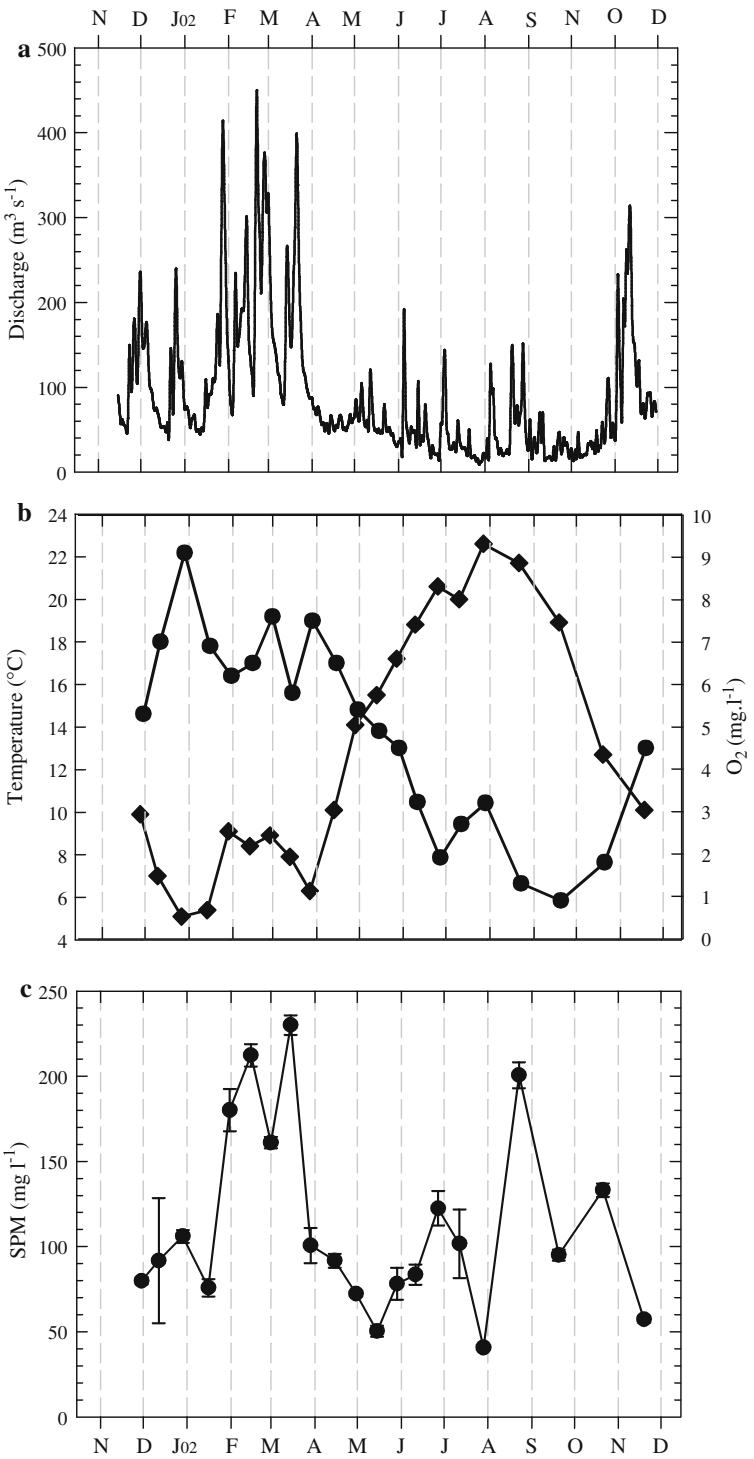

Fig. 2 Seasonal variation of (**a**) discharge (Taverniers 2001, 2002), (**b**) temperature (diamonds) and [O_2] (circles) and (**c**) [SPM] (± 1 SD) at the freshwater station S1 of the Scheldt estuary (2001–2002)

lowest NH_4^+ concentrations (< 1 μmol l^{-1}). In August, $\delta^{15}N_{SPOM}$ shortly decreased to +7.8‰ to increase again to a maximum of +11.5‰ in September 2002. On from September, $\delta^{15}N$ of SPOM gradually decreased to a value of +4.6‰ in November 2002.

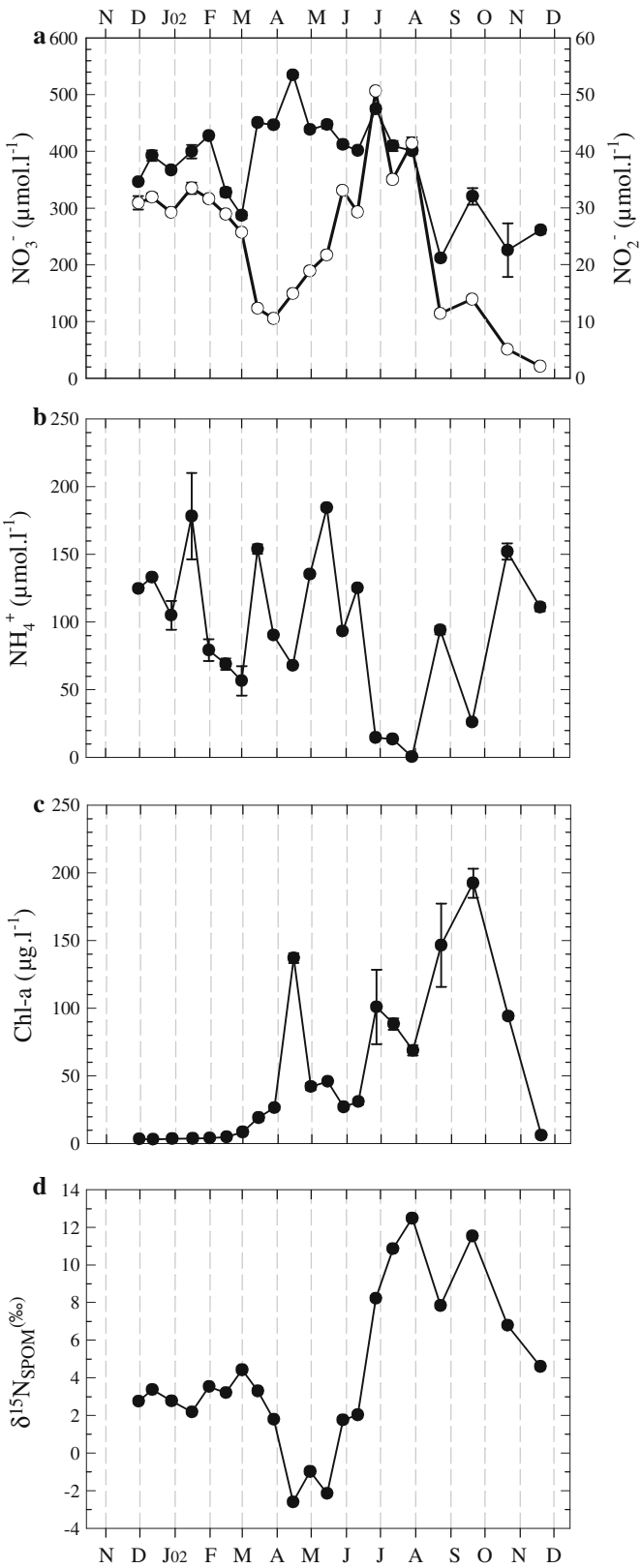

Fig. 3 Seasonal variation of (**a**) [NO_3^-] (black) and [NO_2^-] (white), (**b**) [NH_4^+], (**c**) [Chl-*a*] and (**d**) $\delta^{15}N_{SPOM}$ at the freshwater station S1 of the Scheldt estuary (2001–2002). Error bars = 1 SD

$\delta^{15}NH_4^+$

The $\delta^{15}N$ signature of NH_4^+ varied considerably over the course of the study period (Fig. 4). $\delta^{15}NH_4^+$ averaged $+11.4 \pm 2.0‰$ during winter (30 November 2001 till 29 March 2002). During spring, $\delta^{15}NH_4^+$ increased twice to maxima of +21.8‰ and +28.1‰ in mid-April and late-May, respectively. In summer, $\delta^{15}NH_4^+$ values increased sharply to a first maximum of +70‰ in mid-July. In August, $\delta^{15}NH_4^+$ sharply decreased to +31‰ where after a second maximum of +66‰ was reached in September. In October and November 2002, $\delta^{15}NH_4^+$ values decreased again but were higher than the average winter value of the previous year. The maximum $\delta^{15}NH_4^+$ values (up to +70‰) we observed are, to our knowledge, the highest values reported in literature.

Discussion

Our results show the fortnightly to monthly variation of $\delta^{15}NH_4^+$ at an estuarine freshwater station over a full year cycle and under a variety of conditions. $\delta^{15}NH_4^+$ values were initially stable during winter, but increased dramatically during the spring and summer bloom.

Variation in the $\delta^{15}N$ composition of NH_4^+ in aquatic systems is mainly caused by NH_4^+ consuming and producing processes involving isotopic discrimination which changes the $^{15}N/^{14}N$ ratio of the NH_4^+ pool. An overview of the most important NH_4^+ flux pathways in the Scheldt estuary and their isotope fractionations is shown in Fig. 5. In general, NH_4^+ consumption increases and NH_4^+ production decreases the $\delta^{15}N$ of the NH_4^+ pool. Changes in $\delta^{15}NH_4^+$ must subsequently be interpreted in function of the different, seasonally variable microbial processes affecting the NH_4^+ pool.

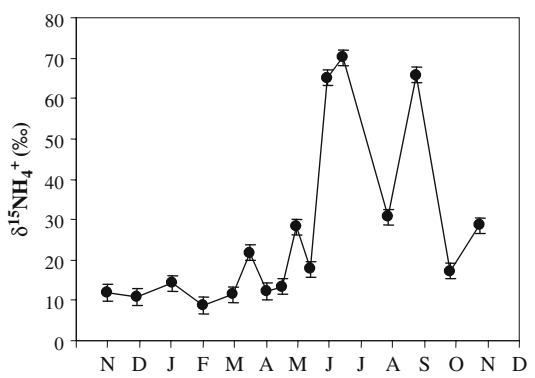

Fig. 4 Seasonal variation in the $\delta^{15}N$ signature of NH_4^+ at the freshwater station S1 of the Scheldt estuary (2001–2002). Error bars = 1 SD

Winter $\delta^{15}NH_4^+$ signatures

During winter, longitudinal transects upstream of our sampling site show only minor changes in NH_4^+ concentration (S. Van Damme, personal communication) suggesting that NH_4^+ losses due to nitrification and biological uptake and inputs through mineralization are of minor importance because of the low water temperature. A conservative behavior during winter might explain why winter (November–March 2001) $\delta^{15}NH_4^+$ values varied only slightly (range: +8.7‰ to +14.2‰; average and standard deviation $+ 11.4 \pm 2.0‰$), with small variations in $\delta^{15}NH_4^+$ likely reflecting minor fluctuations in relative importance of NH_4^+ consuming or producing processes. Indeed, microbial processes are considerably reduced during winter, but do not completely halt (Brion, unpublished results).

$\delta^{15}NH_4^+$ during the bloom months

During the bloom period (April–October 2002), $\delta^{15}NH_4^+$ values ranged between +12.2‰ and

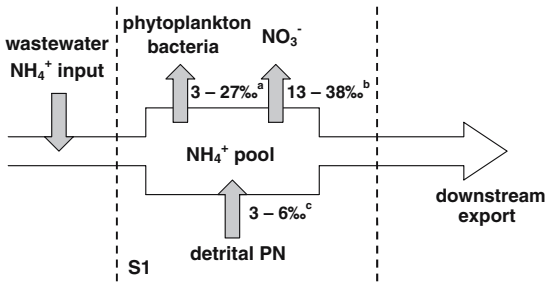

Fig. 5 Schematic representation of the major NH_4^+ fluxes and associated isotope fractionation in the water column of the freshwater station S1 of the Scheldt estuary. Isotope fractionation values are reported in Wada (1980); Cifuentes et al. (1989); Velinsky et al. (1991); Pennock et al. (1996) for microbial NH_4^+ uptake ([a]), Mariotti et al. (1981); Horrigan et al. (1990); Casciotti et al. (2003) for nitrification ([b]) and Montoya et al. (1992) for mineralization ([c])

+70.1‰, suggesting that NH_4^+ does not longer behave conservatively. Recent measurements of NH_4^+ consumption and production rates at S1 during spring and summer showed that nitrification and biological uptake exceed mineralization (Brion, unpublished results), what is also reflected in the sharp decrease in NH_4^+ concentrations over June–July (Fig. 3b). In case of net NH_4^+ consumption $\delta^{15}NH_4^+$ may increase linearly or exponentially with time depending on the relative importance of NH_4^+ consumption and production. In general, for open systems with a continuous NH_4^+ input and where NH_4^+ consumption exceeds NH_4^+ input (Sigman et al. 1999) or for closed systems with considerable back-reaction (mineralization) (Macko et al. 1986), $\delta^{15}NH_4^+$ increases linearly with decreasing fraction f of NH_4^+ remaining in the system. Under these circumstances, the isotopic composition of the NH_4^+ pool can be described as (Macko et al. 1986; Sigman et al. 1999):

$$\delta^{15}NH_{4\,(t)}^{+} = \delta^{15}NH_{4\,(0)}^{+} + (1-f)\times\varepsilon \qquad (1)$$

$\delta^{15}NH_{4(t)}^+$ and $\delta^{15}NH_{4(0)}^+$ denote the $\delta^{15}N$ composition of NH_4^+ at time t and time 0, f denotes the fraction of NH_4^+ remaining in the system at time t and ε denotes the overall isotope fractionation involved with NH_4^+ consumption/production processes, with the isotope fractionation ε for a reaction 'Source ↔ Product' defined as:

$$\varepsilon = \left[\frac{R_{\text{source}}}{R_{\text{product}}-1}\right]\times 1000 \approx \delta^{15}N_{\text{source}} - \delta^{15}N_{\text{product}} \qquad (2)$$

In closed systems where NH_4^+ consumption strongly exceeds mineralization, $\delta^{15}NH_4^+$ increases exponentially with decreasing fraction of NH_4^+ remaining in the system (Mariotti 1981; Fry 2003). In this case the variation in $\delta^{15}NH_4^+$ can be described by the Rayleigh equation:

$$\delta^{15}NH_{4\,(t)}^{+} = \delta^{15}NH_{4\,(0)}^{+} - \varepsilon\times\ln f \qquad (3)$$

These models allow calculating the apparent fractionation factor for NH_4^+ consumption in the Scheldt estuary. In the following section we will investigate which of both models describes the observed change in $\delta^{15}NH_4^+$ best.

Calculation of the remaining NH_4^+ fraction

The fraction f of NH_4^+ remaining in the system at time t is:

$$f = \frac{NH_{4\,(t)}^{+}}{NH_{4\,(0)}^{+}} \qquad (4)$$

The fraction (f) of NH_4^+ remaining in the system at S1 at any time requires knowledge of the initial NH_4^+ concentration ($NH_{4(0)}^+$) of the water parcel sampled. This initial ammonium concentration is set by the upstream point source delivery of ammonium to the river, followed by dilution as tuned by variable river discharge. The point source of ammonium consists essentially of domestic waste water and we assume this source function remains relatively constant over the year. Therefore, during the winter period, when microbial activity is minimal, it is likely that measured ammonium at S1 reflects this initial ammonium content, more or less diluted depending on discharge. It follows that during winter ammonium should correlate inversely with river discharge (Struyf et al. 2004). Following the findings of Struyf et al. (2004), we verified if a relationship of the kind

$$\ln(Q_{S1}+1) = a\ln([NH_4^+]+1) + b \qquad (5)$$

applies for the winter period at S1 (November to March 1999–2002). Freshwater discharge at S1 (Q_{S1}) was calculated as the sum of the discharge at station Melle (M in Fig. 1) and the discharge of the Dender tributary. A highly significant linear relationship is observed: $y = -0.61$ (SE = 0.11) x + 7.69 (SE = 0.50), (SE = standard error); (Fig. 6, $r^2 = 0.66$; $P < 0.001$).

During the other seasons, however, microbial processes increase or decrease the ammonium concentration so that actual NH_4^+ concentrations no longer reflect initial NH_4^+ values. However, for each time point, we can estimate the initial NH_4^+ concentration by assuming that the linear dependency between Q_{S1} and $NH_{4(0)}^+$ (Eq. 5) also

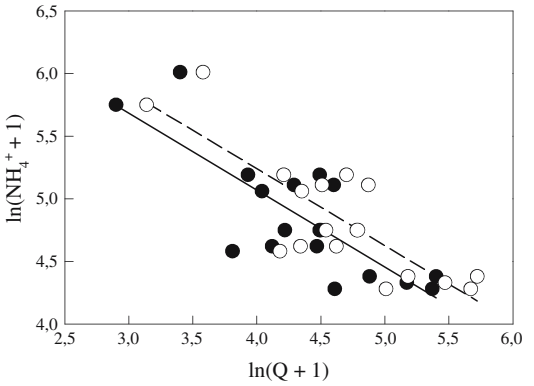

Fig. 6 Ordinary least square regressions of the natural logarithm of discharge measured at S1 (white circles, dashed line) and at M, the head of the estuary (black circles, solid line) and at S1 during the winter period (December–March) of 1999, 2000, 2001 and 2002. [Source $[NH_4^+]$: S. Van Damme and T. Maris, (University of Antwerp), unpublished results; source Q: Taverniers (1999, 2000, 2001, 2002)]

holds for the non-winter months. Thus, for the bloom period we calculate the initial NH_4^+ concentrations for the condition where microbial alteration of the NH_4^+ concentration is minimal. The fraction of NH_4^+ consumed (produced) at each moment is then represented by the ratio between the actual NH_4^+ concentration, $NH_{4(t)}^+$, and the calculated initial NH_4^+ concentration $NH_{4(0)}^+$ (Eq. 4).

However, a complication arose from the fact that discharge data for the Dender tributary (located just upstream from S1) during summer months were fragmentary, thus prohibiting recalculation of $NH_{4(0)}^+$ at S1 for every month sampled. Therefore, we investigated whether it was possible to use the regression between freshwater discharge at station M (Q_M) and $[NH_4^+]$ at S1 to estimate $NH_{4(0)}^+$. In other words, we verified whether it was possible to replace (Eq. 5) by:

$$\ln(Q_M + 1) = a' \ln([NH_4^+] + 1) + b' \qquad (6)$$

$\ln(Q_M + 1)$ and $\ln([NH_4^+] + 1)$ also correlate significantly: $y = -0.62$ (SE = 0.12)$x + 7.52$ (SE = 0.52) (Fig. 6; $r^2 = 0.61$; $P < 0.001$) with Q_M explaining 61% of the winter time NH_4^+ variation at S1. We subsequently compared the $NH_{4(0)}^+$ values obtained using either Q_M or Q_{S1}. The resulting regression is highly significant (bivariate least square regression, $P < 0.001$) and the hypothesis that the slope = 1 and the intercept is not significantly different from 0 is accepted at the 99% confidence level. This justifies the use of the winter linear regression between $\ln(Q_M + 1)$ and $\ln([NH_4^+] + 1)$ to estimate $NH_{4(0)}^+$ at S1. The fraction f of remaining ammonium was subsequently calculated using Eq. 4.

It must be noted that variation in the discharge Q_M explained only 61% of the variation in $[NH_4^+]$. Part of the remaining variation can be attributed to fluctuations in the balance between NH_4^+ consumption and production rates. Also, Q_M-values used in the calculations are those prevailing on the days station S1 (40 km downstream of station M) was sampled, and these Q_M values may be slightly different from those prevailing on the (unknown) date when the NH_4^+ pool, now sampled at S1, was actually present at station M. All further calculations will take this uncertainty into account.

Fractionation associated with NH_4^+ consumption in the Scheldt estuary

Both the open and closed system approaches result in highly significant correlations ($P < 0.001$) between $\delta^{15}NH_4^+$ and the fraction of substrate remaining. For the open system (Eq. 1) the apparent fractionation (ε_{app}) associated with overall NH_4^+ consumption (i.e., nitrification + biological uptake) as given by the slope of the regression of $\delta^{15}NH_4^+$ vs. $(1 - f)$ is +91.3 (SE = 10.2)‰, while the intercept for zero NH_4^+ consumption is −19.6 (SE = 9.6)‰. This value for ε_{app} appears to be much higher than values reported in literature for nitrification and biological uptake (Cifuentes et al. 1989; Horrigan et al. 1990; Velinsky et al. 1991; Fig. 5). Also, the $\delta^{15}NH_{4(0)}^+$ value of −19.6‰ estimated with Eq. 1 is much lower than the observed winter $\delta^{15}NH_4^+$ value of 11.4 (SD = 2.0)‰, casting doubt on the correctness of the former value.

For the closed system approach (Rayleigh model, Eq. 3) the apparent fractionation factor

(ε_{app}) given by the slope of the regression of $\delta^{15}NH_4^+$ vs. ln f is 18.4‰ (SE = 2.0)‰. Such a value fits well within the ranges reported in literature. Furthermore, the intercept of the Rayleigh equation curve (+12.5‰, SE = 5.8‰; Fig. 7) representing the initial $\delta^{15}NH_4^+$ signature, is close to our measured average value for the winter period (+11.4‰, SD = 2.0‰). It thus appears that the Rayleigh model provides a more realistic estimate of the fractionation associated with ammonium utilization in the freshwater section during the bloom period. This suggests also that the freshwater section of the Scheldt estuary acts as a semi-closed system where mineralization is relatively a minor process during the bloom months.

The relative contributions of nitrification and uptake in setting ε_{app} can, however, vary over the course of the bloom period. The bloom period consisted of 3 events (Fig. 3c), a spring bloom in April (maximum [Chl-*a*] of 137 μg l^{-1}), a first summer bloom in June–July (maximum [Chl-*a*] of 101 μg l^{-1}) and a second summer bloom in August–September (maximum [Chl-*a*] of 192 μg l^{-1}). Extremes in [Chl-*a*] generally coincided with extremes in $\delta^{15}NH_4^+$ (compare Figs. 3c and 4).

For the spring period, reports of high nitrification rate (0.5 μmol l^{-1} h^{-1}; Verlinden 2002) and high NH_4^+ uptake rate (1 μmol l^{-1} h^{-1}, Anderson et al. in preparation) clearly indicate that both processes are major controllers of the spring NH_4^+ concentration.

The July bloom was characterized by elevated NO_2^- concentrations (maximum [NO_2^-] of 51 μmol l^{-1}) and ammonium values < 20 μmol l^{-1}, which indicates intense nitrification. If occurring under low O_2 conditions, nitrification can result in NO_2^- accumulation, since nitrite oxidizing bacteria appear to be more sensitive to low O_2 conditions than ammonium oxidizing bacteria (Brion and Billen 1998). High nitrification rates (0.6 μmol l^{-1} h^{-1}) at S1 during summer (July 2004) were confirmed recently (Anderson et al. 2006). In addition, the $\delta^{15}N$ values for NO_3^- in the upstream estuary during summer reported by Middelburg and Nieuwenhuize (2001) (+8.8‰) are close to the winter $\delta^{15}NH_4^+$ signatures reported here (range: +8.7‰ to +14.2‰), suggesting that NH_4^+ can be almost entirely converted to NO_3^- as a result of seasonal nitrifier activity. Hence, nitrification (resulting in nitrite accumulation) is probably the major sink of NH_4^+ during the first half of the summer bloom.

The second summer bloom resulted in higher Chl-*a* concentrations than the first one, while NO_2^- concentrations were considerably lower (maximum [NO_2^-] of 14 μmol l^{-1}). Except for a higher value in August (90 μmol l^{-1}), ammonium stayed low (< 30 μmol l^{-1}). The very low O_2 concentrations prevailing during this second bloom (0.9 mg l^{-1}) might have suppressed nitrification rates completely by now inhibiting also the ammonium oxidizing bacteria. As a result biological uptake would have been the major remaining sink for NH_4^+ during the second summer bloom.

Despite the fact that the dominant NH_4^+ utilization processes were probably different during both summer bloom events, the coinciding $\delta^{15}NH_4^+$ maxima are, however, remarkably similar. This would imply that fractionation factors for nitrification and biological uptake are of similar magnitude in the freshwater part of the Scheldt estuary.

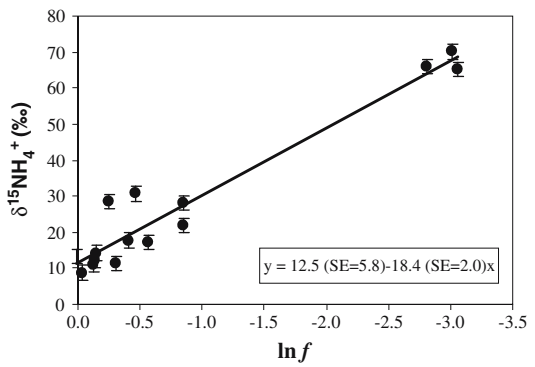

Fig. 7 Scatter plot showing the linear relationship between ln f and $\delta^{15}NH_4^+$ (bivariate least square regression; $r^2 = 0.99$, $P < 0.001$)

Effect of $\delta^{15}NH_4^+$ variability on the $\delta^{15}N$ of SPOM

Ammonium is taken up by microorganisms and converted to particulate organic nitrogen. As a consequence, it has been shown that variations in the $\delta^{15}N$ of SPOM are mainly mediated through the uptake of NH_4^+ by phytoplankton

(Mariotti et al. 1984; Cifuentes et al. 1988; Montoya et al. 1991) and bacteria (Caraco et al. 1998). In this section we will compare measured values for $\delta^{15}N_{SPOM}$ with a calculated $\delta^{15}N$ signal for microbial biomass (i.e. $\delta^{15}N_{MB}$, MB = bacteria and phytoplankton) in an attempt to evaluate the effect of variable $\delta^{15}NH_4^+$ signatures on $\delta^{15}N_{SPOM}$. $\delta^{15}N_{MB}$ can be calculated using the following equation (Mariotti et al. 1981; Fry 2003):

$$\delta^{15}N_{MB} = \varepsilon_{ass} \times \frac{f \ln f}{1-f} + \delta^{15}NH_{4\,(0)}^{+} \quad (7)$$

$\delta^{15}N_{MB}$ is the $\delta^{15}N$ composition of the accumulated microbial biomass in the water column, ε_{ass} is the fractionation associated with NH_4^+ assimilation by bacteria and phytoplankton, f is the NH_4^+ fraction remaining of the original NH_4^+ pool (Eq. 4) and $\delta^{15}NH_{4(0)}^+$ is the initial $\delta^{15}N$ signature of NH_4^+. To solve Eq. 7 we will, in first approximation, assume, that the apparent fractionation factor (ε_{app}) assessed above mainly reflects fractionation associated with NH_4^+ uptake. Furthermore, it must be noted that $\delta^{15}N_{MB}$ calculated using Eq. 7 will represent only the microbial organic nitrogen metabolized from NH_4^+. Microbial organisms can also assimilate other N-sources such as NO_3^- and dissolved organic nitrogen and this will affect their final $\delta^{15}N$ signature.

From Fig. 8 it appears that $\delta^{15}N_{MB}$ and $\delta^{15}N_{SPOM}$ are significantly correlated (bivariate least square regression: $\delta^{15}N_{MB} = 0.99$ (SE = 0.22) $\times$ $\delta^{15}N_{SPOM}$ − 1.98 (SE = 1.81); $P < 0.001$) with $\delta^{15}N_{MB}$ explaining about 69% of the variation observed in $\delta^{15}N_{SPOM}$. Our findings are in accordance with those of Cifuentes et al. (1989) for the Delaware estuary (USA), relating the $\delta^{15}N_{SPOM}$ decrease in spring and the

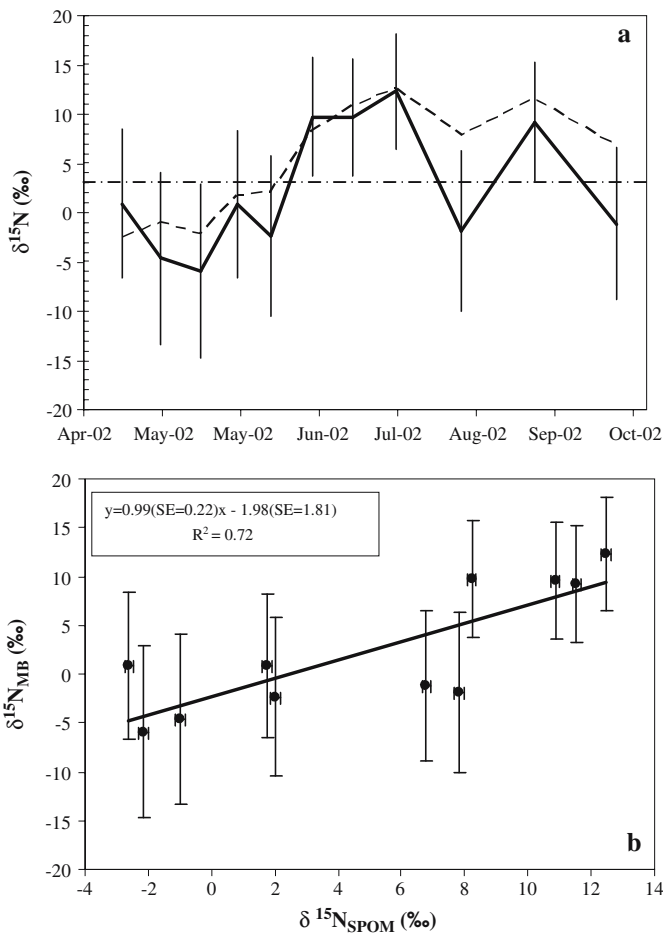

Fig. 8 (**a**) Comparison between $\delta^{15}N$ signatures of SPOM (dashed line) and calculated accumulated microbial biomass (solid line) during the spring and summer phytoplankton bloom (April 2002 till October 2002). The +3‰ line indicates the average $\delta^{15}N$ signature of terrestrial and sewage inputs. Error bars = 1 SD. (**b**) Relationship between measured $\delta^{15}N$ of SPOM and calculated $\delta^{15}N$ of accumulated microbial biomass. Error bars = 1 SD

subsequent increase in summer to phytoplankton growing on NH_4^+ with variable $\delta^{15}N$ signatures.

Long-term trends in $\delta^{15}NH_4^+$ in the freshwater Scheldt estuary

The $\delta^{15}NH_4^+$ values reported in the present study are considerably higher than the ones reported for a Scheldt freshwater section (summer 1982) by Mariotti et al. (1984). $\delta^{15}NH_4^+$ values observed during the early eighties fluctuated around +10‰, a value similar to the one observed here during winter (+11.4‰). Since Mariotti et al. report relatively constant NH_4^+ concentrations for the freshwater part during summer 1982, we can assume that NH_4^+ behaved conservatively (Soetaert et al. 2006). During the early eighties nitrification was suppressed in the oxygen depleted waters (Soetaert et al. 2006), leaving biological uptake as the only moderator of NH_4^+ removal. This situation is probably similar to the one observed during the second summer bloom in the present study. The difference in the effect on $\delta^{15}NH_4^+$ is probably due to present day lower ammonium levels and/or higher phytoplankton biomass compared to the early eighties (both reported for the upstream part of the Scheldt estuary by Soetaert et al. 2006) which results in a decreased fraction of NH_4^+ remaining in the system.

Mariotti et al. (1984) report a sharp decrease in $[NH_4^+]$ in the oligohaline sections of the estuary during the 80-ies due to increasing oxygen levels seawards. We can now back-calculate the $\delta^{15}N$ signature of NH_4^+ for this section of the Scheldt estuary using the ε_{app} and $\delta^{15}NH_{4(0)}^+$ values from the present study (18.4‰ and 11.4‰, respectively), while the value for f is taken as 0.3, which is roughly the fraction of NH_4^+ remaining in the NH_4^+ pool during the summer of 1982 (see Fig. 4 in Mariotti et al. 1984). We obtain a $\delta^{15}NH_4^+$ value of +34.6‰, which is only slightly higher than +29‰, the value reported for that river section during the early 80-ies by Mariotti et al. (1984).

Summary

The $\delta^{15}N$ of ammonium was relatively stable during winter, when NH_4^+ showed a rather conservative behavior. During the spring and summer bloom period, $\delta^{15}NH_4^+$ showed large and dynamic variation with maximum values up to +70‰. The large increase in $\delta^{15}NH_4^+$ was induced by NH_4^+ consumption with an apparent overall fractionation of 18.4‰ (SE 2.0‰) which integrates effects of nitrification and NH_4^+ uptake. During the bloom, the freshwater estuary acted as a semi-closed system where mineralization was inferior to NH_4^+ consumption. The seasonal variation of $\delta^{15}NH_4^+$ was partially (69%) transmitted to the $\delta^{15}N$ of the particulate organic N pool via ammonium-utilizing microbial organisms.

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