Assessing the role of dissolved organic nitrogen in different aquatic ecosystems

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Zusammenfassung

Gelöster organischer Stickstoff (engl.: dissolved organic nitrogen, DON) repräsentiert in Gewässern oft einen der größten Anteile an reaktiven Stickstoff. Allerdings sind die Bedeutung und die Rolle des DON im aquatischen Stickstoffkreislauf bisher noch nicht hinreichend geklärt. Bis vor einiger Zeit war die vornehmliche Meinung, dass DON ein für Organismen ungeeigneter, abbauresistenter Stickstoffpool sei. Dieser Standpunkt hat sich in den letzten Jahren geändert und DON ist zunehmend in den Fokus gerückt, eine wichtige Quelle und aktiver Teil in Umsetzungsprozessen von reaktivem Stickstoff zu sein. Untersuchungen der Verteilung stabiler Stickstoffisotope in DON sowie in Umsetzungsprozessen, die mit ausgeprägten Isotopenfraktionierungen einhergehen, haben ihren Anteil dazu beigetragen.

In der vorliegenden Arbeit beurteile ich anhand von Konzentrationsmessungen und anhand der Analytik stabiler Stickstoffisotope in kombiniertem DON und Ammonium, sowie zusätzlich von partikulärem Stickstoff (particulate nitrogen, PN) und Nitrat, die Rolle von DON im Stickstoffkreislauf zweier sehr unterschiedlicher aquatischer Ökosysteme. Die Elbe und das Elbeästuar repräsentieren hierbei ein eutrophes Ökosystem, charakterisiert durch einen sehr großen Nitratüberschuss, der die Umsetzung von DON durch Phytoplankton und heterotrophe Organismen unnötig machen sollte. Diese beiden Szenarien werden in den Kapiteln 3 (Elbeästuar) und 4 (Elbe) betrachtet. Im Gegensatz dazu ist das östliche Mittelmeer ein extrem oligotrophes Ökosystem, von dem vermutet wird, dass DON dort eine quantitativ signifikante Rolle als Stickstoffspeicher und –quelle einnimmt; Ergebnisse aus diesem System werden in Kapitel 5 präsentiert.

Im Elbeästuar wurden Proben analysiert, die zu verschiedenen Jahreszeiten (Oktober 2005, Juni 2006 und Dezember 2006) entlang des Salzgradienten von 0 bis 32 genommen wurden. Die daraus resultierenden Daten zeigen, dass DON, im Gegensatz zu Nitrat, nicht-konservatives Mischungsverhalten im Salzgradienten des Ästuars aufweist. Im Zusammenhang mit sich ebenfalls verändernden Isotopen-Verhältnissen ist dies ein Hinweis auf Umsetzungsprozesse im Ästuar und auf die Existenz von Quellen und Senken für DON. Adsorptions- und Desorptionsprozesse sind vermutlich die Ursache für den signifikanten Konzentrationsabfall und das Absinken der Isotopen-Verhältnisse in der ästuaren Trübungsmaximumszone (TMZ) und dem steilen Anstieg dieser beiden Parameter auf der

flussabwärts gelegenen Seite der TMZ. Dieses Verhaltensmuster konnte in allen beobachteten Jahreszeiten nachgewiesen werden. Bei Vergleichen mit Daten älterer Studien zeigt sich, dass DON-Frachten im Elbeästuar in den letzten 25 Jahren unverändert geblieben sind.

Am Geesthachter Wehr wurden gelöste und partikuläre Stickstofffrachten und ihre Isotopenverhältnisse in der Tide-freien Elbe über mehr als zwei Jahre in monatlicher Auflösung analysiert (Juni 2005 bis Dezember 2007). Die Daten für Nitrat weisen einen Jahreszyklus mit zwei Perioden auf, bedingt durch saisonal variierende biologische Aktivität. Die Daten für kombiniertes DON und Ammonium zeichnen sich durch einen komplexeren Zyklus mit vier Perioden pro Jahr aus. Das Auswaschen von organischen Düngemitteln aus den Böden im Elbumland wird als Ursache für diese Abweichung von dem erwarteten natürlichen Jahreszyklus angenommen. Die ähnlichen Verläufe der δ^{15}DON- und δ^{15}PN-Daten weisen dabei auf eine enge Verbindung dieser beiden Stickstoffgruppen hin.

Das östliche Mittelmeer ist ein nährstoffarmes oligotrophes Ökosystem mit im Vergleich zu anderen Meeren ungewöhnlich niedrigen Isotopenverhältnissen in reaktiven Stickstoffverbindungen. Im Januar und Februar 2007 wurden Proben an verschieden Stationen des östlichen Mittelmeeres genommen und auf Isotopenverhältnisse in Nitrat, gesamt gelösten reduzierten Stickstoff (total dissolved reduced nitrogen, TRN, d.h. hauptsächlich DON) und suspendiertem partikulären Stickstoff analysiert. Im Tiefenwasser war Nitrat stärker an ^{15}N abgereichert als in anderen vergleichbaren Meeren. Da TRN und PN an ^{15}N angereichert waren, wird extensive Mineralisation von PN als Ursache für die Isotopenzusammensetzungen des reaktiven Stickstoffs im Tiefenwasser angenommen. Das Isotopenverhältnis von TRN lässt darauf schließen, dass TRN zum einen ein Nebenprodukt dieser Mineralisation im Tiefenwasser ist, und zum anderen in der euphotischen Zone als Nebenprodukt von Phytoplankton-Assimilation bzw. Phytoplankton-Fraß entsteht.

Abstract

Dissolved organic nitrogen (DON) is often one of the largest pools of reactive nitrogen in aquatic environments, but the significance and role of DON in the aquatic nitrogen cycle is still under debate. Until recently, the prevailing view was that DON is a recalcitrant nitrogen pool unavailable to organisms; this view changed in recent years, and DON is increasingly recognised as an important pool and active participant in reactive nitrogen turnover processes. Part of this recognition originated from investigations into stable nitrogen isotope distributions in DON and transformation processes that are associated with distinct isotope fractionation.

In this thesis I used concentration and stable isotope analyses of combined DON and ammonium, and in addition of particular nitrogen (PN) and nitrate to assess the role of DON in the nitrogen cycle of two very different aquatic ecosystems. The Elbe River and estuary represent a eutrophic ecosystem with a large nitrate surplus that may make the utilisation of DON by phytoplankton or heterotrophic organisms unnecessary; these two settings are studied in Chapter 3 (Elbe estuary) and Chapter 4 (Elbe River). The eastern Mediterranean Sea, in contrast, is an example for a highly oligotrophic ecosystem where DON is supposed a play a quantitatively significant role as an N repository and source; results for this system are presented in Chapter 5.

In the Elbe estuary, measurements were made on samples taken along a salinity gradient from 0 to 32 during different seasons (October 2005, June 2006, and December 2006). These data show that in contrast to nitrate DON exhibits non-conservatively mixing in the salinity gradient of the estuary. Combined with changing isotope ratios, this is an indicator for transformation processes and for the existence of sources and sinks of DON in the Elbe estuary. Adsorption and desorption processes are proposed to be the reason for the significant decrease of DON concentrations and isotope ratios in the estuarine turbidity maximum zone (TMZ) and the sharp increase of these parameters at the salty side of the TMZ, since these patterns could be observed in all seasons sampled. Compared to older data, the DON load did not change in the last 25 years.

At the weir of Geesthacht, the dissolved and particulate N-loads and their isotopic composition in the non-tidal part of the Elbe River has been monitored for more than two years (June 2005 to December 2007) at monthly resolution. Nitrate data reveal a two-period annual cycle due to seasonally varying biological productivity, data of combined DON and ammonium a more complex four-period annual cycle. Elution of organic fertilizers from soils in the catchment of the Elbe River is the likely reason for observed differences from expected natural DON and ammonium cycles. The similarities of the trends of δ^{15}DON and δ^{15}PN indicate a close coupling of these nitrogen pools.

The eastern Mediterranean Sea (EMS) is a nutrient-poor oligotrophic ecosystem with unusually low isotope ratios in reactive nitrogen pools compared to other oceanic environments. In January and February 2007, samples were taken at different stations across the EMS and analysed in terms of isotope ratios in nitrate, total dissolved reduced nitrogen (TRN, i.e. mainly DON) and suspended PN. In deep water, nitrate was more depleted in ^{15}N than in comparable oceanic environments. Since PN and TRN were ^{15}N-enriched, extensive mineralisation of PN may be the cause of the isotopic makeup of reactive nitrogen in deep water, and isotopic properties of TRN suggest that it is a by-product of these mineralisation processes in deep water, and generated as a by-product of phytoplankton assimilation or phytoplankton grazing in the euphotic zone.

Contents

1. Introduction

1.1 Dissolved organic nitrogen – general information

The study of marine nutrient cycles is an important part of biogeochemistry. One of the most important one is the marine nitrogen cycle, since nitrogen is an essential element for all organisms. This cycle contains both inorganic and organic compounds of nitrogen, in which the organic fraction can be separated into dissolved organic nitrogen (DON) and particular nitrogen.

DON is the nitrogen-bearing part of the dissolved organic matter (DOM) in aquatic environments, and it is defined as that part of the organic nitrogen that can be filtered through a filter with 0.45 µm pore-size. The contribution of DON to total dissolved nitrogen (TDN) in natural environments varies between 15% and 90%: Lowest DON concentrations are found in the deep ocean and high concentrations in rivers, where highest DON dominance characterise unpolluted rivers (Wiegner et al., 2006). DON forms a potential source of nitrogen, carbon, and energy (Bronk, 2002), which are essential for organisms. But even though the DON contribution to most N-pools in marine and freshwater ecosystems is relatively high, it has been considered to be a largely inert pool of heterogeneous composition without any relevant function as a nitrogen source (William and Druffel, 1987). This point of view changed over the last years with numerous studies that specifically dealt with biological processes in which DON was involved. Stepanauskas et al. (1999 a, b) found that a substantially larger fraction of DON is assimilated by marine than by freshwater bacterioplankton, and Bronk et al. (2007) investigated the influence of DON on harmful algal blooms in seasons when the inorganic nitrogen pools are exhausted. Seitzinger and Sanders (1997) and Seitzinger et al. (2002) studied the bioavailability of DON and observed that the proportion of DON that is utilizable by phytoplankton varies by source and land use pattern in the catchment.

Compositionally, DON is a heterogeneous mixture of biologically labile moieties (turnover times on the order of days to weeks) and refractory components which persist for months to hundreds of years. While the refractory matter dominates quantitatively, the labile forms are supposed to be relevant nitrogen sources for biological systems. The composition of DON varies, and a considerable part is still unidentified. The main identified compounds are urea with a proportion of ~ 5% of DON, dissolved free amino acids (DFAA, ~ 6%), dissolved combined amino acids (DCAA, ~ 7%), and humic and fulvic acids. The DCAA fraction can be divided into three categories (Keil and Kirchman, 1993). The first category with the lowest contribution of less than 10% of DCAA includes proteins, similar to that freshly extracted from phytoplankton, with turnover times of hours to days. In eutrophic waters proteins are the dominant form of DCAA (Billen, 1991). Proteins kinetically similar to abiotically glycosylated proteins are part of the second category. They account for almost 50% of DCAA; the turnover time of this category is much slower than that of biotically produced proteins. Nonproteinaceous DCAA belong to the last category with an amount of almost 50% of DCAA. These amino acids are linked to humic or fulvic compounds (Lytle and Perdue, 1981) or adsorbed on clays or other materials (Hedges and Hare, 1987). They are characterized by resistance to standard liquid hydrolysis and by unknown turnover times.

The fraction of humic and fulvic acids, the most hydrophobic components of DON, contains organic acids with molecular weights of 500 to 10000 MW, and can also be separated into three categories. The first category encompasses humic acids, which are not soluble at a pH of less than 2. This category represents 10% to 20% of DOM. Fulvic acids, the second category, are hydrophilic acids and are soluble under natural pH conditions. This is the biggest fraction of DOM with a contribution of equal or more than 50% (Thurman, 1985). Humins, which are mostly insoluble, but may from colloids (Ishiwatari, 1992), form the third category. In addition, other organic compounds have been identified, like nucleic acids, purines, pyrimidines, pteridines, methylamines and creatine (Antia et al., 1991). However, these compounds represent only a small fraction of DON.

In many studies DON is separated by ultracentrifugation through a 1-kDa ultrafilter and thus is differentiated in two fractions: low molecular weight DON (LMW DON, < 1-kDa) and high molecular weight DON (HMW DON, >1-kDa). Most labile DON belongs to LMW DON, and the recalcitrant part of DON is part of HMW DON (Benner et al., 1992, 1997; Kerner and Spitzy, 2001).

1.2 DON as part of the nitrogen cycle

The marine nitrogen cycle includes a whole series of processes and pathways (Fig. 1.1), but the role of DON is underrepresented, because it is not entirely clear what role DON does play. The main processes affecting DON are assimilation of dissolved inorganic nitrogen (DIN, i.e. nitrate, nitrite and ammonium) by phytoplankton, which is in part excreted as DON, and ammonification as a sink for DON. But by this description the role of DON in the marine nitrogen cycle is not well represented, since many different sources (Fig. 1.2) and sinks (Fig. 1.3) of DON do exist.

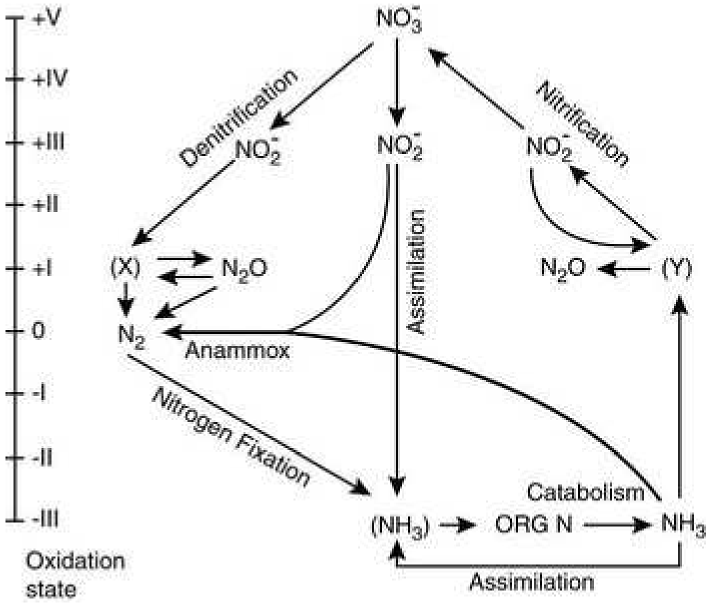


Figure 1.1: The marine nitrogen cycle. X and Y represent intracellular intermediates that are not accumulated in the water column (from Codispoti et al., 2001).

In Fig. 1.2, only the biotic sources of DON are presented. Not shown is the terrestrial input, i.e. DON transport via overland runoff and groundwater (Valiela et al., 1990; Tobias et al., 2001) or the atmospheric inputs (Cornell et al., 1995). The biotic sources are extracellular phytoplankton production, bacterial release, release from micro- and macrozooplankton, and viral release (Bronk, 2002).

For the direct DON release from phytoplankton, two different models have been proposed: the active release (outflow model) and the passive diffusion model (Fogg, 1966). Active release includes different processes, such as release of excess photosynthates (exudation; Fogg, 1983), release due to osmotic changes (for example at changing salinity in an estuarine salinity gradient), or release of DON in response to elevations in light (Lomas and Glibert, 1999). The passive diffusion model includes permeation of metabolites through cell membranes due to large concentration gradients existing between intra- and extracellular pools (Bronk, 2002). Two other processes involving phytoplankton as a source of DON are lysis due to viral infection (Fuhrmann, 1999; Gobler et al. 1997) and during sloppy feeding of zooplankton (Dagg, 1974; Lampert, 1978; Bronk, 2002). Also N_2-fixation by *Trichodesmium*, a cyanobacterium, can be a source of DON, since up to 50% of fixed N_2 is released directly as DON during growth in natural populations of *Trichodesmium* in the Atlantic Ocean and Caribbean Sea (Capone et al., 1994; Glibert and Bronk, 1994).

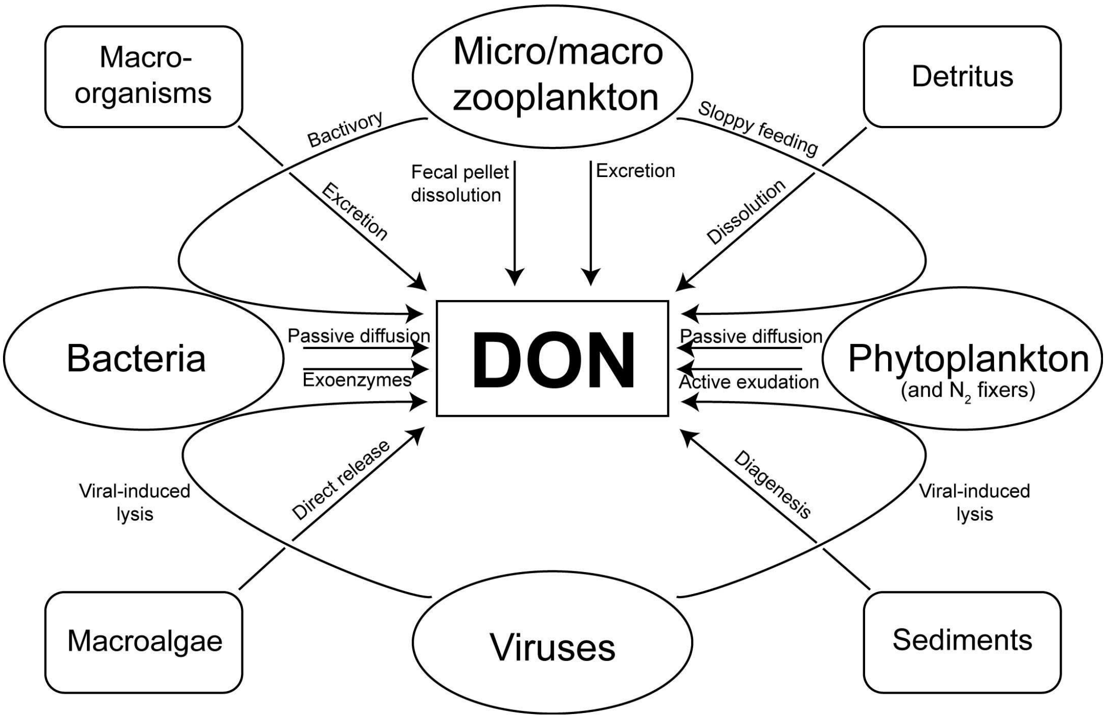


Figure 1.2: Conceptual diagram of biotic processes involved in DON release in aquatic systems (from Bronk, 2002).

Bacterial release is primarily related to LMW DON such as urea (Berman et al., 1999), and two mechanisms exist: Active release of exoenzymes or passive diffusion. Release of DON through mineralization of organic aggregates by bacteria has also been observed (Smith et al., 1992).

Processes involving micro- and macrozooplankton as a DON source are release via sloppy feeding (Dagg, 1974; Lampert, 1978; Bronk, 2002), via bactivory, when dissolved intracellular compounds are released due to broken cells (Bronk, 2002), via release of waste containing DON (excretion; Miller and Glibert, 1998), or via diffusion away from or the dissolution of fecal pellets (Jumars et al., 1989).

Viral release is a DON source because in the final stages of a viral infection, when the cell bursts due to an increase of phage, cellular contents are released. Due to this mechanism, viruses do not only affect the quantity of DON, but also the quality (Fuhrmann, 1999, 2000).

Other sources of DON include the release from excretion of macroorganisms (Tupas and Koike, 1990), the direct release from macroalgae (Mann, 1982; Branch and Griffith, 1988), as well as release from detrital particles via dissolution (Carlson, 2002) or the diagenetic release from sediments (Burdige, 2002).

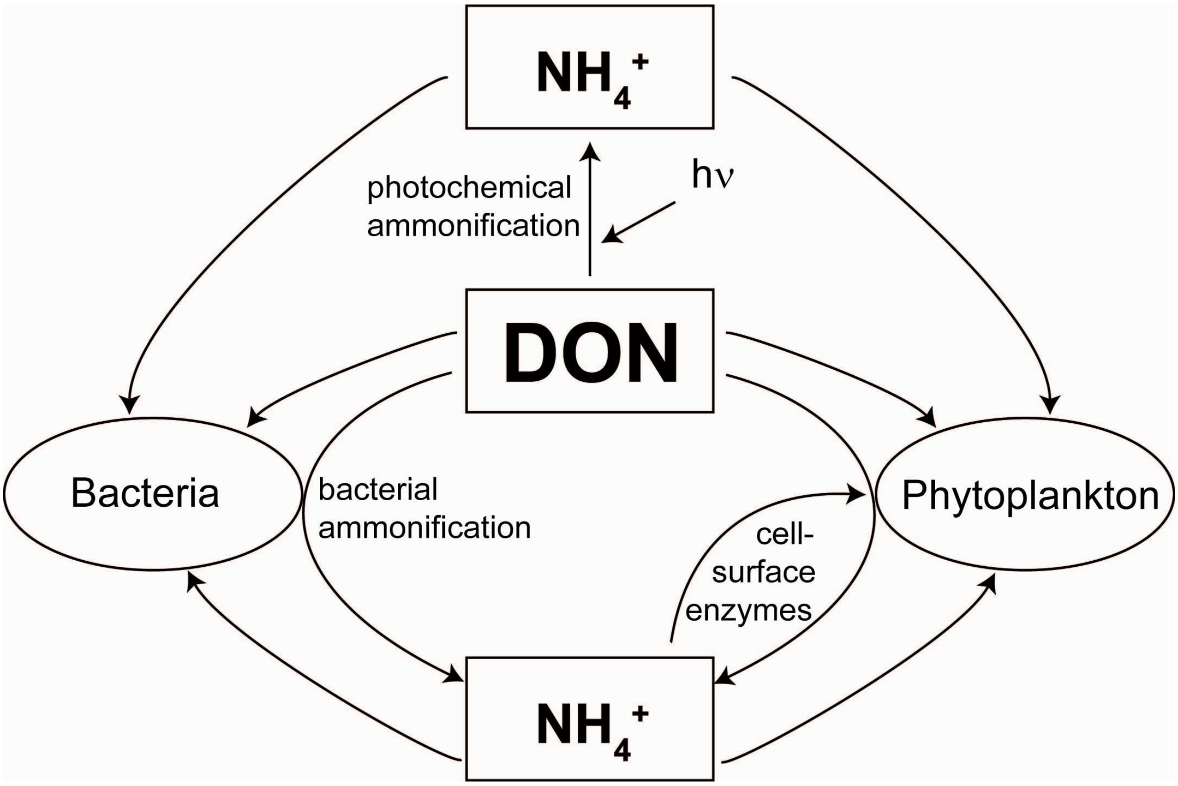


Figure 1.3: Conceptual diagram of processes involved in DON utilization in aquatic systems (from Bronk, 2002)

In Fig. 1.3, the main sinks for DON are presented: Heterotrophic uptake, autotrophic uptake and abiotic photochemical decomposition (Bronk, 2002).

Heterotrophic bacteria possess the ability for the utilization of dissolved proteins, DCAA and DFAA for growth (Carlson, 2002; Veuger et al., 2004), but most bacteria can take up only small organic components (Antia et al., 1991). For the utilization of bigger DON compounds, extracellular hydrolysis of these compounds is necessary (Münster and De Haan, 1998).

Two possible mechanisms of DON utilization by autotrophs exist: One is bacterial degradation of DON followed by phytoplankton uptake of the released nitrogen. The second is the direct incorporation of DON compounds via surface enzymes (Bronk, 2002). Several studies dealt with the bacterial DON degradation (Berman et al., 1991; Antia et al., 1991;

Palenik and Hensen, 1997). When the process results in the release of ammonium, it is known as ammonification.

For the direct uptake of DON the presence of cell surface enzymes like amine oxidases is necessary to cleave amino groups from amino acids and primary amines (Palenik and Morel, 1990a, b; 1991). A number of dinoflagellate species have the ability to use organic nutrients either via cell surface enzymes (Palenik and Morel, 1990a, b), or direct assimilation (Butler et al., 1979; Berg et al., 1997).

A third observed sink of DON is photochemical decomposition, which can promote the release of labile nitrogen components from DOM (Bushaw et al., 1996). This photoproduction of ammonium, DFAA, DCAA and nitrite has been studied predominantly in fresh or brackish water environments, but this process is not ubiquitous (Bertilsson et al., 1999). Especially the aromatic humic substances with their ability to absorb UV light are important substrates for photochemical decomposition (Valiela and Teal, 1979; Stevenson, 1994).

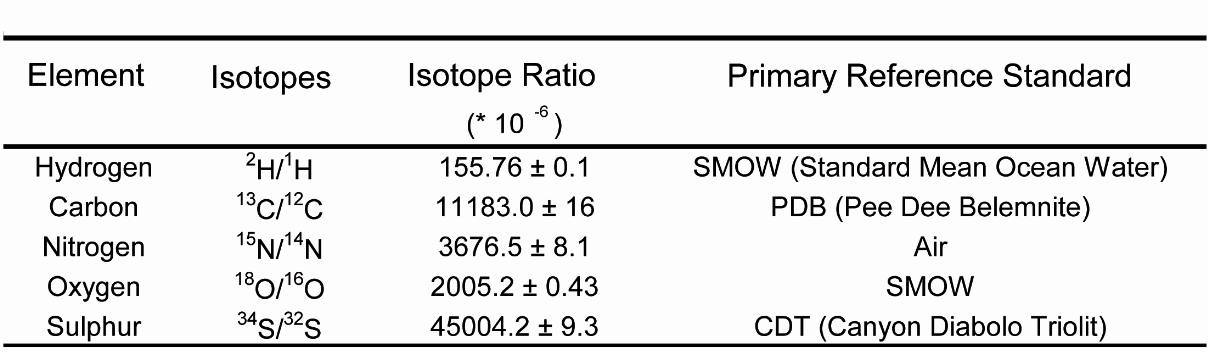
1.3 DON – Analytical problems and stable isotopes

To study DON and its role in the nitrogen cycle it is necessary to use a suitable method to measure DON concentrations with high precision (Bronk et al., 2000), but it is difficult to measure DON directly, since DIN (i.e. ammonium, nitrate and nitrite) may interfere with most analytical methods. So the current method is to determine the concentration of total dissolved nitrogen (TDN) and to subtract the concentrations of separately measured ammonium and combined nitrate/nitrite, with the residual being defined as DON (Bronk, 2002).

$$c(DON) = c(TDN) - c(NH_4^+) - c(NO_3^- / NO_2^-) \qquad \text{(Eq. 1.1)}$$

For the measurement of TDN concentration three different methods are common: ultraviolet oxidation (Armstrong et al, 1966), high-temperature oxidation (Sharp, 1973) and persulfate oxidation (Menzel and Vaccaro, 1964; Sharp, 1973; Solórzano and Sharp, 1980). In a broad community comparison of the three methods, no one emerged as clearly superior (Sharp et al., 2002).

Concentration changes in natural systems are often inconclusive as indicators for sources, sinks, and internal turnover, and the measurement of stable isotope ratios has greatly increased our ability to identify processes and turnover rates in N cycling. Table 1.1 lists elements and their isotopes that are often used in ecosystem studies. Many elements exist in different isotopes, i.e. they differ in the number of their neutrons, but in chemical reactions they react identically. However, a slight discrimination against the heavier isotope may occur in non-equilibrium reactions due to their lower reactivity, since their vibrational frequencies are lower and thereby their molecular bonds are stronger (Kendall, 1998). This slight discrimination leads to a fractionation of the stable isotopes during many biological processes, so that substrates and products differ slightly in the isotopic ratios.



Element	Isotopes	Isotope Ratio (* 10 $^{-6}$)	Primary Reference Standard
Hydrogen	$^{2}H/^{1}H$	155.76 ± 0.1	SMOW (Standard Mean Ocean Water)
Carbon	$^{13}C/^{12}C$	11183.0 ± 16	PDB (Pee Dee Belemnite)
Nitrogen	$^{15}N/^{14}N$	3676.5 ± 8.1	Air
Oxygen	$^{18}O/^{16}O$	2005.2 ± 0.43	SMOW
Sulphur	$^{34}S/^{32}S$	45004.2 ± 9.3	CDT (Canyon Diabolo Triolit)

Table 1.1: In ecosystem studies commonly used elements and their stable isotopes. Listed are the two most abundant stable isotopes of each element, the corresponding isotope ratio and the international calibration standards. Today, primary standards are exhausted and have been replaced by secondary standard materials (Hayes, 1983).

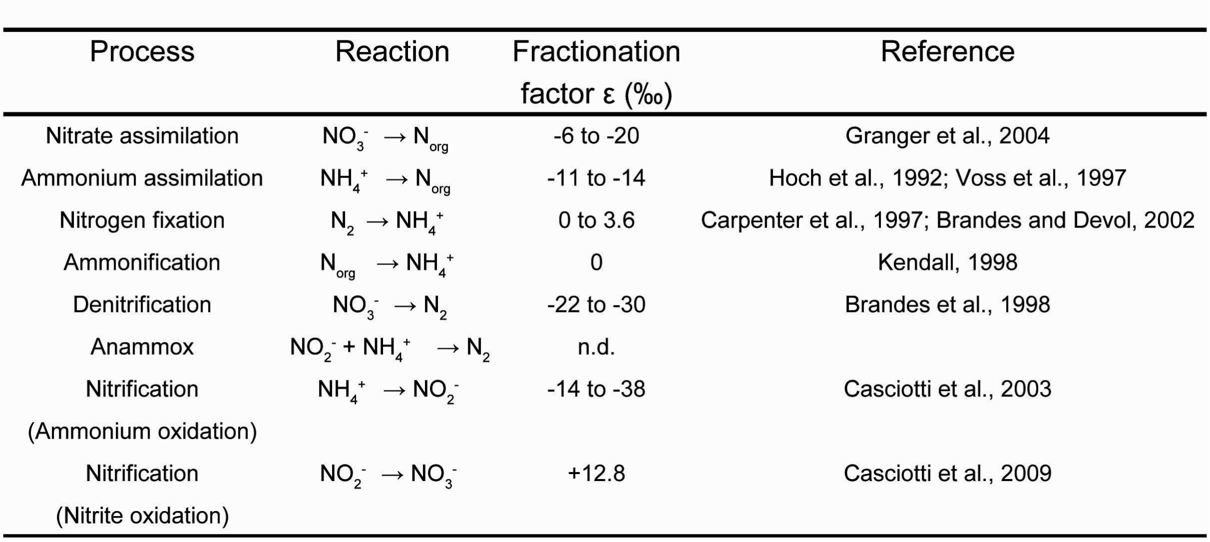
The changes of the ratio of the two most abundant isotopes of an element compared to a standard reference material are expressed in the delta notation. In this study, the main focus lies on nitrogen isotopes, with atmospheric nitrogen N_2 as the standard reference material.

$$\delta^{15}N = \left(\frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{std}} - 1 \right) * 1000 \qquad \text{(Eq. 1.2)}$$

δ^{15}N of atmospheric nitrogen as the standard reference material is defined as 0‰.
The degree of a fractionation depends on the involved process and is characterized by the fractionation factor ε, which in the case of nitrogen stable isotopes is defined as

$$\varepsilon = \left(\frac{R_{product}}{R_{substrate}} - 1 \right), \text{ with } R = \frac{^{15}N}{^{14}N} \qquad \text{(Eq. 1.3)}$$

In Table 1.2 the main processes of the marine nitrogen cycle and the corresponding fractionation factors are listed. Hence, different nitrogen pools can be characterized by their own specific isotopic signature due to the different biological processes that are involved.



Process	Reaction	Fractionation factor ε (‰)	Reference
Nitrate assimilation	$NO_3^- \rightarrow N_{org}$	-6 to -20	Granger et al., 2004
Ammonium assimilation	$NH_4^+ \rightarrow N_{org}$	-11 to -14	Hoch et al., 1992; Voss et al., 1997
Nitrogen fixation	$N_2 \rightarrow NH_4^+$	0 to 3.6	Carpenter et al., 1997; Brandes and Devol, 2002
Ammonification	$N_{org} \rightarrow NH_4^+$	0	Kendall, 1998
Denitrification	$NO_3^- \rightarrow N_2$	-22 to -30	Brandes et al., 1998
Anammox	$NO_2^- + NH_4^+ \rightarrow N_2$	n.d.	
Nitrification (Ammonium oxidation)	$NH_4^+ \rightarrow NO_2^-$	-14 to -38	Casciotti et al., 2003
Nitrification (Nitrite oxidation)	$NO_2^- \rightarrow NO_3^-$	+12.8	Casciotti et al., 2009

Table 1.2: Fractionation factors ε for major transformation processes in the marine nitrogen cycle. ε of anammox has not been published yet.

Some nitrogen pools and the corresponding isotopic signatures are presented in Fig. 1.4.

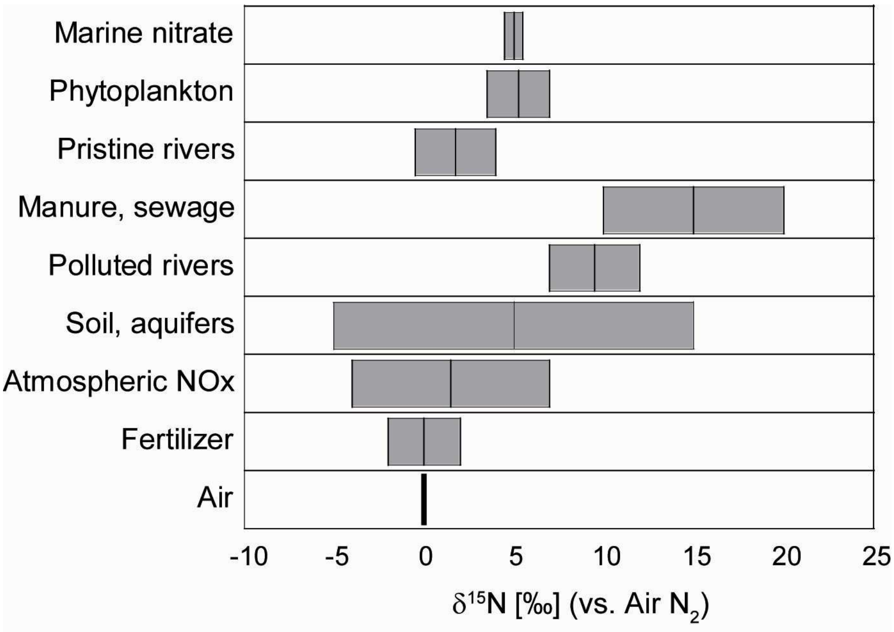


Figure 1.4: Isotopic signatures of $\delta^{15}N$ in different nitrogen sources (values and ranges from Kendall, 1998)

The measurement of the isotope ratio $\delta^{15}N$ of DON ($\delta^{15}DON$) is subject to the same difficulties that inhibit the direct DON concentration measurement. Therefore the determination of $\delta^{15}DON$ is also only possible with the workaround of the determination of $\delta^{15}N$ of TDN and subsequent calculation using equation 1.4:

$$\delta^{15}DON = \frac{\delta^{15}TDN * c(TDN)}{c(DON)} - \left[\frac{\left(\delta^{15}N - NO_3^- * c(NO_3^-)\right)}{c(DON)} + \frac{\left(\delta^{15}NH_4^+ * c(NH_4^+)\right)}{c(DON)} + \frac{\left(\delta^{15}Blank * c(Blank)\right)}{c(DON)} \right]$$

(Eq. 1.4)

Since the method for the determination of $\delta^{15}NH_4^+$ was not yet available during this study, and the expected ammonium concentration was low, the term in eq. 1.4 concerning ammonium was neglected and combined $DON+NH_4^+$ was determined throughout the entire study.

2. Study areas and thesis outlines

For a better understanding of the role of DON in the aquatic nitrogen cycle, this thesis investigates concentrations and $\delta^{15}N$ of combined DON and ammonium (DON+NH_4^+; in chapter 5 termed total reduced dissolved nitrogen; TRN) in aquatic ecosystems of different trophic level to identify differences and/or similarities. Relations to other forms of reactive nitrogen like nitrate and particular nitrogen (PN), their correlations, anti-correlations or the absence of these relationships should help to clarify the mechanisms DON is involved.

2.1 A eutrophic ecosystem using the example of the Elbe River / NW Europe

A eutrophic ecosystem is characterized by high nutrient concentrations, either from natural or anthropogenic sources, leading to an increase in primary production over pristine conditions. Many ecological effects can arise from stimulating primary production, but some effects may strongly influence the entire ecosystem: Among these are decreased biodiversity and toxicity effects (Horrigan et al., 2002; Anderson, 1994).

In aquatic ecosystems, the increase in nutrient supply leads to an increase in algal population growth and biomass produced. On the one hand, nutrient-stimulated algal blooms limit the sunlight available to bottom-dwelling organisms, and on the other hand cause the oxygen demand of sinking organic matter to rise, often resulting in decreases in the amount of dissolved oxygen in deeper water. Oxygen is required by all respiring plants and animals and it is produced in daylight by photosynthesizing algae. Under eutrophic conditions, dissolved oxygen in the surface layer increases strongly during daylight, but it is also strongly reduced after dark by the respiring algae and by microorganisms that respire the increasing mass of dead algae. When dissolved oxygen levels decline to hypoxic levels, fish and other marine animals suffocate. As a result, creatures such as fish, shrimp, and especially immobile bottom dwellers die off (Horrigan et al., 2002).

Some algal blooms are toxic to animals and plants (so called harmful algal blooms), and the toxic compounds produced may be passed up through the food chain, resulting in animal mortality. When the algae die or are eaten, their toxins are released which can kill animals and may also pose a threat to humans (Lawton and Codd, 1991; Martin and Cooke, 1994).

The Elbe River with a length of 1094 km is the second largest river discharging into the North Sea. The estuary extends over 142 km and enters into the German Bight, the south-eastern part of the North Sea, at the city of Cuxhaven. This part of the Elbe is continuously dredged and channelled to enable access for large container ships to the port of Hamburg, one of the largest container ports in the world. The tidal Elbe estuary is separated from the upstream river system by the only barrage along the entire river, the weir at the city of Geesthacht at stream kilometre 585. The average fresh water discharge is 861m^3/s at the Elbe mouth (Cuxhaven) and 700m^3/s at the weir of Geesthacht. The water residence time in the estuary ranges from ~ 10 days during mean high water flow (2000 m^3/s) and ~ 72 days during mean low water flow (300 m^3/s) with ~ 32 days during mean tide level (IKSE, 2005). Nearly 25 million people live in the entire catchment area of 148 268 km^2 (Behrendt et al., 2004). The Elbe River is the largest source of total dissolved nitrogen (TDN) and nitrate of the inner German Bight (Brockmann and Pfeiffer, 1990), which is that part of the North Sea most affected by eutrophication (OSPAR, 2008).

2.2 An oligotrophic ecosystem using the example of the eastern Mediterranean Sea

An oligotrophic ecosystem is characterized by low nutrient concentrations and therefore only low primary production. Thus, there are only small populations of flora and fauna in oligotrophic ecosystems due to very low food supply.

The eastern Mediterranean Sea is an oligotrophic ecosystem (Antoine et al., 1995; Béthoux, 1989) with relatively fast turn over rates of limiting nutrients and a primary production level of approximately half the level observed in the Sargasso Sea or the Northeast Pacific (Krom et al., 2003). The low productivity is due to the special flow conditions in this basin: Modified Atlantic Water flows in through the Strait of Sicily at the surface and sinks in the eastern part of the basin. The high temperature, high salinity Levantine Intermediate Water at depths between 200 and 500 m is fed by this flow that leaves the basin as a westward current through the Sicily Strait. Mineralisation products sinking out of the mixed layer are collected and transported by this Levantine Intermediate Water. The deep water below this circulation cell is depleted in nutrients compared to deep water in all other parts of the global ocean (Malanotte-Rizzolli and Bergamasco, 1989; Krom et al., 1991).

Another remarkable feature of the eastern Mediterranean Sea is the δ^{15}N levels of deep-water nitrate, suspended matter and surface sediments, because they are unusually depleted compared to values in other oceanic ecosystems (Coban-Yildiz et al., 2006; Pantoja et al., 2002; Struck et al., 2001). The low interexchange of Mediterranean Deep Water with other oceanic water masses increases the impact of regional sources of reactive nitrogen.

2.3 Thesis outline

This thesis is based on three publications, which are presented in the following chapters.

Chapter 3

Schlarbaum, T., Dähnke, K., and Emeis, K., 2010: Turnover of combined dissolved organic nitrogen and ammonium in the Elbe estuary / NW Europe: Results of nitrogen isotopes investigations. *Mar. Chem.* **119**, 91-107

Chapter 4

Schlarbaum, T., Dähnke, K., and Emeis, K., 2010: Dissolved and particulate reactive nitrogen in the Elbe River / NW Europe: a 2-year N-isotope study. *Biogeoscience Discuss.* **7**, 7543-7574, doi:10.5194/bgd-7-7543-2010

Chapter 5

Emeis, K.-C., Mara, P., Schlarbaum, T., Möbius, J., Dähnke, K., Struck, U., Mihalopoulos, N., and Krom, M., 2010: N-isotope ratios of nitrate, dissolved organic nitrogen and particulate nitrogen in the Eastern Mediterranean Sea. In press in *Journal of Geophysical Research – Biogeochemistry*

My contribution to chapter 5 was recovery and filtration of the nutrient samples, digestion and analysis plus interpretation of the TDN samples, and determination of the isotope ratios $\delta^{15}N$ and $\delta^{18}O$ in nitrate (parts of the data set).

3. Turnover of combined dissolved organic nitrogen and ammonium in the Elbe estuary/NW Europe: results of nitrogen isotope investigations

Abstract

Dissolved organic nitrogen (DON) is often the dominant form of reactive nitrogen transported from land to sea by rivers, but is considered to be largely recalcitrant and behaves conservatively in many estuaries. We measured the concentration and the isotope ratio $\delta^{15}N$ of combined DON and ammonium (δ^{15}DON+NH_4^+) in the Elbe River estuary (SE North Sea, NW Europe) by a combination of a modified persulfate digestion and the denitrifier method. Measurements were made on samples taken along the salinity gradient from 1 to 32 during different seasons, in order to gauge the effects of internal biological processes and external signatures (such as pollution). Combined DON and ammonium concentrations ranged from 20 to 60 µM, and δ^{15}DON+NH_4^+ from 0 to 11‰. The results show that DON+NH_4^+ contributes < 20% to total reactive nitrogen in the river end-member and rises to 50% in the outer estuary. By comparison with older data, the DON load in the Elbe River did not change since the 1980´s, when nitrate and phosphate pollution was maximal. We find evidence that DON and/or ammonium or reactive components in DON are both consumed and produced in the estuary, indicated by changing isotope ratios and non-conservative mixing gradients. The estuarine turbidity maximum zone (TMZ) at salinities <5, which today is a significant source of nitrate from nitrification, coincides with significantly decreased DON+NH_4^+ concentrations and δ^{15}DON+NH_4^+ in all seasons sampled. Whether this is due to selective absorption/desorption of ^{15}N enriched moieties onto particle surfaces, or to selective heterotrophic assimilation and nitrification is yet unclear, and the loss of DON+NH_4^+ does not balance the added nitrate. Because DON+NH_4^+ concentrations and δ^{15}DON+NH_4^+ rise sharply seaward of the TMZ, we consider adsorption/desorption processes most likely. In the salinity gradient 5 to 30, DON+NH_4^+ behaves conservatively in both concentration and isotopic composition.

Key words: Dissolved organic nitrogen, stable isotopes, estuary, particulate nitrogen

3.1 Introduction

Dissolved organic nitrogen (DON) is a major (between 15% and 90%) component of total dissolved nitrogen (TDN) transported to the coastal ocean by rivers, with highest DON dominance in unpolluted rivers (Wiegner et al., 2006). DON has until recently been considered to be a largely inert pool of heterogeneous composition that is not a relevant N-source for estuarine and coastal ecosystems. This view is changing: Although only parts of the heterogeneous DON pool in estuarine environments apparently are bioavailable (Bronk et al., 2007; Seitzinger and Sanders, 1997; Seitzinger et al., 2002), labile moieties of DON are now seen as potentially important nitrogen sources for assimilatory and dissimilatory biological processes in coastal environments, and in particular in those situations, when dissolved inorganic nitrogen (DIN) is exhausted (Bronk, 2002). Significantly, there is some evidence that a decrease in the DIN/DON ratio of coastal waters (due to decreasing ratios in river loads or to seasonal depletion of DIN) may be favourable to dinoflagellates and cyanobacteria and may be implicated in the triggering of harmful algal blooms (Bronk et al., 2007).

Our interest here is to investigate the fate of DON in the extended estuarine salinity gradient of the Elbe River/NW Europe between a well-defined riverine (weir at Geesthacht) and marine end member in the German Bight/southeastern North Sea (Fig. 1). The estuary has been monitored continuously over the last decades for changes in water quality (ARGE, 2008), and data are available that offer a long-term view of the estuarine biogeochemistry of DON. The role of DON may have changed as a result of pollution reduction measures in the watershed, and since the mid-1980´s, TDN and DIN loads of the river have decreased by 30% (Radach and Paetsch, 2007). The first objective of the present study was to investigate whether the combined concentration of DON and ammonium and the NO_3^-/DON+NH_4^+ ratio in this river have changed as well.

Secondly, we present (to our knowledge) a first systematic and seasonally resolved data set on the $^{15}N/^{14}N$ composition of combined DON and ammonium (expressed as the δ value in ‰ = $[(R_{sample}/R_{standard})-1]*1000$, $R = {}^{15}N/^{14}N$ in DON+NH_4^+ and in the international standard atmospheric dinitrogen) of an estuary, allowing us to better evaluate whether or not DON+NH_4^+ behaves conservatively in the mixing zone between marine and fresh waters than concentrations alone. This was motivated by the fact that decreased DIN loads were accompanied by significant changes in estuarine nitrate turnover: The estuary changed from being a nitrate sink to being a nitrate source (Dähnke et al., 2008), and the nitrate source was associated with the estuarine turbidity maximum zone (TMZ) that forms at salinities between 0.4 and 2 (Bergemann, 1995). That nitrate increase was most pronounced in June 2006, when the internal addition doubled the original riverine nitrate concentration (Dähnke et al., 2008). Based on a concomitant decrease in $\delta^{18}O$ of nitrate, the source of the added nitrate was most likely nitrification of ammonium: The increase in nitrate concentrations was not associated with substantial changes in $\delta^{15}N\text{-}NO_3^-$, but only by a pronounced drop in $\delta^{18}O$. Because ammonium levels in the Elbe estuary are too low to account for an input of this magnitude, it was suggested that local nitrification of ammonium derived from the degradation of organic matter and its subsequent rapid oxidation by particle-associated nitrifying bacteria within the TMZ caused the nitrate peak. This would involve either DON or particulate nitrogen (PN) and should thus be accompanied by changes in concentrations and/or isotopic composition of DON and PN.

In summary, our objectives here are to 1) test if the NO_3^-/DON+NH_4^+ ratio in the Elbe river changed, and whether this is due to enhanced DON+NH_4^+ production, or decreasing nitrate levels alone over the last decades, 2) to examine concentration and isotopic changes versus salinity in the estuary for evidence of DON+NH_4^+ turnover, and in particular turnover in the TMZ, and 3) to establish whether seasonal differences in DON+NH4+ turnover are apparent in the estuary.

3.2 Materials and Methods

Our approach is based on determinations of combined DON and ammonium concentrations and the isotope ratio δ^{15}DON+NH_4^+ along the salinity gradient during different seasons in the Elbe estuary. Mixing diagrams of salinity versus DON+NH_4^+ concentrations, or versus δ^{15}DON+NH_4^+, help to determine whether DON+NH_4^+ behaves conservatively in the estuary or not. The mixing behaviour of dissolved compounds can be assessed by using a mixing model for calculating concentrations (Liss, 1976):

$$c_{mix} = f * c_r + (1 - f) * c_m \qquad \text{(Eq. 3.1)}$$

The indexes r and m denote riverine and marine concentrations, and f indicates the fraction of freshwater in each sample calculated from salinity, with 32 as salinity of the marine end member for German Bight water (f = (32- salinity)/32).

For isotopic values a calculation of mixing with concentration-weighted isotopic values is used (Fry, 2002):

$$\delta_{mix} = \frac{\left[f * c_r * \delta_r + (1-f) * c_m * \delta_m\right]}{c_{mix}} \qquad \text{(Eq. 3.2)}$$

Conservative mixing leads to linear mixing paths in the case of concentrations, whereas salinity-based isotope mixing diagrams usually show curvilinear mixing behaviours, reflecting concentration-based weighting of end member isotopic contributions.

3.2.1 Study site

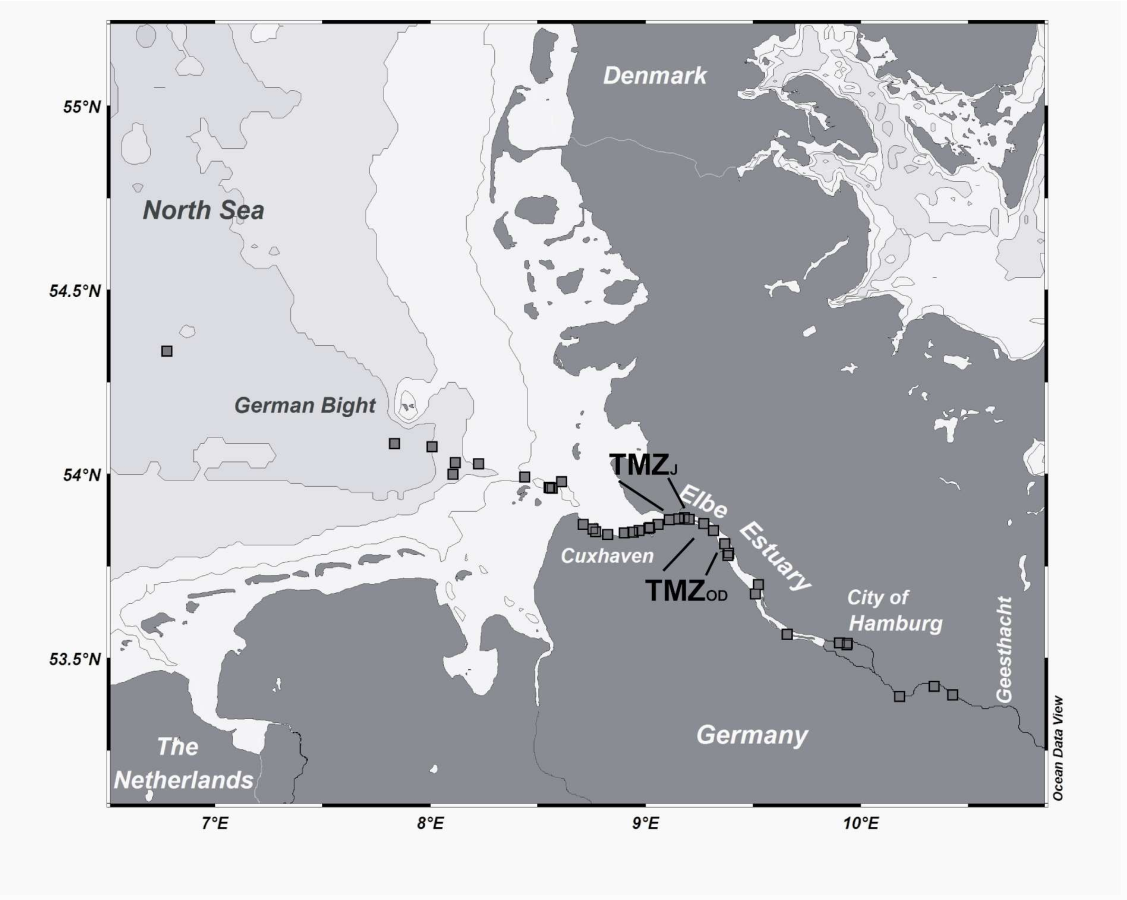


Figure 3.1: Sample stations in the Elbe estuary and location of places referred to in the text.

The Elbe River is 1094 km long and the second largest river discharging into the North Sea (Fig. 3.1). The tidal estuary extends over 142 km from the weir at the city of Geesthacht (stream kilometre 585) to the port of Cuxhaven into the German Bight, the south-eastern part of the North Sea. The average fresh water discharge at the Elbe mouth (Cuxhaven) is 861

m^3/s; nearly 25 million people live in the entire catchment area of 148 268 km^2. The Elbe River is the largest source of total dissolved nitrogen (TDN) and nitrate of the inner German Bight (Brockmann and Pfeiffer, 1990), which is that part of the North Sea most affected by eutrophication (OSPAR, 2008).

The Elbe tidal estuary is continuously dredged and channelled to enable access for large container ships to the port of Hamburg, one of the largest container ports in the world. The water residence time in the estuary ranges from ~ 10 days during mean high water flow (2000 m3/s) and ~ 72 days during mean low water flow (300 m3/s) with ~ 32 days during mean tide level (IKSE, 2005).

3.2.2 Sampling

On three occasions, samples were collected along the salinity gradients from <5 to >28. The sampling campaigns were conducted in October 2005, June 2006 and December 2006 on board the R/V Ludwig Prandtl (Table 3.1). Samples from the open North Sea were taken on board of the R/V Gauss and R/V Walther Herwig. In October 2005 and June 2006, water was collected at each station from surface waters (1 – 2 m), from 5 m depth and from 1 m above the ground for depth profiles in the Elbe estuary by using a multiprobe with rosette sampler, which was also used to measure the salinity in-situ.

	No. of		c(NO_3^-) [µM]		c(DON+NH_4^+) [µM]		δ^{15}N-NO_3^- [‰]		δ^{15}DON+NH_4^+ [‰]		δ^{15}PN [‰]	
Date	stations	Salinity	mean	range	mean	range	mean	range	mean	range	mean	range
Oct. '05	13	0.43–27.5	132.3± 61.3	22.1–190.0	35.0 ± 7.3	27.0–55.6	11.6 ± 0.3	10.9–12.1	5.1 ± 1.9	1.6 – 9.0	8.9 ± 0.8	7.3 – 9.9
Jun. '06	17	0.35–31.3	107.7± 69.9	7.5 – 227.3	35.7 ± 10.4	19.8–57.6	12.1 ± 2.0	9.4–17.1	5.9 ± 2.2	0.1 – 9.0	7.0 ± 3.3	1.6 – 14.2
Dec. '06	22	0.52–27.2	142.4± 65.3	33.9–231.7	38.6 ± 8.7	24.5-60.1	10.2 ± 0.7	8.0–10.9	8.4 ± 2.7	1.6 – 11.3	8.2 ± 0.3	7.7 – 9.3

Table 3.1: Synopsis of analytical results for sampling campaigns in the Elbe estuary from October 2005 to December 2006.

Since results from the first two campaigns showed that concentrations and δ^{15}N were homogeneous over water depth with constant salinity, in December 2006 water was only collected from surface water at different salinity by using the ship's membrane pump, since salinity well reflects the degree of mixing between marine and riverine water. Water samples (200 - 1000 ml, volumes depending on suspended particulate matter concentrations; SPM) were immediately filtered through precombusted GF/F filters (4.6 cm diameter, at constant vacuum) and stored in PE bottles that had been soaked in acid (10% HCl) overnight and rinsed with deionised water (DIW). Each bottle was rinsed with sample water before being filled. Water samples and filters were frozen on board at -18°C until analysis. Before analyses, filters were dried at 40-50°C and stored at 4°C.

3.2.3 Analytical Methods

Glassware was washed with deionised water (DIW), soaked in soap and 10% HCl baths, and washed again with DIW after each single step. All non-volumetric glassware was combusted at 450°C for at least 4 hours. Volumetric glassware and PE-bottles were washed with DIW, soaked in a 10% HCl bath overnight, washed again with DIW, and dried at 50°C.

Concentration of total dissolved nitrogen (TDN) in DIW was determined using the “persulfate oxidation method” (Knapp et al., 2005; Koroleff, 1976; Solórzano and Sharp, 1980) (see below) and yielded concentrations of < 1µM.

3.2.3.1 Nitrate and Nitrite concentrations

Concentrations of nitrate and nitrite were determined with an AutoAnalyzer3 (Bran+Luebbe, Hamburg) using standard colorimetric techniques (Grasshoff et al., 1999). Measurements showed that nitrite concentrations could be neglected as they contributed less than 1% to the total NO_x pool. For our setup, the detection limit for the combined determination of nitrate and nitrite is 0.05 µM.

3.2.3.2 Isotopic composition of nitrate

Nitrate $\delta^{15}N$ ($\delta^{15}N\text{-}NO_3^-$) was analysed by the “denitrifier method” (Casciotti et al., 2002; Sigman et al., 2001). Nitrate was quantitatively reduced to nitrous oxide (N_2O) by using a strain of denitrifier bacteria that lacks N_2O reductase activity. N_2O was automatically extracted, purified in a Gasbench II (ThermoFinnigan) and analysed by a Finnigan Delta plus XP mass spectrometer. The analysis of the samples were referenced to injections of N_2O from a pure N_2O gas cylinder and then standardised using an internationally accepted nitrate isotopic reference material (IAEA-N3, $\delta^{15}N$ = 4.7‰). We also used an in-house potassium nitrate standard for further validation of our results, which we measured with each batch of samples. The standard deviation for replicate analyses (n = 4) was ± 0.2‰ at the high DON concentrations found here.

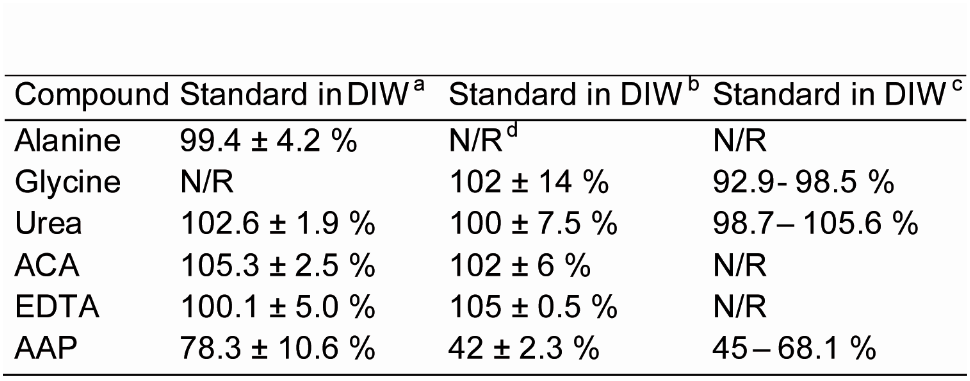
3.2.3.3 DON+NH_4^+ concentration

Since we were not able to separate DON and ammonium for individual isotopic compositions, we determined a combined signal of δ^{15}DON and $\delta^{15}NH_4^+$ (δ^{15}DON+NH_4^+), and also measured the concentration as a combined value. To determine the DON+NH_4^+ concentration and δ^{15}DON+NH_4^+ we used a published method (Knapp et al., 2005), with small modifications. The first step is the oxidation of total dissolved nitrogen (TDN, the sum of nitrate, nitrite, ammonium and DON) to nitrate using the persulfate oxidation method (Knapp et al., 2005; Koroleff, 1976; Solórzano and Sharp, 1980).

For the oxidation of TDN to nitrate, 20 ml of the sample was added to a Teflon bottle, to which 5 ml of a persulfate oxidising reagent (POR) is added. The POR was made up daily with 3.75 g certified ACS-grade sodium hydroxide (NaOH) dissolved in 250 ml of deionised water, followed by 7.5 g certified ACS-grade boric acid (H_3BO_3) and 12.5 g certified ACS-grade potassium persulfate, which was recrystallised three times (Grasshoff et al., 1999). After adding the POR to the sample, Teflon caps were closed tightly and the Teflon bottles were placed in a digestion rotor for microwave digestion. The samples were treated for 13 min in a MLS 1200 Mega digestion microwave oven following an application for acid digestion. To determine the reagent blank, each batch of samples included one Teflon bottle filled with only 5 ml POR. After digestion this bottle was filled with 20 ml deionised water. Concentration and $\delta^{15}N$ of the POR sample was treated as the reagent blank for the water samples of the same batch. The reagent blank was always <2 µM.

DON+NH_4^+ concentrations were calculated by subtracting the concentrations of nitrate and nitrite from analysed nitrate concentration of persulfate digested samples. Reagent blanks were also subtracted and dilution steps considered by corrections.

To evaluate the yield of this procedure, we experimented with several standard materials. Table 3.2 lists common DON standards (glycine, alanine, urea, 6-amino caproic acid (ACA), EDTA and 4-aminoantipyrine (AAP) and the degree of completeness of oxidation to nitrate using our digestion procedure. For comparison, other published values are also listed (Bronk et al., 2000; Knapp et al., 2005).



Compound	Standard in DIW[a]	Standard in DIW[b]	Standard in DIW[c]
Alanine	99.4 ± 4.2 %	N/R[d]	N/R
Glycine	N/R	102 ± 14 %	92.9- 98.5 %
Urea	102.6 ± 1.9 %	100 ± 7.5 %	98.7– 105.6 %
ACA	105.3 ± 2.5 %	102 ± 6 %	N/R
EDTA	100.1 ± 5.0 %	105 ± 0.5 %	N/R
AAP	78.3 ± 10.6 %	42 ± 2.3 %	45 – 68.1 %

Table 3.2: Results of tests on the oxidation efficiencies of persulfate digestion for different standard materials. DIW: Deionized water, ACA: 6-amino caproic acid, EDTA: ethylenediamine-tetraacetate, AAP: 4-aminoantipyrine. Notes: a) Standard in DIW is as calculated from a 10, 20, 50, 100, 200, and 400 µM concentration series of each standard (this study); b) Standard in DIW is as calculated from a 0.0, 2.5, 5.0, 7.5, and 10.0 µM concentration series of each standard (Knapp et al., 2005); c) same standard series as under b) reported by Bronk et al. (Bronk et al., 2000). d) N/R= not reported.

Oxidation efficiencies have previously been reported for concentrations from 0 to 10.0 µM in DIW only. In rivers, we expected higher concentrations of DON+NH_4^+, so we tested the oxidation efficiencies of the POR for concentrations of 10, 20, 50, 100, 200 and 400 µM of each standard in DIW. For most concentrations, oxidation efficiency was in the range of 98 – 105%, except for the highest concentration of 400 µM, which yielded only 70-90% of the standards. Because of the molecular structure, oxidation efficiency for AAP was generally lower (between 73 and 84%, and 68% in the case of 400 µM solutions).

3.2.3.4 δ^{15}N analysis of TDN

After the persulfate digestion, δ^{15}N-NO_3^- in oxidised samples and reagent blanks was determined using the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001).

Differing from the published method (Knapp et al., 2005), the digestion employed here resulted in a sample pH value of about 6, so that the addition of HCl was not necessary and a possible source of contamination was eliminated. The volumes of the samples for the bacterial reduction to N_2O were adjusted to 20 nmol nitrate to be injected.

As mentioned above, we did not separate DON and ammonium, so our results for DON-concentration and δ^{15}DON included ammonium. However, studies performed in the course of monitoring water quality in the Elbe River (ARGE, 2001, 2002, 2003, 2004, 2005a, 2005b, 2007a, 2007b) over the last recent eight years show that ammonium concentrations were small during time periods comparable to the periods of our sampling campaigns (Fig. 3.2). Appreciable ammonium concentrations (> 5 µM, reflecting ≥ 10% of measured DON+NH_4^+) occurred only in samples from the port of Hamburg (km 615 – 645) and near Cuxhaven (km 725) seawards of the TMZ. In June 2006, the ARGE Elbe detected no ammonium in the entire estuary (ARGE, 2007b). So in areas with a distinct maximum of measured DON+NH_4^+ concentration (port of Hamburg and seawards the TMZ) we estimate the portion of ammonium in our data to approximately 20%. In all other areas the impact is estimated to be less than 10%.

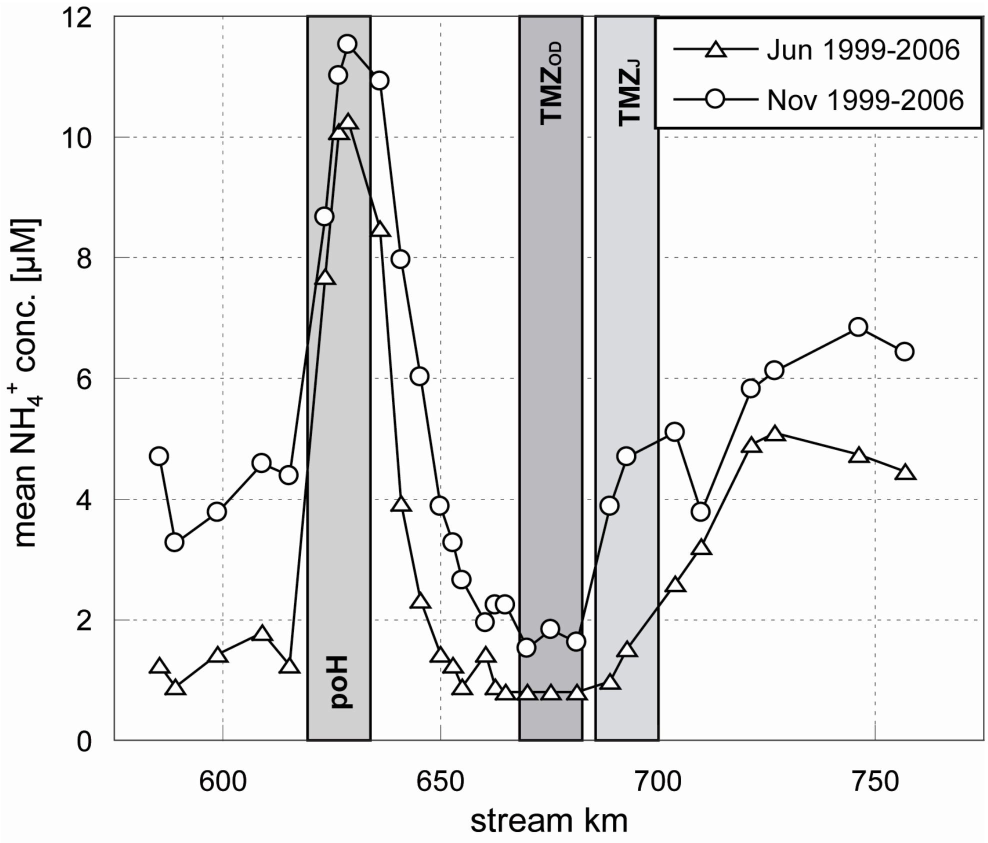


Figure 3.2: Mean ammonium concentration in June and November in the Elbe estuary from 1999 to 2006, measured by the ARGE Elbe (ARGE Elbe, 2001, 2002, 2003, 2004, 2005a, 2005b, 2007a, 2007b)

For calculations of the δ^{15}DON+NH_4^+, mass balance calculations were made using the measured nitrate concentrations and δ^{15}N of the oxidised sample, the reagent blank and the original (not oxidised) sample:

$$\delta^{15} DON + NH_4^+ = \frac{\delta^{15} TDN * c(TDN)}{c(DON + NH_4^+)} - \left[\frac{\left(\delta^{15} N - NO_3^- * c(NO_3^-)\right)}{c(DON + NH_4^+)} + \frac{\left(\delta^{15} Blank * c(Blank)\right)}{c(DON + NH_4^+)} \right]$$

(Eq. 3.3)

We tested the combination of persulfate digestion and denitrifier method to measure δ^{15}DON+NH_4^+ by parallel analyses of urea standard solutions of different concentrations. The δ^{15}N of solid urea was measured by using a Flash EA 1112 elemental analyzer combined with a Finnigan Delta plus XP mass spectrometer, yielding a δ^{15}N value of 0.5‰ ± 0.2‰. Measurements of the urea solutions after preparation as described above yielded in δ^{15}N values of 0.4 ± 0.2‰ (n = 21) after blank correction.

Repeated measurements of replicate water samples demonstrated the reproducibility of our method for δ^{15}DON+NH_4^+ analyses. The mean measured standard deviation of δ^{15}TDN and δ^{15}N-NO_3^- was 0.3‰ (3 to 4 repetitions). The mean standard deviation for TDN and nitrate concentration was 1 µM. Because of error propagation, the calculated standard deviation of δ^{15}DON+NH_4^+ ranged from 0.3 to 2.0‰ with a mean value of 0.9‰, and the calculated mean standard deviation for DON+NH_4^+ concentration was 1.7 µM.

3.2.3.5 Particulate nitrogen (PN)

δ^{15}PN was analysed with a Flash EA 1112 elemental analyzer coupled to a Finnigan Delta plus XP mass spectrometer. Analysed samples were standardised using the isotopic reference materials “High organic sediment standard OAS” (Cat.no. B2151, Batch no. 2824, δ^{15}N = +4.4‰ ± 0.19‰), “Low Organic Content Soil Standard OAS” (Cat.no. B2153, Batch no. 2822, δ^{15}N = + 6.7‰ ± 0.15‰) and IAEA-N1 (δ^{15}N = + 0.4‰). The standard deviation for replicate analysis was 0.2‰ (n=3).

3.3 Results

The analytical results on samples of all three cruises are plotted in Figs. 3.3 to 3.10. Because of poor weather conditions in October 2005 and December 2006, samples were taken only up to a salinity of 28 at the Elbe mouth near Cuxhaven. In June 2006, samples were taken also in the river plume in the German Bight up to a salinity of 32, representing typical German Bight coastal water.

Fig. 3.3 plots the measured salinity (a) and light transmission data (b) (an uncalibrated measure of turbidity) in the Elbe River against stream kilometre. Note that the salinity front moves up and down the estuary, depending on the tidal action, and influences the location of the TMZ, which also is mobile depending on the tidal wave and the current speed. On our

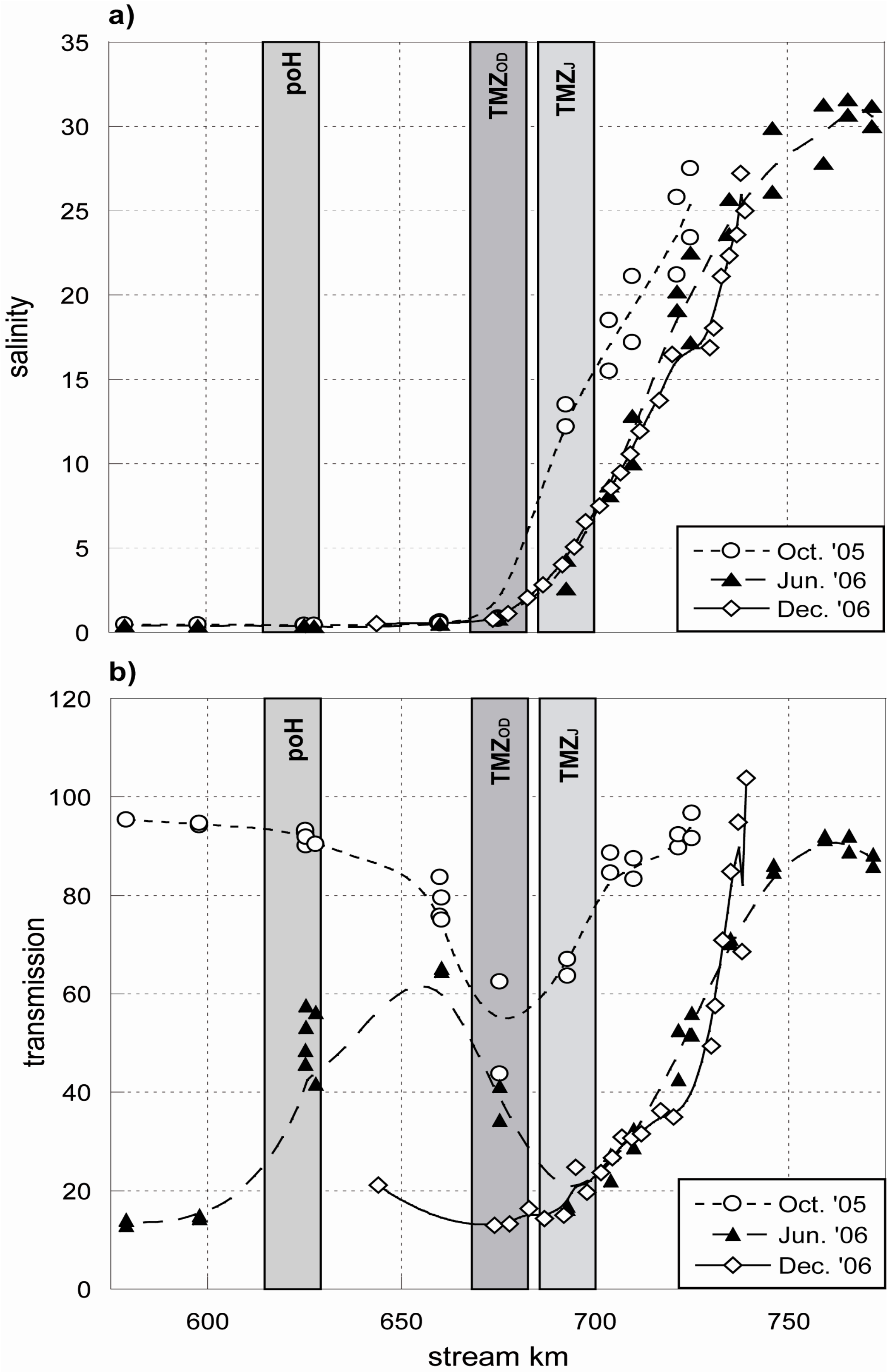


Figure 3.3: Salinity (a) and transmission (b) data in the Elbe estuary against stream kilometre. Indicated are the Port of Hamburg (poH), and the location of the turbidity maximum zone in October 2005 and December 2006 (TMZ_{OD}) and in June 2006 (TMZ_J). Lines in this and the following figures are best-fit curves using the locally weighted Least Squared error method.

sampling campaigns, the salinity front was always located near the town of Glückstadt (~stream km 675), moving slightly downstream only in June 2006. During all sampling campaigns, the turbidity maximum was situated between stream km 670 and 700, corresponding to the salinity range from 0.5 to 2 (Fig. 3.3b).

Depth profiles at single stations taken during the campaigns in October 2005 and June 2006 showed no significant changes in the $\delta^{15}N$ values of nitrate, TDN, and $DON+NH_4^+$ when salinity profiles were homogeneous. Differences were small and in the range of measurement uncertainty. Only when salinity changed with depth due to the mixing of denser marine water from the German Bight near the bottom with buoyant river water in the brackish water zone (Bergemann, 1995) $\delta^{15}N$ of at least one of the analysed parameters (NO_3^-, $DON+NH_4^+$) was observed to change.

3.3.1 $DON+NH_4^+$ concentrations

Concentrations of $DON+NH_4^+$ varied between 20 and 60 µM, with no significant seasonal differences (Fig. 3.4). Unlike nitrate (Fig. 3.5), the $DON+NH_4^+$ concentration differences between freshwater and saltwater end members are small, and the salinity vs. $DON+NH_4^+$ plot gives no indication of conservative mixing for $DON+NH_4^+$ in the entire Elbe estuary from Geesthacht to the German Bight. Only at salinities higher than 10, DON mixed nearly conservatively with German Bight water, but the slope of the mixing trend is much less linear than that of nitrate concentrations (compare Figs. 3.4-3.7). We found maximum $DON+NH_4^+$ concentrations in the port of Hamburg (35-58 µM at salinities < 1; km 615 to 635) and at salinities >7 seaward of the TMZ (40-50 µM in December and October, 30 µM in June). In the TMZ between stream km 650 and 700, $DON+NH_4^+$ concentrations fluctuated around 30 µM.

Upstream of Hamburg, $DON+NH_4^+$ concentrations ranged between 30 and 35 µM and corresponded to the river concentration determined landward of the weir at Geesthacht (Schlarbaum, unpublished data). Concentrations in the German Bight waters at salinities >32 were higher in winter (18.3 µM) than in summer (6.4 µM).

On average, estuarine $DON+NH_4^+$ concentrations in winter (38.6 ± 8.7 µM) were slightly higher than in summer or autumn (35.7 ± 10.4 µM and 35.0 ± 7.3 µM, respectively).

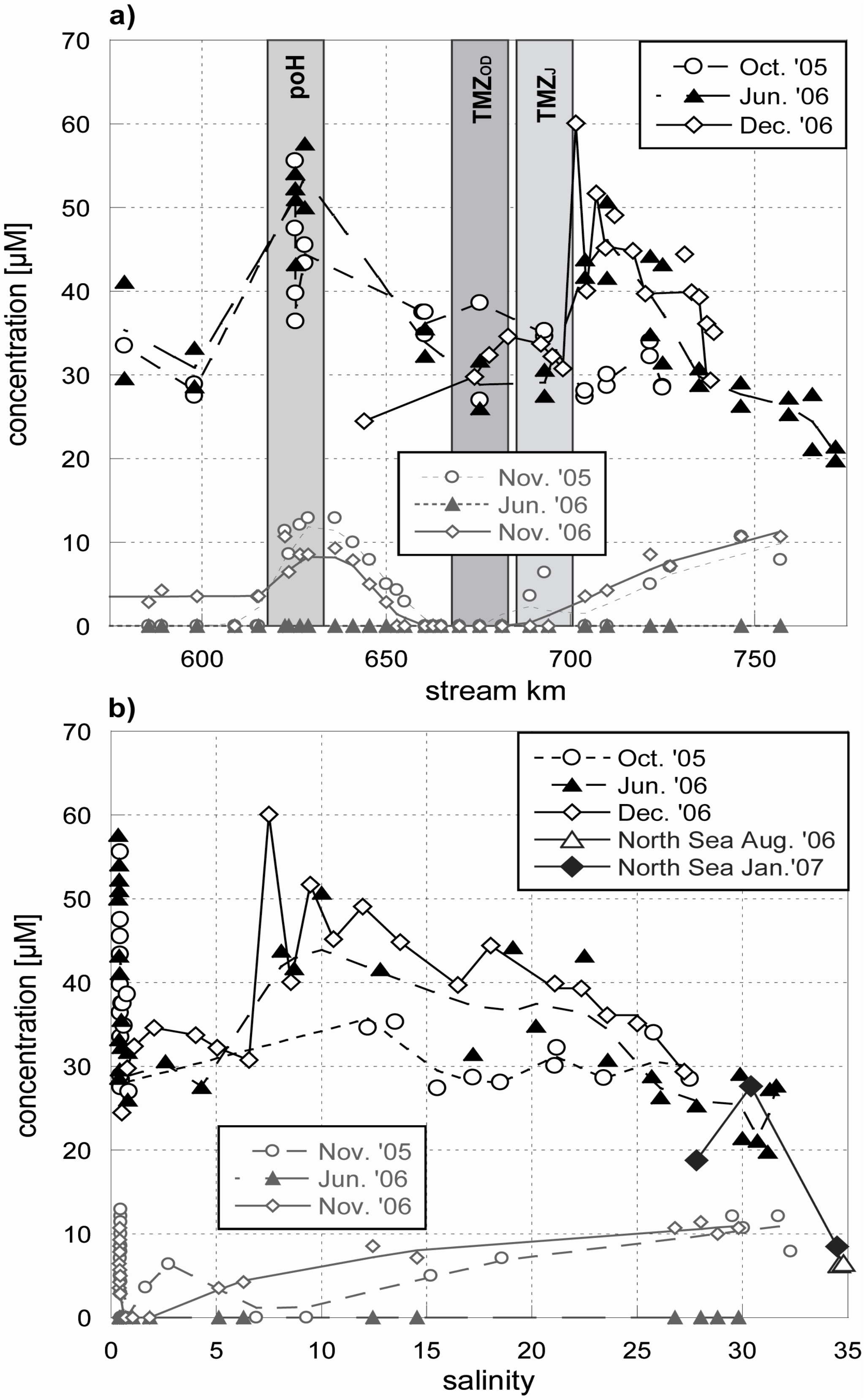


Figure 3.4: DON+NH_4^+ (black symbols) and ammonium (grey symbols) concentrations in the Elbe estuary versus stream kilometre (a) and versus salinity (b).

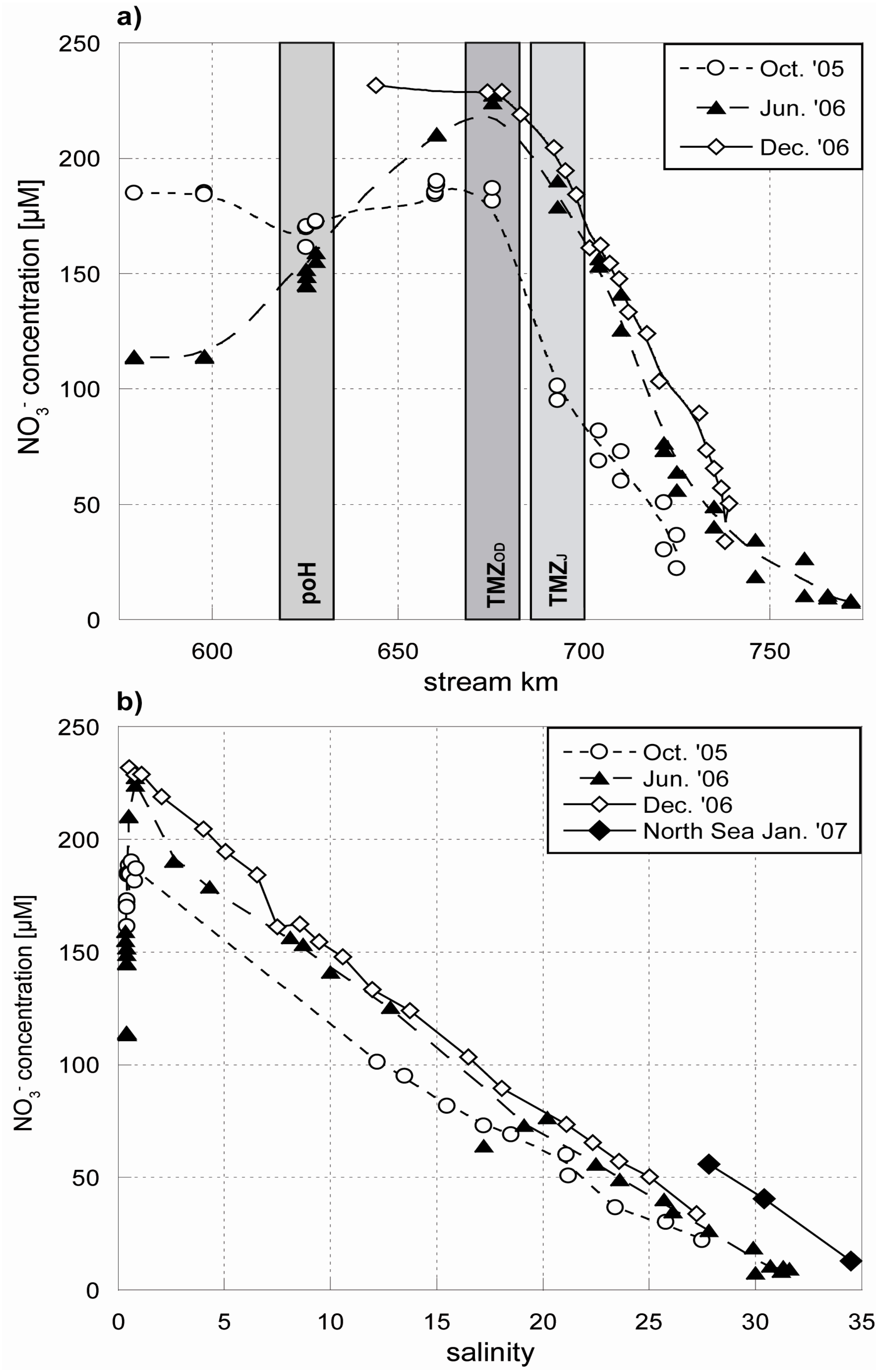


Figure 3.5: Nitrate concentrations in the Elbe estuary versus stream kilometre (a) and versus salinity (b).

3.3.2 Isotopic composition of DON+NH_4^+

The δ^{15}DON+NH_4^+ ranged from 0.1‰ to 11.3‰ (Fig. 3.6) and seasonal variations were subtle. The data also suggest that both DON+NH_4^+ production and consumption occurred in the estuary. The river end member δ^{15}DON+NH_4^+ ranged from 2 to 5‰, samples from the North Sea taken at salinities >30 had uniform δ^{15}DON+NH_4^+ values in summer and winter situations (range 3 - 4.5‰).

In a statistical evaluation of the entire data set (all data pooled), we sought for correlations amongst parameters (see also Table 3.3 for an analysis of data for each season separately). The δ^{15}N of DIN does not appear to significantly covary with δ^{15}DON+NH_4^+ (Fig. 3.9), but a weak positive (0.44, n=78) correlation exists between DON+NH_4^+ concentrations and δ^{15}DON+NH_4^+ on the one hand, and δ^{15}DON+NH_4^+ is negatively correlated with both PN concentrations (-0.47; n=53) and the δ^{15}PN (-0.52; n=49) on the other hand; all these are significant on the 0.01 level.

Correlation coefficients (r) in the salinity gradient (salinity > 0.8)	Oct. '05	No. of stations	Jun. '06	No. of stations	Dec. '06	No. of stations
δ^{15}DON+NH_4^+ / δ^{15}PN	0.431	4	-0.475	7	**-0.710**	20
δ^{15}DON+NH_4^+ / $\delta^{15}NO_3^-$	-0.367	11	-0.364	20	-0.439	20
δ^{15}PN / δ^{15}TDN	0.438	4	-0.174	7	-0.336	20
δ^{15}PN / $\delta^{15}NO_3^-$	-0.519	4	0.312	7	0.136	20

Correlation coefficients (r) (all stations)	Oct. '05	No. of stations	Jun. '06	No. of stations	Dec. '06	No. of stations
δ^{15}DON+NH_4^+ / δ^{15}PN	0.047	12	-0.230	18	**-0.710**	20
δ^{15}DON+NH_4^+ / $\delta^{15}NO_3^-$	-0.179	25	-0.228	34	-0.439	20
δ^{15}PN / δ^{15}TDN	-0.245	12	-0.482	18	-0.336	20
δ^{15}PN / $\delta^{15}NO_3^-$	-0.221	12	-0.278	18	0.136	20

Table 3.3: Correlation coefficients (r) of variables in the salinity gradient >0.8 and for all stations sampled. Significant values ($\alpha = 0.05$) are printed in bold numbers.

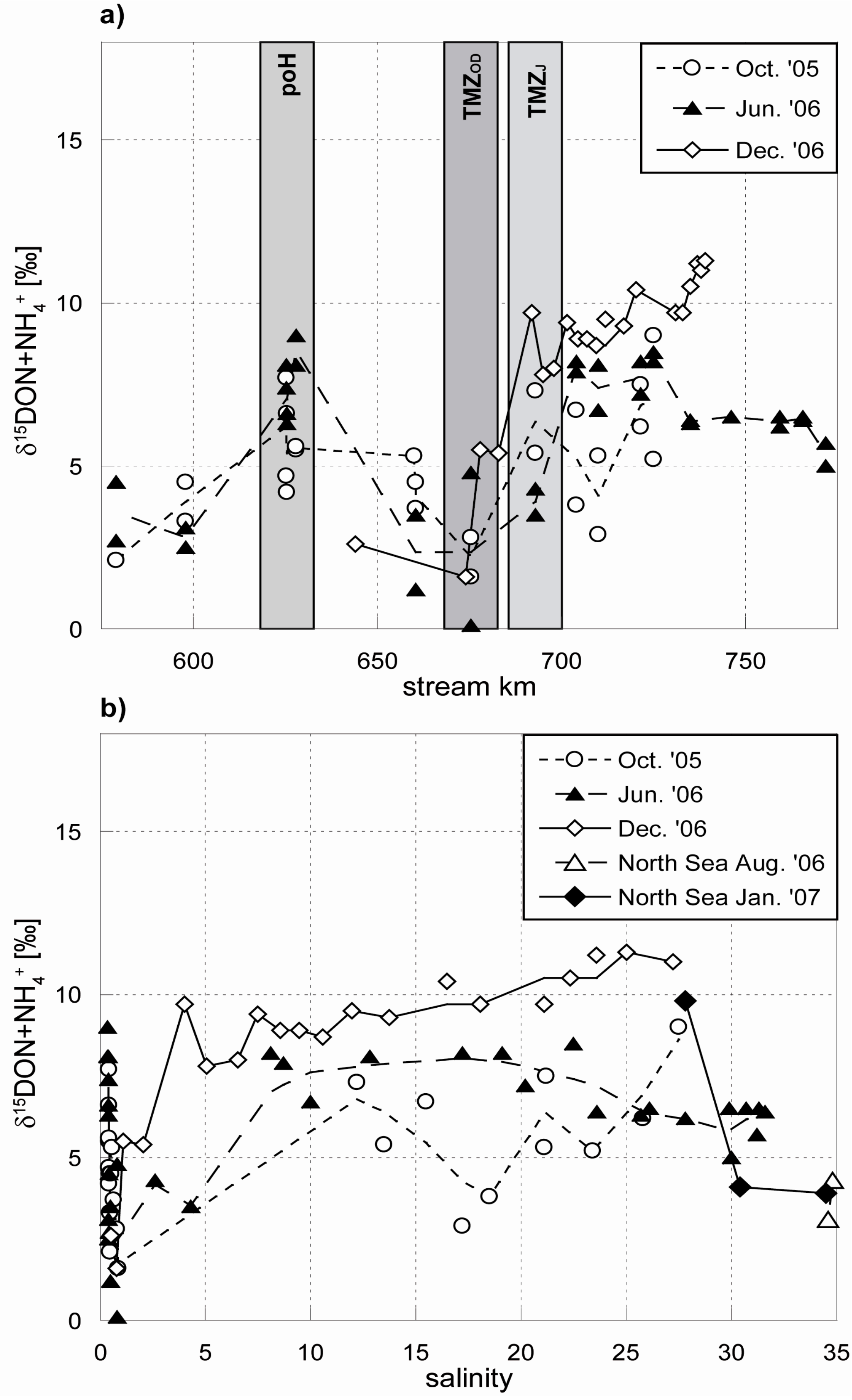


Figure 3.6: $\delta^{15}DON+NH_4^+$ in the Elbe estuary versus stream kilometre (a) and versus salinity (b).

3.3.3 Seasonal patterns

In December 2006, δ^{15}DON+NH_4^+ in the Elbe estuary was significantly enriched (about 2-5‰) over summer or autumn values (Fig. 3.6). This may due to the fact, that ammonium concentrations were slightly higher in winter than in summer. In comparison to river waters over the whole salinity gradient, DON+NH_4^+ in samples from the North Sea was more depleted in ^{15}N (3 – 4‰ compared to 3 – 9‰ in October 2005, 4 – 8‰ in June 2006 and 5 – 11% in December 2006, respectively), but in the same range as in samples from the river water end member at the weir of Geesthacht at stream kilometre 585 (2‰ in October 2005, 2.5 – 3‰ in June 2006). In June 2006, δ^{15}DON+NH_4^+ was constant between 7 and 8‰ in the salinity interval from 7 to 22. At higher salinity (23 – 30) δ^{15}DON+NH_4^+ was between 6 and 7‰, at lower salinity (1 – 5), δ^{15}DON+NH_4^+ was 3 – 4‰. In the port of Hamburg (salinity ~0.5), local sources of DON and/or ammonium resulted in elevated δ^{15}N values between 6 and 9‰. Most depleted isotopic values for DON+NH_4^+ characterized the TMZ during all seasons, which is also coupled to a pronounced drop in DON+NH_4^+ concentration (Figs. 3.5 and 3.6).

In the salinity range between 5 and 23, no clear deviation from conservative mixing could be seen for the samples of June and December 2006. In the samples of October 2005 a large scatter with no clear trend was found. Lowest mean δ^{15}DON+NH_4^+ values were measured in October 2005, highest values occurred in December 2006; in all three seasons (June 2006 was intermediate) we see a tendency to increasing δ^{15}DON+NH_4^+ with increasing salinity. In June 2006 we found a minimum in δ^{15}DON+NH_4^+ at salinity > 23, approaching the values measured in offshore waters with values below 5‰ (Fig. 3.6).

3.3.4 δ^{15}N of particulate nitrogen

The isotopic composition of particulate nitrogen was remarkably uniform and nearly constant throughout the entire river in both October 2005 and December 2006 (range of δ^{15}PN from 7 to 10‰; Fig. 3.8). The largest range in δ^{15}PN was observed in samples from the June 2006 expedition: In the salinity interval from 1 to 15, δ^{15}PN was ~ 8 to 9‰, whereas at higher salinities, δ^{15}PN values ranged between 8 and 14‰. Values < 5‰ were measured in samples taken in the port of Hamburg, but adjacent samples upstream and downstream of the harbour

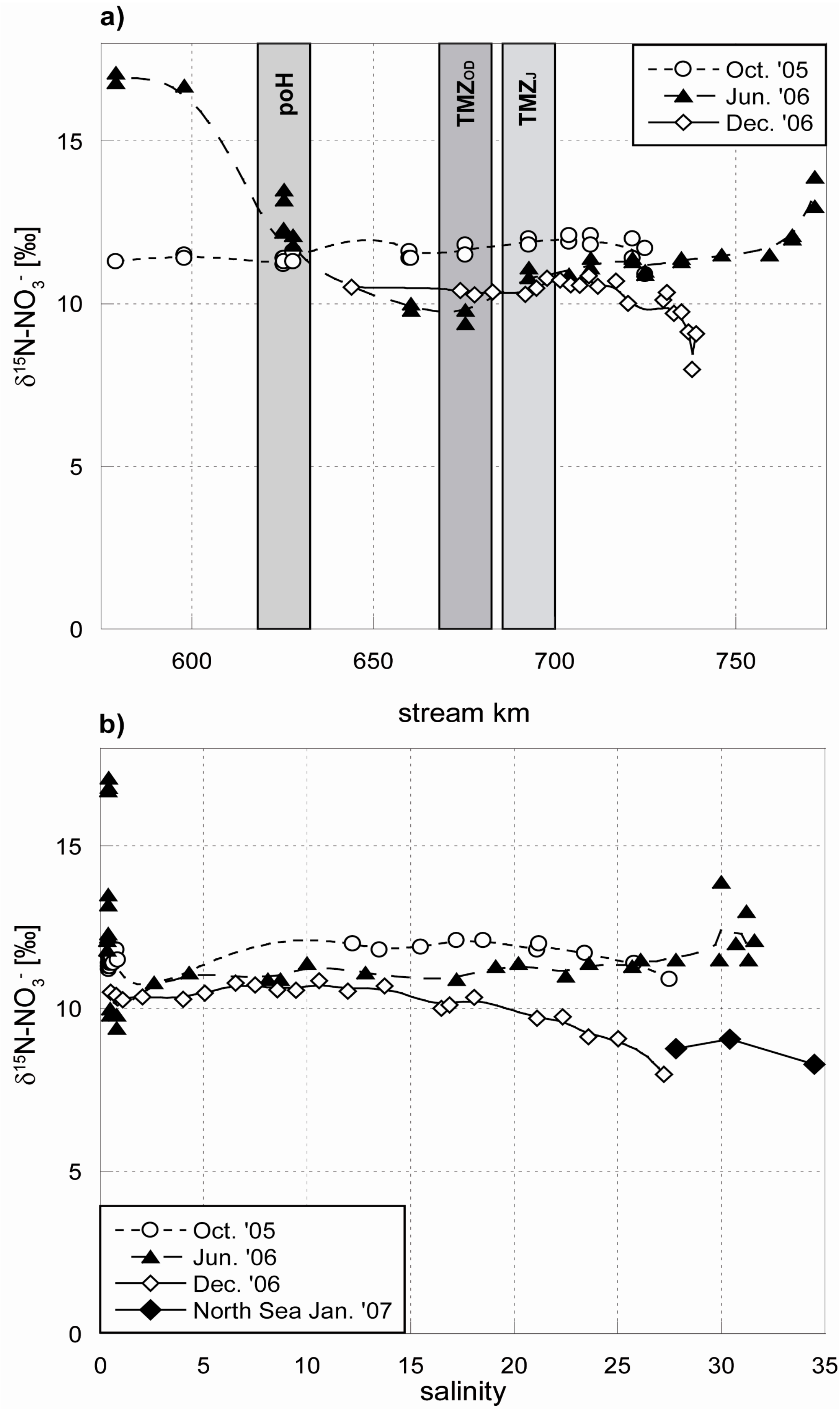


Figure 3.7: $\delta^{15}NO_3^-$ in the Elbe estuary versus stream kilometre (a) and versus salinity (b).

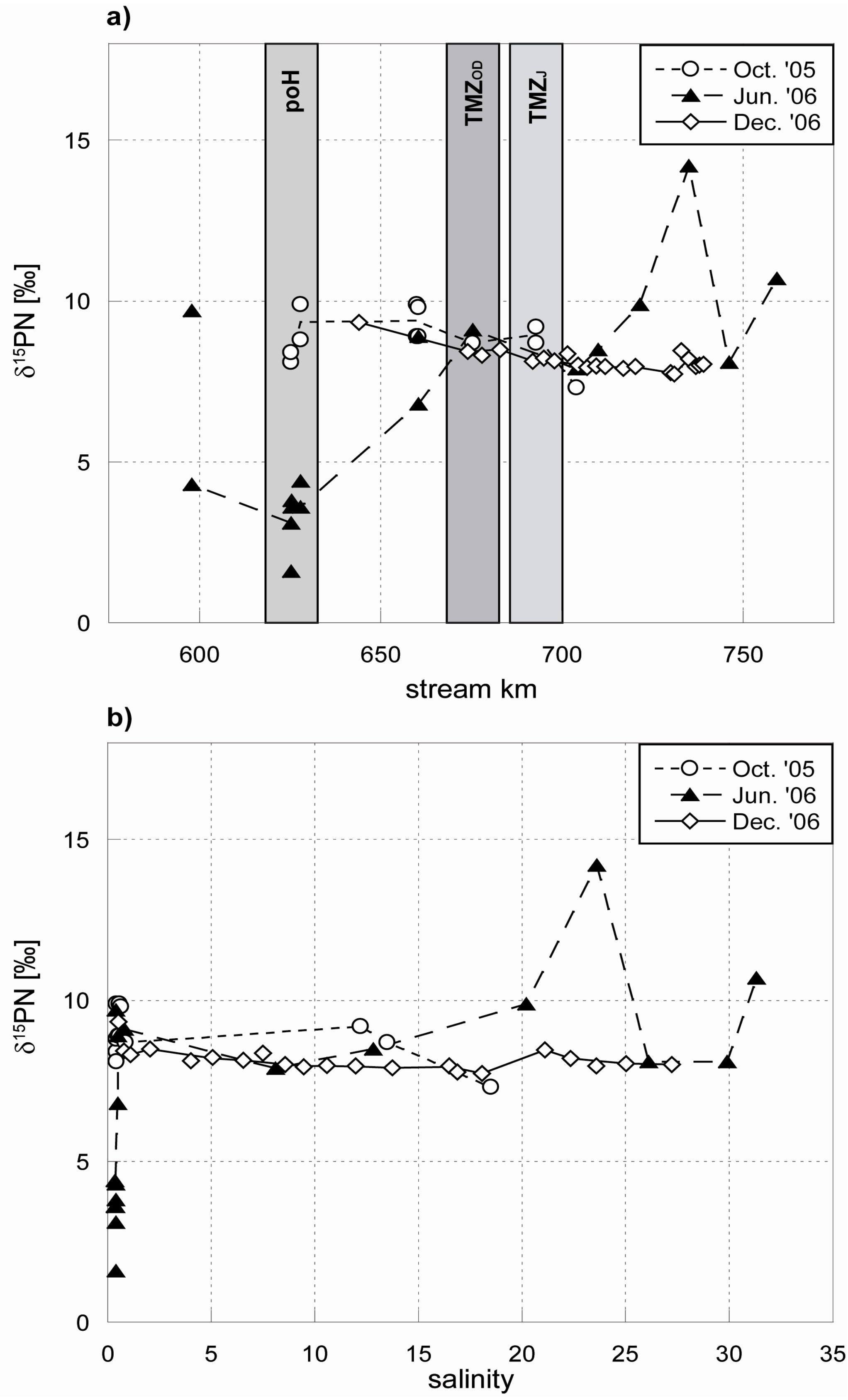


Figure 3.8: δ^{15}PN in the Elbe estuary versus stream kilometre (a) and versus salinity (b).

had δ^{15}PN between 9 and 10‰. We thus assume that PN here is influenced by local sedimentation and resuspension, or assimilation of different N sources during the summer season. It is likely that a large sewage treatment plant in the port of Hamburg has an effect and may also affect N species differently at different seasons due to different concentrations and different isotopic compositions of the sewage-derived nitrogen forms.

Besides the lack of seasonal variability, there also is a lack of correlation between the isotopic composition of DIN, DON+NH_4^+ and PN. As shown in Table 3.3 and Fig. 3.9, the correlation coefficients for the data of δ^{15}DON+NH_4^+ and δ^{15}PN indicate a significant (α=0.05) negative correlation for the winter situation, and no correlation during the other seasons. The significant negative correlation in winter may be due to the fact that all samples at that time were taken in the brackish water interval, in which DON+NH_4^+ and PN are more or less conservatively mixed (at salinities > 10, which accounts for approximately half of the samples from December 2006). An important reason for the lack of correlation between PN and DON+NH_4^+ may be that throughout the estuary, different processes contribute as sources or sinks to either of these pools, so that samples from the port of Hamburg have a completely different composition than those from the TMZ.

3.3.5 NO_3^-/DON+NH_4^+ratios

In Fig. 3.10, the ratios of nitrate to DON+NH_4^+ of our cruises and those of earlier studies of the Elbe estuary are presented. In October 2005, the ratios varied between 3.2 and 6.6 in the low salinity part of the Elbe estuary, with a minimum of 3.2 in the port of Hamburg. With increasing salinity, the ratio decreased to 1.0. In summer 2006 the ratio ranged between 2.8 and 3.7 in the low salinity areas of the Elbe estuary, but with only a slight minimum in the port of Hamburg. In contrast to the data from October 2005, the ratio increased rapidly in the TMZ to 7.9, before decreasing to 0.4 in high salinity waters of the German Bight. Highest ratios of 9.5 were determined in December 2006 at a salinity of 0.52, the ratio then decreased with increasing salinity to 1.4. Overall, the ratios range from < 1 to almost 10.

Studies from the 1980’s and 1990’s (Brockmann and Pfeiffer, 1990; Brockmann, 1994; Kerner and Spitzy, 2001) showed comparable ratios (Fig. 3.10). Only in January 1986 Brockmann and Pfeiffer (1990) found very low ratios due to high ammonium concentrations. In the same study the ratio did not decrease with increasing salinity in April 1987.

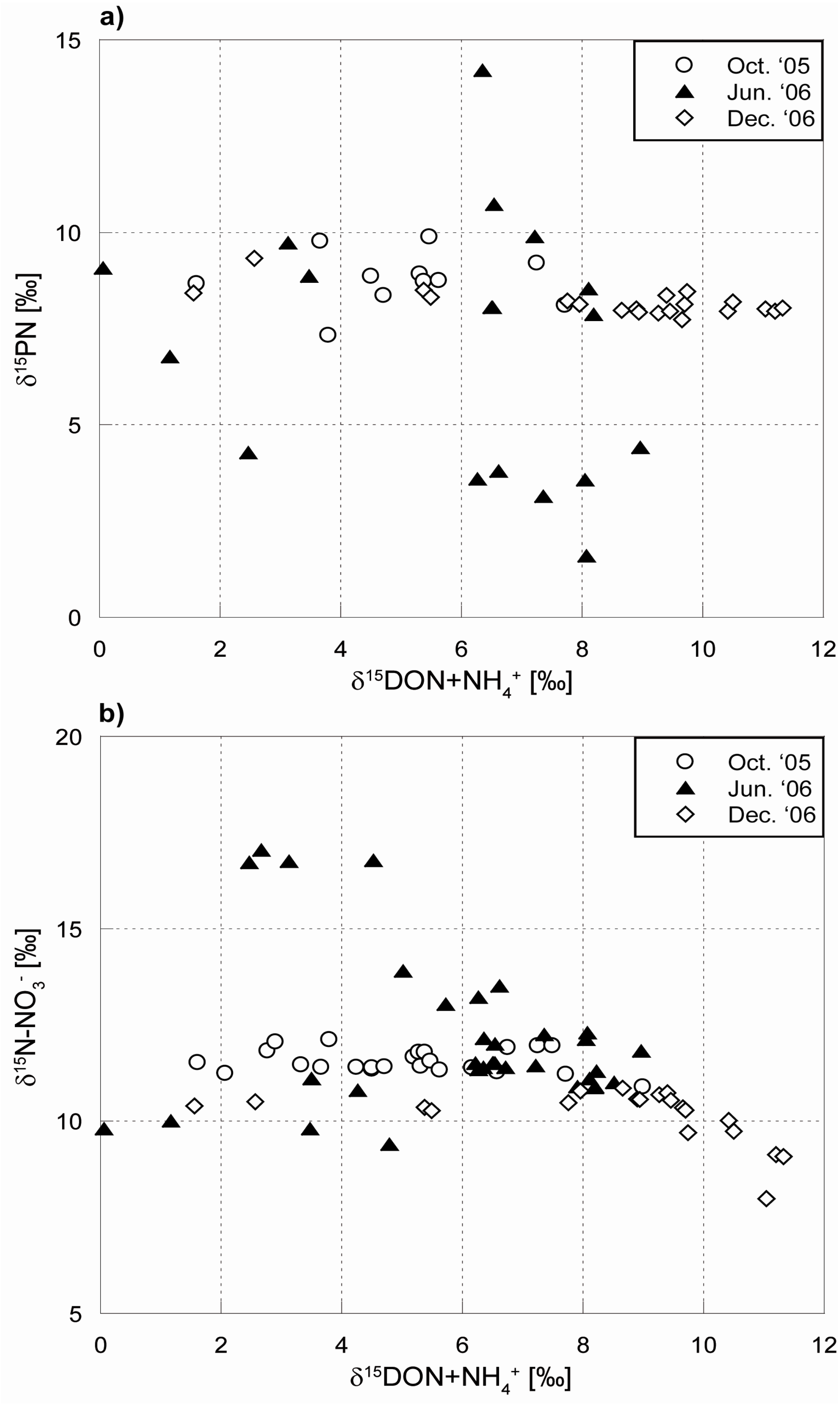


Figure 3.9: Cross plots of δ^{15}PN versus δ^{15}DON+NH$_4^+$ (a) and δ^{15}NO$_3^-$ versus δ^{15}DON+NH$_4^+$ (b).

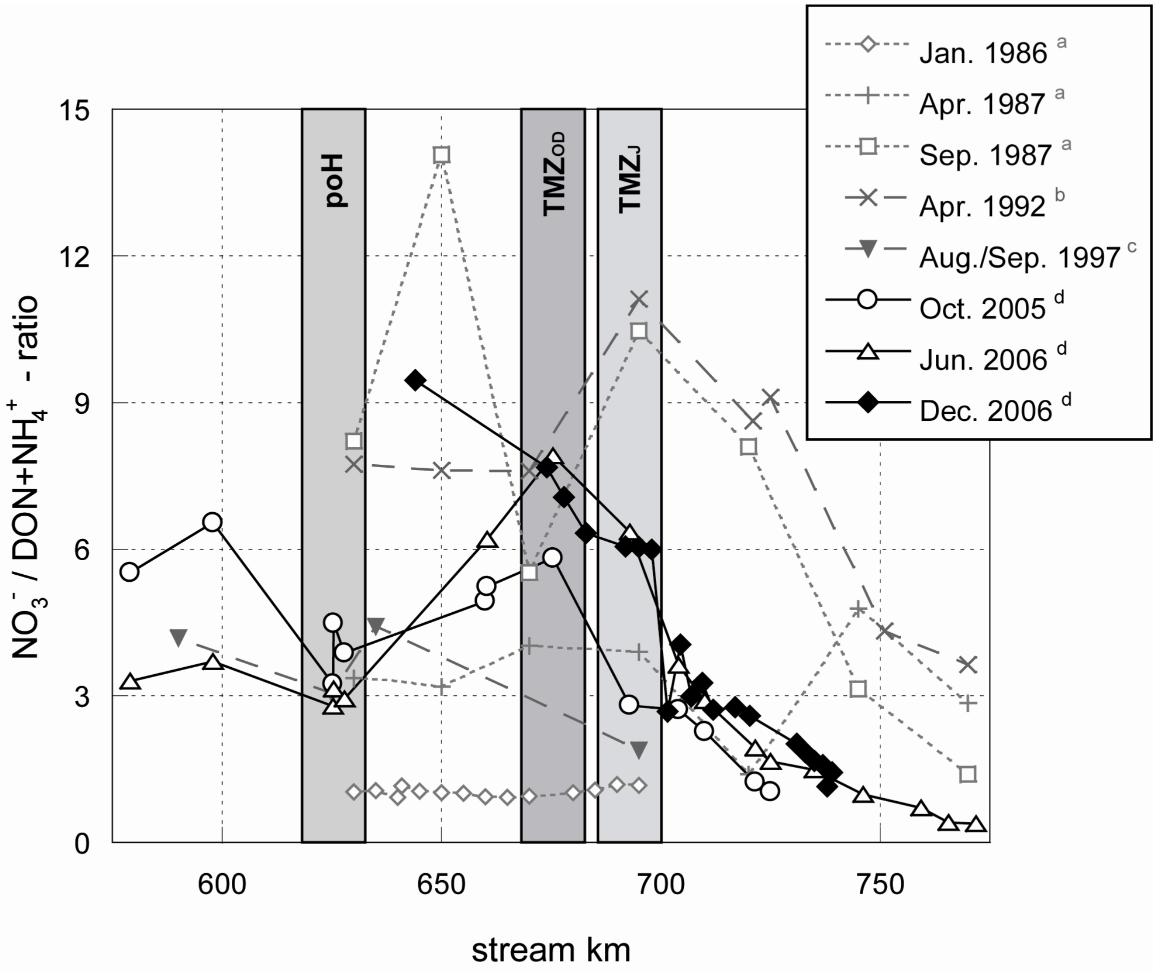


Figure 3.10: NO_3^-/DON+NH_4^+ - ratios in the Elbe estuary. January 1986 to September 1987 (indexed a) data are from (Brockmann and Pfeiffer, 1990); April 1992 (indexed b) data are from (Brockmann, 1994); August/September 1997 data (indexed c) are from (Kerner and Spitzy, 2001); October 2005 to December 2006 data (indexed d) are from this study.

3.3.6 The influence of ammonium on combined DON+NH_4^+

With the method used here we only were able to measure $\delta^{15}N$-NO_3^- and δ^{15}TDN, so that a combined signal of DON and ammonium is reported. However, we can estimate the contribution of ammonium from published long-term monitoring data. Measurements of the ARGE-Elbe (ARGE, 2001, 2002, 2003, 2004, 2005a, 2005b, 2007a, 2007b) in the years since 1999 show that the mean ammonium concentration of the entire estuary is about 3.1 ± 4.1 µM in June/July and 5.0 ± 4.3 µM in November (Fig. 3.2). Compared to the data of combined DON+NH_4^+ concentration in our study, the average share of ammonium in the combined values is estimated as about 9% in summer, 14% in autumn and 13% in winter, over the entire estuary.

Ammonium concentrations in the Elbe estuary show the same characteristics as the combined DON+NH_4^+ concentrations (Figs. 3.2 and 3.4a), but the increase of ammonium concentration does not account for the increase of the combined DON+NH_4^+ signal in the port of Hamburg and downstream of the TMZ.

In the port of Hamburg the mean annual ammonium concentration increases to a maximum of 10.3 ± 4.9 µM in June/July and 11.5 ± 3.1 µM in November, which corresponds to an ammonium fraction of 11% in summer and 18% in autumn. In winter we did not measure near the city of Hamburg due to poor weather conditions.

Downstream of the TMZ the mean ammonium concentration has a maximum of 5.1 ± 3.7 µM in June/July and 6.8 ± 3.8 µM in November, representing ammonium contributions of 13% in summer, 16% in autumn and 12% in winter in this area of the Elbe estuary.

3.4 Discussion

Our objectives for this study were to see if DON+NH_4^+ concentrations have changed over the past decades and to evaluate if DON+NH_4^+ is either conservatively mixed, or affected by non-conservative processes. We also wished to identify distinct sources or sinks for DON+NH_4^+ in the Elbe estuary, and whether seasonal differences in DON+NH_4^+ load and cycling are evident.

3.4.1 The influence of ammonium on combined DON+NH_4^+

For a rough estimate of the likely influence of $\delta^{15}NH_4^+$ on the combined δ^{15}DON+NH_4^+ signal, we turn to data of $\delta^{15}NH_4^+$ generated by other authors.

Ahad et al. (2006) measured $\delta^{15}NH_4^+$ in the Tyne and the Tweed estuaries in northeastern England. Even if these estuaries are much smaller than the Elbe estuary, the climatic conditions and catchment characteristics are similar and especially the data of the Tyne estuary should represent the conditions in the Elbe estuary.

In the Tyne estuary, Ahad et al. (2006) measured $\delta^{15}NH_4^+$ signatures from 7.9 to 11.3‰ in summer and 7.3 to 10.4‰ in winter with an average of 10.2 ± 0.9‰ and 9.6 ± 1.7‰ during summer and winter, respectively. In the Tweed estuary, $\delta^{15}NH_4^+$ values also were slightly lower in winter (5.3 to 7.3‰) than in summer (7.8 to 10.1‰), with averages of 6.9 ± 0.6‰ in winter and 9.5 ± 0.7‰ in summer.

Middelburg and Nieuwenhuize (2001) measured $\delta^{15}NH_4^+$ in two other European estuaries: the Loire estuary in France and the Thames estuary in England. $\delta^{15}NH_4^+$ values ranged from -4 to 17‰ in the Loire and from 14 to 43‰ in the river Thames. Mariotti et al. (1984) studied the isotopic composition of ammonium and nitrate in the Scheldt estuary in Belgium and the Netherlands and found $\delta^{15}NH_4^+$ values of 10 to 29‰. In both the Scheldt estuary and the Thames estuary there were mid-estuarine maxima of $\delta^{15}NH_4^+$, which coincided with a decrease in $\delta^{15}NO_3^-$. This combination was interpreted to be due to nitrification (Mariotti et al.,1984; Middelburg and Nieuwenhuize, 2001). The low range of $\delta^{15}NH_4^+$ in the Loire estuary was explained by a lack of external ammonium supply.

If we take the mean ammonium concentration in the Elbe estuary measured by the ARGE Elbe over the last eight years (ARGE, 2001, 2002, 2003, 2004, 2005a, 2005b, 2007a, 2007b) and the average $\delta^{15}NH_4^+$ values from the Tyne and the Tweed estuary (Ahad et al., 2006) for a rough calculation of δ^{15}DON in the Elbe estuary, we see a decreased signal compared to the combined $\delta^{15}DON+NH_4^+$ (Fig. 3.11), but the decrease of 0.36 ± 0.29‰ in summer and 0.21 ± 0.79‰ in winter for the $\delta^{15}NH_4^+$ data of the Tyne estuary is smaller than the standard deviation calculated by error propagation of 0.9‰ (see Section 3.2.3.4). For the $\delta^{15}NH_4^+$ data of the Tweed estuary, the values are even smaller, with a decrease of 0.27 ± 0.26‰ in summer and an increase of 0.15 ± 0.61‰ in winter.

Even if this is only a rough estimation, the influence of ammonium on the combined $\delta^{15}DON+NH_4^+$ in the Elbe estuary is likely small and lies in the range of the standard deviation of the used method.

3.4.2 Long-term changes in DON loads and ratios

How do combined $DON+NH_4^+$ concentrations in 2005/2006 compare to data available from previous studies of DON in the Elbe estuary? Fig. 3.12a presents the combined DON and ammonium concentrations measured in the Elbe estuary between Hamburg and the German Bight in different studies over the last 20 years; Fig. 3.12b plots the mean DON and the mean ammonium concentration in the same studies. $DON+NH_4^+$ concentrations were between 50 µM and almost 90 µM in samples collected in summer and autumn of 1997

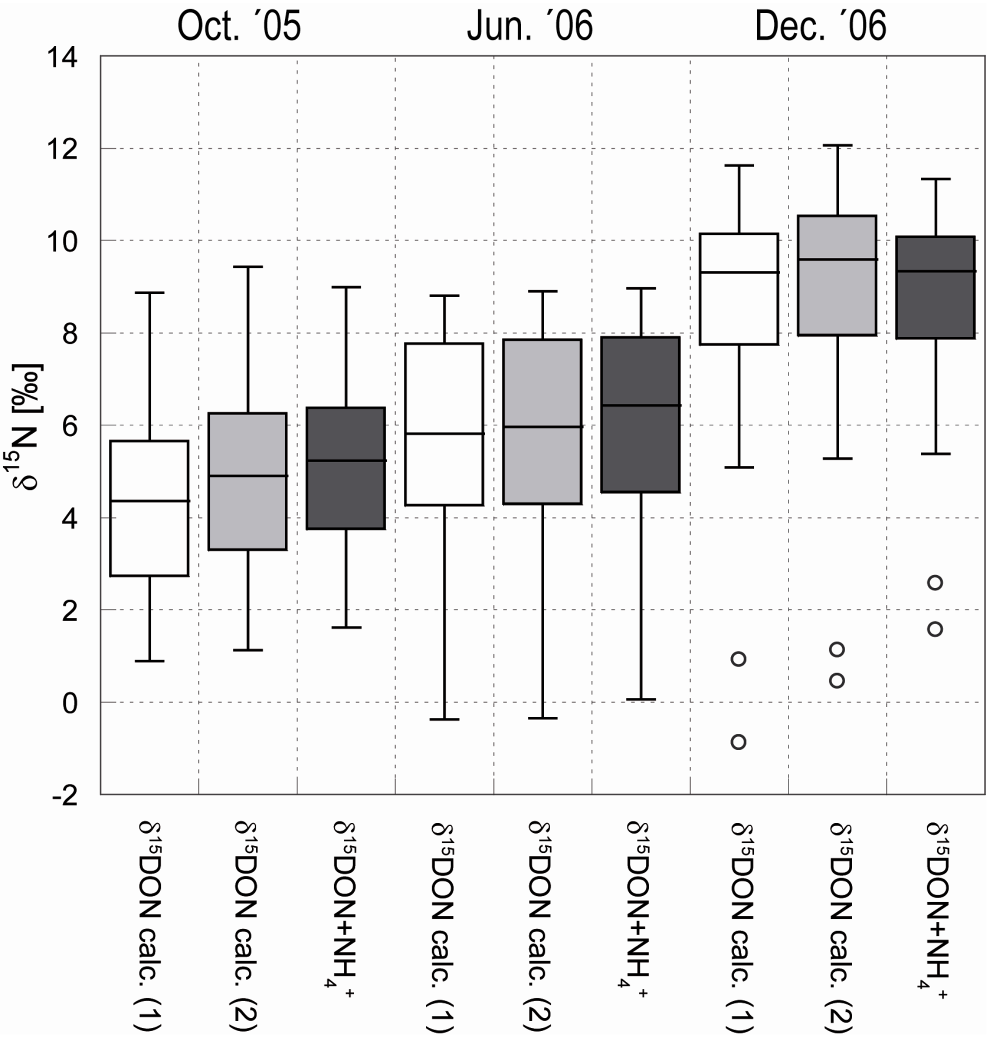


Figure 3.11: Box-and-whisker plots of calculated δ^{15}DON values in the Elbe estuary in comparison with measured δ^{15}DON+NH_4^+ values. For the calculation of mean NH_4^+ concentrations and isotopic contribution, we used data from the ARGE Elbe (ARGE Elbe, 2001, 2002, 2003, 2004, 2005a, 2005b, 2007a, 2007b) and $\delta^{15}NH_4^+$ values of the Tyne estuary (1) and the Tweed estuary (2) (Ahad et al. 2006)

(Kerner and Spitzy, 2001), which is twice the concentration determined here approximately 10 years later. But in contrast to all other studies, no samples from stations with higher salinity seawrds the TMZ have been analysed in the study of Kerner and Spitzy (2001). Earlier data on DON+NH_4^+ concentrations in the Elbe estuary are from different seasons in 1986 and 1987 (Brockmann and Pfeiffer, 1990), when much higher ammonium concentrations than today were found, typically with highest values in samples collected in spring and winter (Fig. 3.12a). That was a time when TDN and DIN transports in the Elbe peaked due to eutrophication (Radach and Paetsch, 2007). In April 1992, DON+NH_4^+ concentrations reached up to 60 µM (Brockmann, 1994). Since then, the DON+NH_4^+ load of

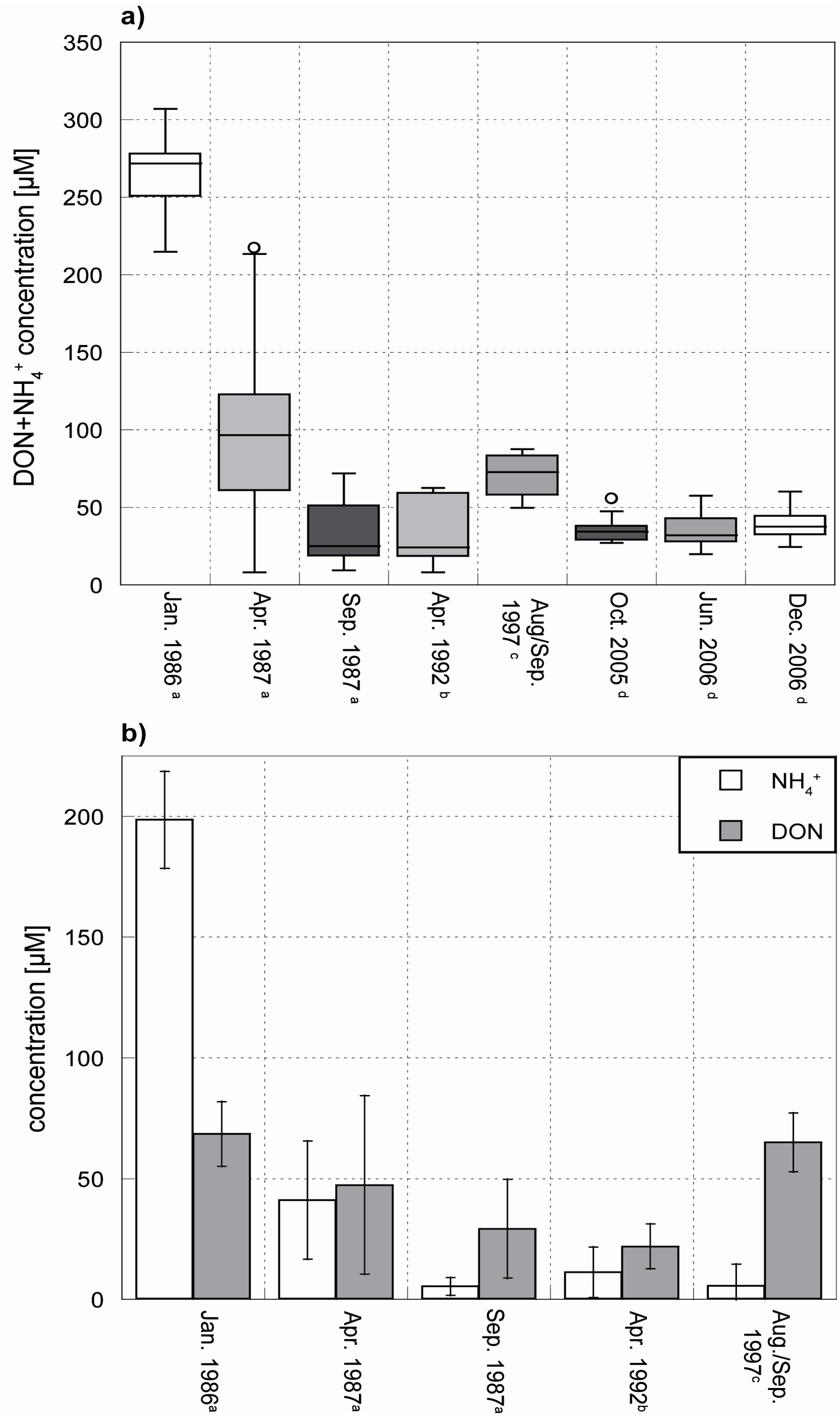


Figure 3.12: Box-and-whisker plots of data on combined DON+NH$_4^+$ concentrations (a) and mean ammonium and DON concentration (b) in the Elbe estuary. Data sources as in Fig. 3.10.

the Elbe apparently has not changed systematically, but overall DON+NH_4^+ loads have decreased from > 250 µM to < 50 µM in the period from 1986 to 1992. This decrease is due to decreased ammonium concentrations, because a comparison of the pure DON concentrations from these studies shows no systematic change in DON concentrations over the last twenty years (Fig. 3.12b). The decrease in combined DON and ammonium concentration also coincides with an intermittent, but significant decrease in TDN loads of the Elbe, accompanied by an even more drastic and lasting increase in dissolved N:P ratios (Radach and Paetsch, 2007) around 1987 due to the ban of phosphates in detergents.

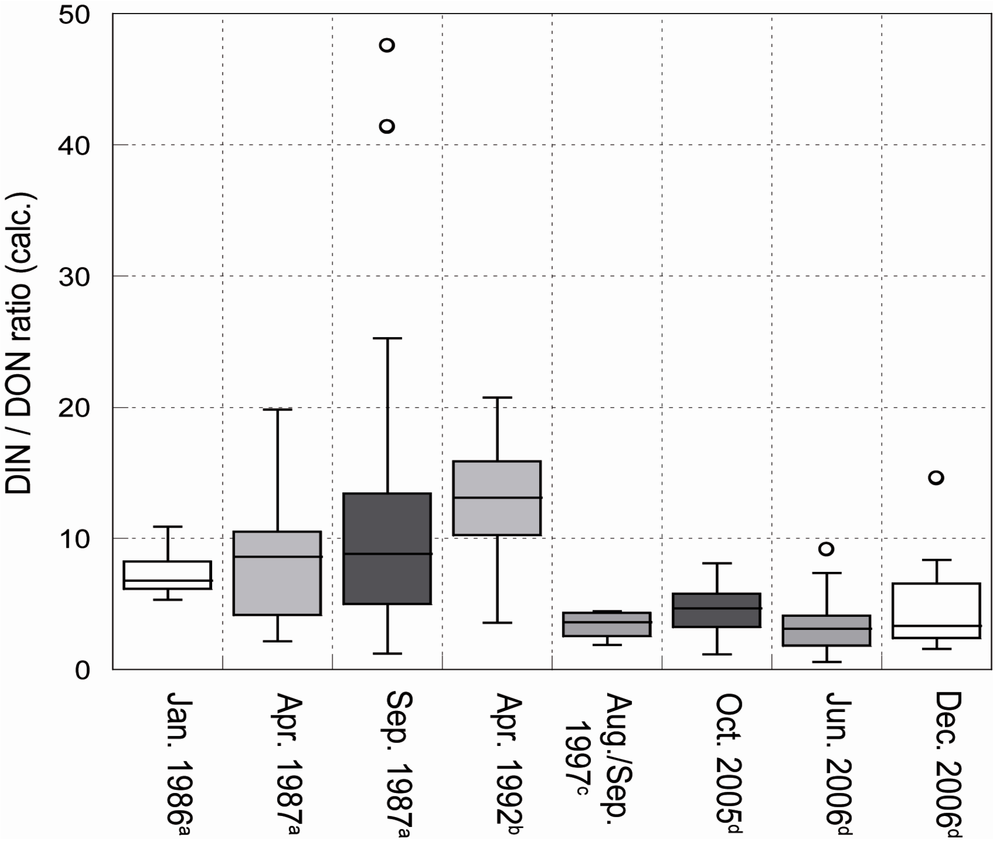


Figure 3.13: Box-and-whisker plots of calculated DIN/DON ratios in the Elbe estuary over the last two decades. Data sources as in Fig. 3.10.

A compilation of NO_3^-/DON+NH_4^+ratios along the estuary (Fig. 3.10) also indicates no systematic changes over the last two decades. The reduced nutrient input to the Elbe estuary apparently had no effect on this ratio. However, if we look on the DIN/DON ratio (Fig. 3.13, with calculated values for DON and ammonium in our study based on mean ammonium concentration of the ARGE Elbe data set), a decrease may have occurred in the last ten years compared to the 1980’s and early 1990’s. This decrease may be due to reduced nutrient input:

the ammonium concentrations in particular decreased from > 250 µM in winter months in the early 1980's to < 10 µM nowadays (ARGE, 2000). In contrast, DON concentration did not exhibit any systematic changes over the same time period (Fig 3.12b).

In April 1992 DON was determined along the entire gradient of the Elbe estuary (Brockmann, 1994): DON concentrations decreased, from 35 µM DON in the port of Hamburg to less than 10 µM in the high salinity end-member of the German Bight. The combined DON+NH_4^+ concentration decreased from 60 µM in the port of Hamburg to approximately 12 µM in the German Bight. These concentrations are in the same range as ours for the estuary, but lower for the saline water of the German Bight, which showed seasonal variability in other studies (Brockmann et al., 1999). Both previous studies (Brockmann, 1994; Brockmann and Pfeiffer, 1990) and ours found decreasing DON+NH_4^+ concentrations in the lower estuary due to dilution with marine water, consistent with conservative mixing. As we will discuss below, however, the added information from δ^{15}DON+NH_4^+ indicate a turnover of DON+NH_4^+ in the estuary.

3.4.3 Seasonality

The seasonal trend in DON+NH_4^+ concentrations and δ^{15}DON+NH_4^+ is subdued and was only evident in somewhat higher concentrations and ^{15}N enrichment in winter as compared to autumn and summer in the Elbe estuarine salinity gradient from 5 to 25. This seasonal difference (see Table 3.1) is not inherited from the upstream river, where DON+NH_4^+ has a narrow range of δ^{15}DON+NH_4^+ in samples < stream kilometre 600 (Fig. 3.6). Likewise, significant seasonal variability in δ^{15}PN was only observed in the TMZ, with very low values in June 2006 (Fig. 3.8), which we will address in more detail later.

3.4.4 DON turnover in the Elbe estuary

Nitrate and DON+NH_4^+ behave differently in the estuary of Elbe River. Nitrate clearly increased in the lower estuary and mixed conservatively at increasing salinity in the years 2006 and 2007 (Figs. 3.5 and 3.7). In contrast, it is hard to classify the mixing pattern of DON+NH_4^+ for both mass and isotopic composition. While the overall pattern downstream of a salinity of ~10 may be seen as mostly conservative, gradients in the upper estuary indicate non-conservative behaviour and further pinpoint the TMZ of the Elbe estuary as both a sink and a source for $DON+NH_4^+$.

We detected two zones with maximum $DON+NH_4^+$ concentrations in the Elbe estuary (Fig. 3.4): one lies in the port of Hamburg, which is best explained by input from sewage treatment plants or direct inputs (and high local ammonium concentrations). In the port area, the ammonium concentration accounts for approximately 20% of measured $DON+NH_4^+$ concentrations (ARGE, 2007a; ARGE, 2007b) and thus is expected to have an effect on the $\delta^{15}DON+NH_4^+$ determined by our method. These assumptions are confirmed by isotopic composition, since we also observed an increase of $\delta^{15}DON+NH_4^+$ in the port of Hamburg (Fig. 3.6), which is an indicator for waste water nitrogen input (McClelland and Valiela, 1998). Another mechanism may be responsible for the decrease in PN isotope values and the according increase in $\delta^{15}DON+NH_4^+$: Badr et al (2008) found that in summer, release of DON from bacterioplankton may account for a significant fraction of DON in the Plym estuary in southwest England, Europe. The most pronounced shift in isotope values of both $DON+NH_4^+$ and PN in the port of Hamburg occurred in June 2006, and the observed increase of $\delta^{15}DON+NH_4^+$ and drop in $\delta^{15}PN$ is in accord with DON turnover. $\delta^{15}PN$ increased again further down the estuary, which can be assigned to progressive ammonium uptake and isotope fractionation (Middelburg and Herman, 2007). The other zone with a distinct maximum in $DON+NH_4^+$ concentration was found seaward of the TMZ, at the upper limit of brackish water (Bergemann, 1995).

The role of the turbidity maximum zone

Intriguingly, the landward head of the TMZ consistently coincided with a distinct minimum of $DON+NH_4^+$ concentrations and $\delta^{15}DON+NH_4^+$, coincident with sharply rising DIN concentrations and a drop in $\delta^{15}N\text{-}NO_3^-$ of nitrate (Dähnke et al., 2008). Seaward of the TMZ (from stream km 700), both $DON+NH_4^+$ concentrations and $\delta^{15}DON+NH_4^+$ increased again.

Main potential sinks for DON are uptake by phytoplankton or heterotrophic bacteria (Bronk et al., 2007; Veuger et al., 2004), adsorption to particles (Berman and Bronk, 2003), conversion to ammonium followed by nitrification (Kerner and Spitzy, 2001), and photochemical decomposition. Given that the main sink appears to be coupled to the TMZ, where significant photochemical reactions are unlikely, we regard the latter as an improbable explanation for the removal of DON. Likewise, a parallel decrease of concentrations and isotope values, as we observed it regardless of season at the head of the TMZ, is difficult to explain by assimilation, as it is usually selective in favour of the light isotope. In addition, nitrate is abundant in the estuarine waters and should preclude DON assimilation by phytoplankton.

While the quantitative role of DON as a nitrogen source for autotrophs is debated (Bronk et al., 2007), potential mechanisms of utilization, such as bacterial ammonification and subsequent phytoplankton uptake of the released nitrogen (Antia et al., 1991; Berman et al., 1991), are indicated for many estuarine settings. In a microcosm study on turnover of low- (LMW-DON) and high-molecular weight DON (HMW-DON) in waters from the Elbe estuary, LMW-DON was preferentially removed and nitrified over HMW-DON (Kerner and Spitzy, 2001). However, the concentration changes in either DON compartment associated with this conversion were small, and Kerner and Spitzy (2001) proposed that particulate N is the source of ammonium that is immediately nitrified in the particle-rich environment of the Elbe River TMZ. Support came from a correlation between the nitrification rate and particulate matter content observed in their study, and was explained by the large numbers of nitrifiers associated with the suspended matter. They conclude that heterotrophic degradation of PN and chemoautotrophic processes must be intimately coupled in the particle-rich turbidity zone to cover the N-demand for nitrification on the suspended aggregates.

But why would concentrations and δ^{15}DON+NH_4^+ increase seaward of the TMZ? A likely candidate process should remove DON in the inner Elbe estuary and liberate it again at higher salinity to restore concentrations and isotopic composition. Such a process may be abiotic and may be associated with flocculation and sorption to either detrital particles or high-molecular weight DOC. This occurs at the head of many estuarine salinity gradients and often coincides with the estuarine turbidity maximum (Eisma, 1986; Kranck, 1984), followed by desorption at higher salinity. The DON+NH_4^+ concentration minimum in the Elbe TMZ coincides with a minimum in δ^{15}DON+NH_4^+ in our samples, and both increase rapidly at the saline tail of the TMZ (Fig. 6). Previously, a mechanism which involves adsorption/desorption as a control on DON concentrations in the Delaware Estuary has been proposed (Mannino and Harvey, 1999). By analogy, one possible mechanism for the observed change in DON+NH_4^+ concentrations and δ^{15}DON+NH_4^+ may thus be the selective absorption onto particles in suspension of ^{15}N- enriched DON moieties, which are removed from the dissolved fraction. Although δ^{15}PN is constant in the TMZ (where adsorption is indicated) and in the upper limit of brackish water (where desorption would take place), this may be due to the fact that PN and the adsorbed DON are isotopically similar and the adsorbed mass of DON is small compared to the mass of the particles. A release of particle-bound ammonium with increasing salinity has been found in sediments (Seitzinger et al., 1991), which supports this hypothesis. In addition, the independence of season also argues for a non-biological source of DON or ammonium in the case of the Elbe TMZ at its saline tail. That the δ^{15}DON+NH_4^+ recovered to that of PN at a salinity of 7 to 10 further implies that particle-adsorbed DON may be an important source of dissolved organic nitrogen to the water column in the outer Elbe estuary.

3.5 Summary and Conclusions

Differing from nitrate, DON+NH_4^+ does not behave entirely conservatively in the Elbe estuary. The city of Hamburg and its port in the freshwater interval (salinity <0.2) are a clear source of DON and/or ammonium, possibly due to input from direct discharges, local biogenic sources or sewage treatment plants. While mixing appears to be mostly conservative in the mid- to lower estuary (salinities >10), we see clear signals of input an active cycling in the upper part of the estuary at salinities between 2 and 5. The decrease in DON+NH_4^+ concentration at the freshwater head of the estuarine turbidity maximum zone is best explained by sorption of DON and/or ammonium onto particles in the particle rich environment. Desorption at the salty tail of the TMZ restores concentrations and δ^{15}DON+NH_4^+ to values seen upstream. Seasonal variations in DON+NH_4^+ and PN concentration and δ^{15}DON+NH_4^+ and δ^{15}PN were only small and not significant. To our knowledge, this is the first dataset on the estuarine mixing pattern of δ^{15}DON+NH_4^+ in seasonal resolution. Even though the interpretation is hampered by the lack of data on N-cycling in the TMZ, the main conclusion is that most turnover of DON+NH_4^+ takes place in the TMZ, just as it has been found for the turnover of nitrate.

Acknowledgements

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4. Dissolved and particulate reactive nitrogen in the Elbe River/NW Europe: a 2-year N-isotope study

Abstract

Rivers collect and transport reactive nitrogen to coastal seas as nitrate, ammonium, dissolved organic nitrogen (DON), or particulate nitrogen. DON is an important component of reactive nitrogen in rivers and is suspected to contribute to coastal eutrophication, but little is known about seasonality of DON loads and turnover within rivers. We measured the concentrations and the isotope ratios $^{15}N/^{14}N$ of combined $DON+NH_4^+$ ($\delta^{15}DON+NH_4^+$), nitrate ($\delta^{15}N\text{-}NO_3^-$) and particulate nitrogen ($\delta^{15}PN$) in the non-tidal Elbe River (SE North Sea, NW Europe) over a period of 2 years (June 2005 to December 2007) at monthly resolution. Combined $DON+NH_4^+$ concentrations ranged from 22 to 75 µM and comprised nearly 23% of total dissolved nitrogen in the Elbe River in annual mean; PN and nitrate concentrations ranged from 11 to 127 µM, and 33 to 422 µM, respectively. Combined PN and $DON+NH_4^+$ concentrations were, to a first approximation, inversely correlated to nitrate concentrations. $\delta^{15}DON+NH_4^+$, which varied between from 0.8‰ to 11.5‰, changed in parallel to $\delta^{15}PN$ (range 6 to 10‰), and both were anti-correlated to $\delta^{15}N\text{-}NO_3^-$ (range 6 to 23‰). Seasonal patterns of $DON+NH_4^+$ concentrations and $\delta^{15}DON+NH_4^+$ diverge from those expected from biological $DON+NH_4^+$ production in the river alone and suggest that the elution of organic fertilisers significantly affects the $DON+NH_4^+$ pool in the Elbe River.

Key words: Dissolved organic nitrogen, nitrate, stable isotopes, river, seasonality, particulate nitrogen

4.1 Introduction

Dissolved organic nitrogen (DON) is a major contributor to total dissolved nitrogen (TDN, the sum of nitrate, nitrite, ammonium and DON) discharged from land to the coastal ocean. Meybeck (1993) estimated that nearly 70 % of the nitrogen entering coastal regions via rivers is in the form of DON, and highest relative DON contributions characterize TDN loads of unpolluted rivers (Wiegner et al., 2006). But although DON comprises a smaller fraction than nitrate of the total N load in many eutrophied rivers, anthropogenic sources may significantly increase the natural DON background (Agedah et al., 2009; Howarth, 2004) up to a point where anthropogenic sources exceed wetland-derived DON in low-N streams (Stanley and Maxted, 2008). DON is thought to be an inert pool of heterogeneous composition that is not a relevant N-source for freshwater and estuarine ecosystems (Williams and Druffel, 1987), but recent work suggests that labile fractions of DON are selectively turned over in estuaries (Schlarbaum et al., 2010). When discharged to coastal seas, DON may substitute for dissolved inorganic nitrogen (DIN) as a substrate for phytoplankton assimilation. A substantially larger fraction of DON is assimilated by marine than by freshwater bacterioplankton; the susceptibility of DON to mineralization by bacteria appears to increase with increasing salinity during transport from fresh to marine water (Stepanauskas et al., 1999a, 1999b). In seasons when the inorganic N pools are exhausted DON may in particular promote harmful algal blooms (Bronk, 2002; Bronk et al., 2007).

The lack of knowledge on DON sources and turnover in rivers, as well as the potential influence of coastal ecosystems near river discharge areas motivated our investigation into DON dynamics in the Elbe river, possible seasonality of DON discharge into the adjacent estuary and coastal sea, and use of isotopic indicators for origin, as well as for possible sources and sinks of DON in the river.

As a sequel to a study on combined DON and ammonium dynamics in the Elbe estuary (Schlarbaum et al., 2010), we here present a data set on concentrations and the $^{15}N/^{14}N$ composition of combined DON and ammonium (DON+NH_4^+), particulate nitrogen (PN), and nitrate (expressed as the δ value in ‰ = $[(R_{sample}/R_{standard})-1]*1000$, R = $^{15}N/^{14}N$ in DON+NH_4^+, nitrate, or PN, and in the international standard atmospheric dinitrogen) of the Elbe River. The first objective of this study is to investigate the seasonal pattern of combined DON and ammonium in the river that drains an intensely farmed (70% agriculture) catchment of 148,268 km^2 in central Europe, and in which policy measures, such as a ban on organic fertilizers from beginning of November to the end of January (DüV, 2009), potentially impose an external rhythm on possible external DON and ammonium sources. Secondly, we were interested in links between the DON and PN pools, which both may be products of phytoplankton assimilation of the dissolved inorganic nitrogen (DIN) load, or - in the case of DON - may originate from dissimilation of PN within the river. The data set is of monthly resolution and permits us to assess seasonal variations depending on internal cycling or external inputs, and turnover of reactive N between different pools.

4.2 Materials and Methods

4.2.1 Study site

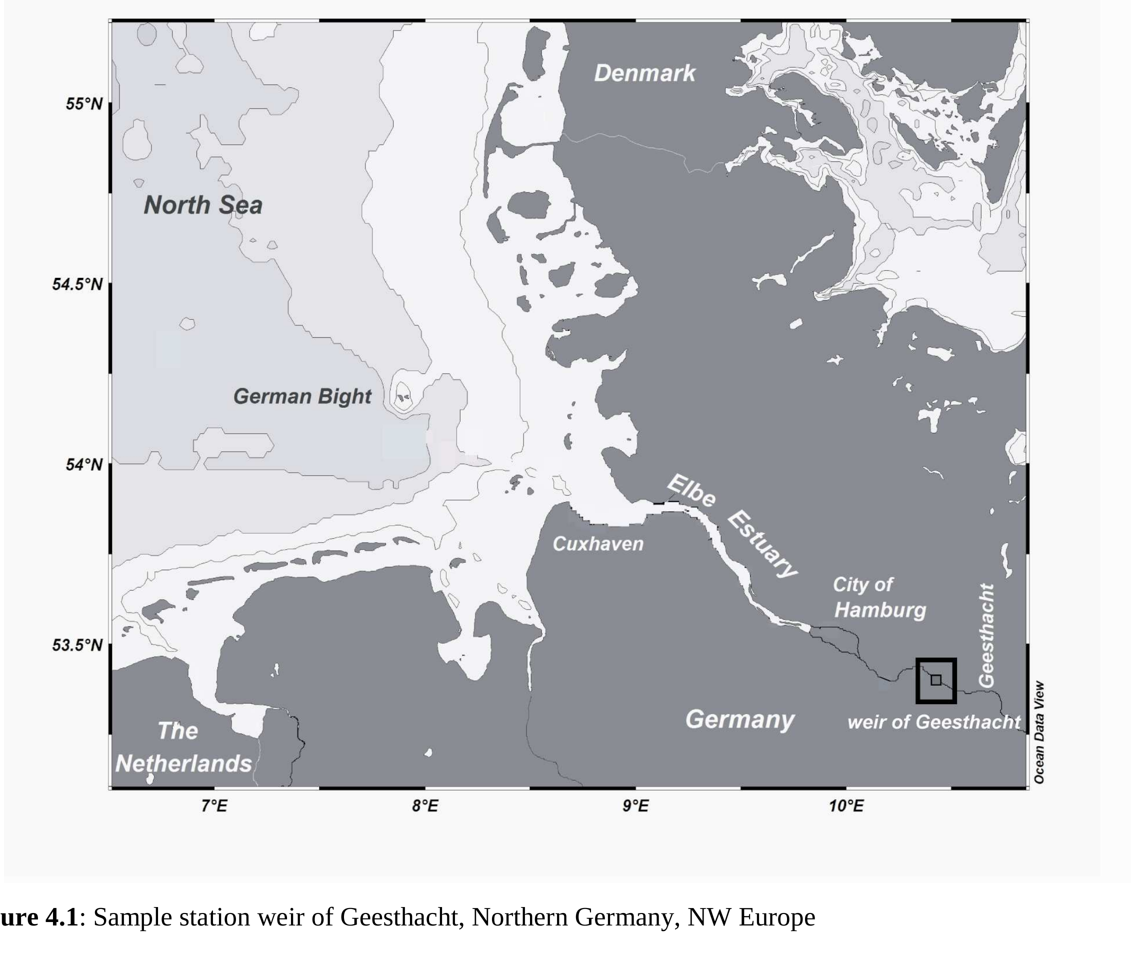


Figure 4.1: Sample station weir of Geesthacht, Northern Germany, NW Europe

The Elbe River is 1094 km long and one of the largest rivers in Germany discharging into the North Sea. The weir at Geesthacht (built 1957 -1959 AD) at stream kilometre 585 is the only barrage along the Elbe River and separates a tidal estuary from the upstream river system (Fig. 4.1). The average fresh water discharge at the weir Geesthacht is 700 m³/s, and can rise to 4000 m³/s during floods; nearly 25 million people live in the entire catchment area of 148,268 km^2 (Behrendt et al., 2004). The Elbe River is the largest nutrient source of the German Bight (Brockmann and Pfeiffer, 1990), which is severely affected by eutrophication (Osparcom, 2008). In 2007, the Elbe discharged 87 kt reactive N (85% nitrate, 15% DON+NH_4^+ + PN) into the estuary (Arge, 2008).

4.2.2 Sampling

From June 2005 to December 2007, monthly water samples were collected at the weir using a Ruttner sampler. Initially, samples were collected at two different water depths (0.5m and 3m), which was reduced to only 1 sample from 1-2 m after initial analyses showed no significant difference between the two depths. The river water was immediately filtered through precombusted GF/F filters and stored in PE bottles that had been soaked in acid overnight and rinsed with deionised water (DIW). Each bottle was rinsed with sample water before being filled. Water samples were frozen at -18°C until analysis. Filters were dried at 60-70°C and stored at 4°C before analyses.

4.2.3 Methods

Glassware was washed with deionised water (DIW), soaked in soap and 10% HCl baths and washed again with DIW after each single step. All non-volumetric glassware was combusted at 450°C for at least 4 hours, volumetric glassware and PE-bottles were washed with DIW, soaked in 10% HCl bath overnight, washed again with DIW and dried at 50°C. Concentration of total dissolved nitrogen (TDN) in DIW was determined using the “persulfate oxidation method” (Solórzano and Sharp, 1980; Koroleff, 1976; Knapp et al., 2005) (see below) with a blank of < 1µmol/l.

4.2.3.1 Concentrations and δ^{15}N analysis of Nitrate and Nitrite

Concentrations of nitrate and nitrite were measured with standard colorimetric techniques (Grasshoff and Anderson, 1999) on an AutoAnalyzer3 by Bran & Luebbe. Concentration of nitrite in the water samples was negligible (below 2% of the nitrate concentration) at a detection limit of 0.05 µM. δ^{15}N-NO_3^- of nitrate was analysed by using the “denitrifier method” (Sigman et al., 2001; Casciotti et al., 2002). Nitrate was quantitatively reduced to nitrous oxide (N_2O) by using a strain of denitrifier bacteria that lacks N_2O reductase activity. N_2O was automatically extracted, purified in a Gasbench (ThermoFinnigan) and analysed on a Finnigan Delta plus XP mass spectrometer. The sample size was adjusted to 20 nmol nitrate in each sample. The samples were referenced to injections of N_2O from a pure N_2O gas cylinder and then standardised using an internationally accepted nitrate isotopic reference material (IAEA-N3, $\delta^{15}N$ = 4.7‰). We used an internal potassium nitrate standard for further validation of our results, which we measured with each batch of samples. The standard deviation for replicate analyses (n = 4) was ± 0.2‰. The method also permits determination of $\delta^{18}O\text{-}NO_3^-$ (Sigman et al., 2001; Casciotti et al., 2002; Dähnke et al., 2008), which is referenced to IAEA-N3 with a $\delta^{18}O\text{-}NO_3^-$ of 22.7‰ versus Vienna Standard Mean Ocean Water (VSMOW) (Böhlke et al., 2003). We note that this value has recently been corrected and is now reported as 25.6‰, but decided to use the formerly assigned value for a better comparison with previous studies (Johannsen et al., 2008; Schlarbaum et al., 2010).

4.2.3.2 Concentration and $\delta^{15}N$ analysis of TDN

To determine the TDN concentration and δ^{15}TDN we used the method of Knapp et al. (2005), with small modifications as described in Schlarbaum et al. (2010). In brief, total dissolved nitrogen is oxidized to nitrate using the “persulfate oxidation method” (Solórzano and Sharp, 1980; Koroleff, 1976; Knapp et al., 2005). Concentration of TDN was determined as nitrate after oxidation by the method described above.

For the determination of δ^{15}TDN nitrate in oxidised samples and reagent blanks was converted to N_2O using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002) as described above.

4.2.3.3 Concentration and $\delta^{15}N$ analysis of combined $DON+NH_4^+$

Concentration of combined $DON+NH_4^+$ was calculated by the difference between TDN and nitrate, because concentration of nitrite was consistently negligible.

For the $\delta^{15}DON+NH_4^+$ mass balance calculations were made using the measured nitrate concentrations and $\delta^{15}N$ values of the oxidised sample, the reagent blank and the unoxidised sample:

$$\delta^{15} DON + NH_4^+ = \frac{\delta^{15}TDN * c(TDN)}{c(DON + NH_4^+)} - \left[\frac{\left(\delta^{15}N - NO_3^- * c(NO_3^-)\right)}{c(DON + NH_4^+)} + \frac{\left(\delta^{15}Blank * c(Blank)\right)}{c(DON + NH_4^+)} \right]$$

(Eq. 4.1)

The combination of persulfate digestion and denitrifier method to measure δ^{15}DON+NH$_4^+$ has been tested by oxidation of urea standard solutions with a concentration range of 10 to 400 µM. The δ^{15}N of solid urea was measured by using a Flash EA 1112 elemental analyzer coupled to a Finnigan Delta plus XP mass spectrometer, yielding a δ^{15}N value of 0.5‰ ± 0.2‰. Measurements of the urea solutions after preparation as above yielded in δ^{15}N values of 0.4‰ ± 0.2‰ after blank correction.

Repeated measurements of the same water sample demonstrated the reproducibility of the δ^{15}N method for δ^{15}DON+NH$_4^+$ analyses. The mean measured standard deviation of δ^{15}TDN and δ^{15}N-NO$_3^-$ was 0.2‰ (3 to 4 repetitions). The mean standard deviation for TDN and nitrate concentration was 1 µM. Because of error propagation, the calculated standard deviation of δ^{15}DON+NH$_4^+$ ranged from 0.1 to 2.8‰ with a mean value of 1.2‰, and the calculated mean standard deviation for combined DON+NH$_4^+$ concentration was 2.2 µM.

The method does not separate DON and ammonium. However, in comparison with DON concentration, ammonium concentrations were near to or below detection limit (< 2.9 µM) except for samples taken during winter seasons and in June 2007. During winter seasons, ammonium concentrations occasionally increased up to 34 µM (February 2006), equalling 50 % of the combined DON+NH$_4^+$ loads; in June 2007, ammonium accounted for approximately 10% of combined DON+NH$_4^+$.

4.2.3.4 Concentration and δ^{15}N analysis of PN

Particulate nitrogen was sampled by filtering the water samples through precombusted (6 hours, 450°C) and tared GF/F filters. After filtration the filters were dried at 60°C and stored dark at 4°C until analysed. The weight of particulate matter on the filters was determined, and C and N weight % were analysed by using a Flash EA 1112 elemental analyzer. The δ^{15}PN was analysed with a Flash EA 1112 elemental analyzer coupled to a Finnigan Delta plus XP mass spectrometer. Results were standardised using the internationally accepted isotopic reference materials “High organic sediment standard OAS”

(Cat.no. B2151, Batch no. 2824, $\delta^{15}N$ = + 4.4‰ ± 0.19‰), “Low Organic Content Soil Standard OAS” (Cat.no. B2153, Batch no. 2822, $\delta^{15}N$ = + 6.7‰ ± 0.15‰) and IAEA-N1 ($\delta^{15}N$ = + 0.4‰). The standard deviation for replicate analysis was 0.2‰ (3 replicates).

4.2.4 Annual and seasonal loads and load-weighted isotopic values

For an estimate of the mass loads of $DON+NH_4^+$, TDN, PN, and nitrate and the average N-isotope composition of these compounds, we used our analytical data and the discharge rates of the sampling dates to calculate annual loads as:

$$L = \sum \left| J_i \right| * c_i * flow_i \qquad \text{(Eq. 4.2)}$$

The entire time interval J of 12 months for annual calculations (6 months for seasonal calculations) was divided in *n* sampling intervals with the duration $|J_i|$, the concentration c_i and the discharge $flow_i$. The annual load L is the sum of the single loads in the sampling intervals J_i (Hebbel and Steuer, 2006; Johannsen et al., 2008).

For the calculation of the load-weighted annual average isotope composition, the isotope values were multiplied with the respective concentration and weighted with the loads according to the formulas:

$$\delta^{15}N_{wml} = \frac{\sum \delta^{15}N_i * c_i * flow_i}{\sum c_i * flow_i} \qquad \text{(Eq. 4.3)}$$

$$\delta^{18}O_{wml} = \frac{\sum \delta^{18}O_i * c_i * flow_i}{\sum c_i * flow_i} \qquad \text{(Eq. 4.4)}$$

where $\delta^{15}N_{wml}$ and $\delta^{18}O_{wml}$ are the load-weighted annual isotope values, $\delta^{15}N_i$ and $\delta^{18}O_i$ are the measured isotope values of individual samples, c_i is the respective concentration, and $flow_i$ the discharge flow.

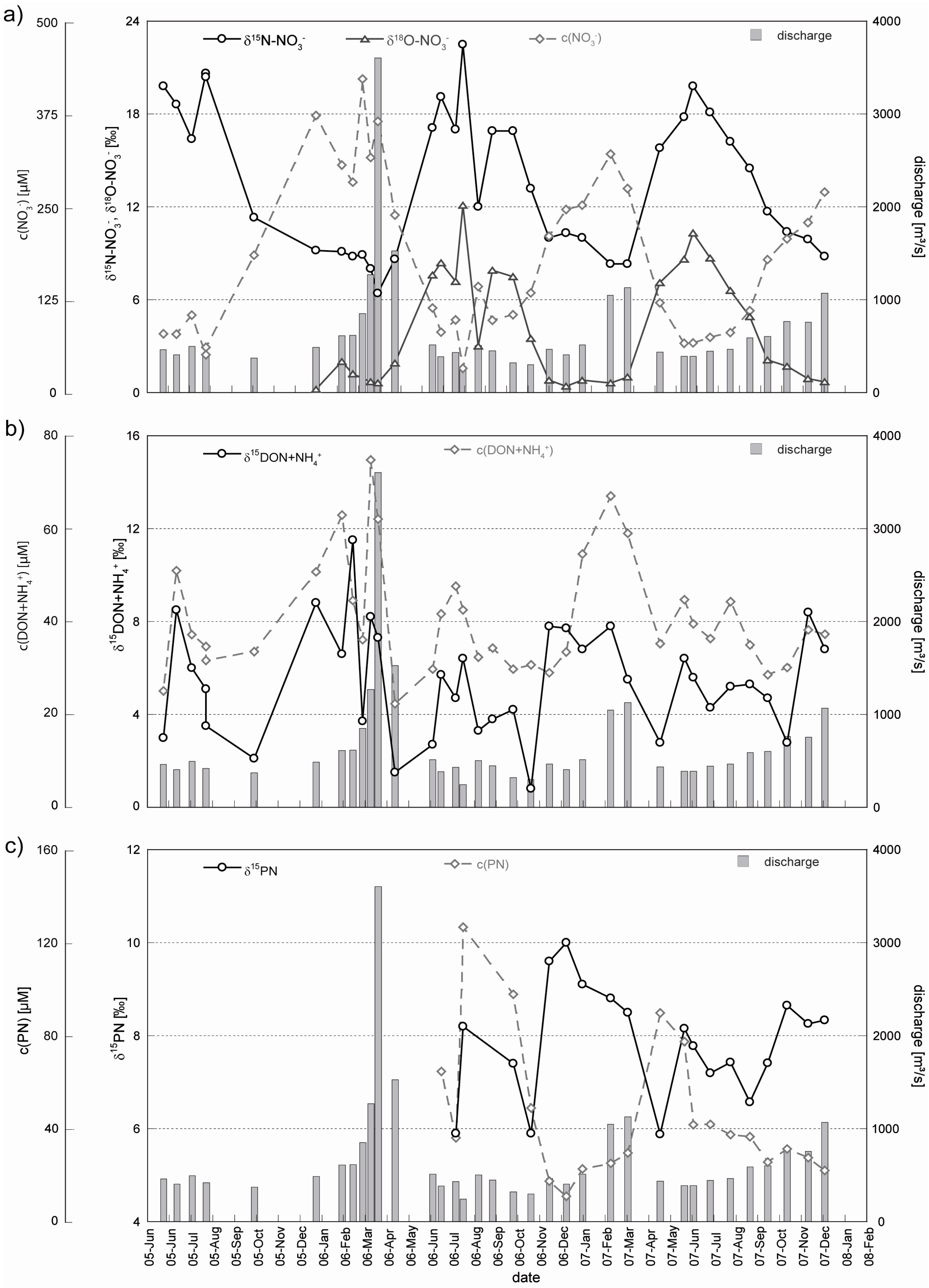


Figure 4.2: concentration and isotopic values in the Elbe River at the weir of Geesthacht, June 2005 – December 2007, bars represent the river discharge. Note the different scales of the y-axes. a) Nitrate concentration, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in the Elbe River. Data from 2006 have been published in Johannsen et al. (2008). b) combined $DON+NH_4^+$ concentration and $\delta^{15}DON+NH_4^+$. c) PN concentration and $\delta^{15}PN$

4.3 Results

The analytical results from sampling in the period June 2005 to December 2007 are plotted in Fig. 4.2. Water discharge for the sampling dates were plotted as bars and show an exceptional spring flood in April 2006. In the first 6 months of sampling, we determined only concentrations and δ^{15}N of nitrate and DON+NH_4^+; the last two years, measurements included also δ^{18}O-NO_3^-, and concentrations and δ^{15}PN (from July 2006 to December 2007).

4.3.1 Nitrate concentrations and isotopic compositions

Throughout the entire sampling period covered in our current study, nitrate displays a clear seasonal trend in concentrations and isotopic composition. δ^{15}N-NO_3^- and δ^{18}O-NO_3^- were both enriched during summer months (maxima 23‰ and 12‰, respectively) and were both depleted during winter times (minima 6‰ and < 1‰, respectively) (Fig. 4.2a) and are anti-correlated ($r^2 = 0.84$, $r = - 0.92$, $\alpha \leq 0.01$) (Table 4.1) to nitrate concentrations (min. 30 µM in summer seasons, max. 420 µM in winter seasons). δ^{15}N-NO_3^- and δ^{18}O-NO_3^- varied almost parallel and are strongly correlated ($r^2 = 0.96$, $r = 0.98$, $\alpha \leq 0.01$, Table 4.1) to each other. A plot of δ^{18}O vs. δ^{15}N shows that the isotope values plot a slope of 0.81 : 1, which is close to a 1 : 1 slope (Fig. 4.3). Nitrate concentration and isotopic composition from January 2006 to December 2006 are also published in Johannsen et al., 2008.

r^2 \ r	c(NO_3^-)	c(TDN)	c(DON+NH_4^+)	c(PN)	δ^{15}N-NO_3^-	δ^{18}O-NO_3^-	δ^{15}TDN	δ^{15}DON+NH_4^+	δ^{15}PN
c(NO_3^-)		0.995	0.462	-0.674	-0.918	-0.897	-0.902	0.369	0.584
c(TDN)	**0.991**		0.547	-0.656	-0.907	-0.868	-0.898	0.414	0.579
c(DON+NH_4^+)	**0.214**	**0.299**		-0.104	-0.359	-0.278	-0.424	0.592	0.193
c(PN)	**0.454**	**0.431**	**0.011**		0.745	0.770	0.655	-0.296	-0.375
δ^{15}N-NO_3^-	**0.843**	**0.822**	**0.129**	**0.555**		0.977	0.964	-0.287	-0.487
δ^{18}O-NO_3^-	**0.805**	**0.754**	**0.077**	**0.593**	**0.955**		0.916	-0.380	-0.496
δ^{15}TDN	**0.813**	**0.807**	**0.179**	**0.429**	**0.930**	**0.840**		-0.275	-0.552
δ^{15}DON+NH_4^+	**0.136**	**0.171**	**0.351**	**0.088**	**0.082**	**0.144**	**0.076**		0.726
δ^{15}PN	**0.341**	**0.336**	**0.037**	**0.141**	**0.237**	**0.246**	**0.305**	**0.528**	

Table 4.1: Correlation coefficients of all measured parameters in the Elbe River at the weir of Geesthacht, June 2005 – December 2007. r^2 in bold, underlined coefficients present a level of significance $\alpha \leq 0.01$.

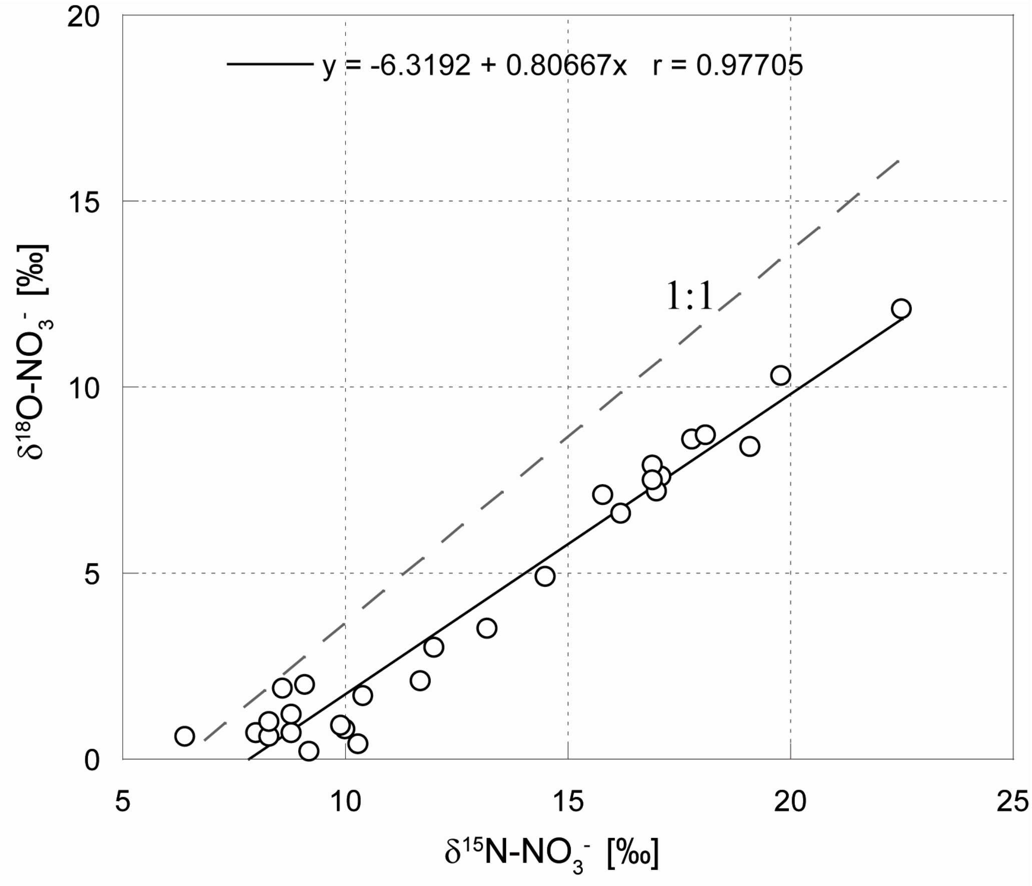


Figure 4.3: Relationship between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ to examine the fractionation factor ratio $\varepsilon^{15}/\varepsilon^{18}$, dashed line represents a 1:1 ratio

4.3.2 DON+NH_4^+ concentrations and isotopic compositions

DON+NH_4^+ concentrations also showed a distinct seasonality: Concentrations varied between 22 µM and 76 µM, δ^{15}DON+NH_4^+ ranged from 1‰ in November 2006 to 12‰ in March 2006 (Fig. 4.2b). We found two distinct maxima in both concentration and δ^{15}DON+NH_4^+ per year. The first maximum with higher values in both concentration and δ^{15}DON+NH_4^+ appeared in winter months (from December to March), the second in summer; minima occurred during spring and autumn seasons. Unlike nitrate, δ^{15}DON+NH_4^+ and DON+NH_4^+ concentration are only loosely correlated ($r^2 = 0.35$, $r = 0.59$, $\alpha \leq 0.01$, Table 4.1).

4.3.3 DON+NH_4^+/TDN ratios

The DON+NH_4^+ contribution to TDN differs through the seasons, with an annual average of DON+NH_4^+/TDN of 23%. The highest DON+NH_4^+/TDN ratio occurred in August

2006 (57%), the lowest in March 2006 (8%) (Table 4.2). On average, the DON+NH_4^+/TDN ratio in summer is about twice as high as in seasons with less biologic activity (33% versus 15% in winter, 18% in spring and 17% in autumn).

	c(NO_3^-) [µM]	c(TDN) [µM]	c(DON+NH_4^+) [µM]	c(PN) [µM]	c(DON+NH_4^+) ratio [%]	δ^{15}N-NO_3^- [‰]	δ^{18}O-NO_3^- [‰]	δ^{15}TDN [‰]	δ^{15}DON+NH_4^+ [‰]	δ^{15}PN [‰]
summer half-year (Apr-Sep)	112 ± 76	150 ± 78	38 ± 9	57 ± 32	29.1 ± 11.4	16.5 ± 4.1	6.5 ± 3.3	12.8 ± 2.3	4.8 ± 1.7	7.2 ± 0.9
winter half-year (Oct-Mar)	258 ± 82	303 ± 91	45 ± 15	33 ± 25	15.1 ± 3.6	10.1 ± 2.2	1.6 ± 1.9	9.5 ± 1.5	6.2 ± 2.8	8.5 ± 1.1
winter (Dec-Feb)	295 ± 48	346 ± 56	51 ± 14	20 ± 6	14.7 ± 2.9	9.3 ± 0.7	0.8 ± 0.6	9.0 ± 0.7	7.4 ± 0.9	9.1 ± 0.7
spring (Mar-May)	261 ± 119	308 ± 125	47 ± 17	66 ± 32	18.0 ± 10.3	10.3 ± 4.1	3.0 ± 3.4	9.3 ± 2.4	5.9 ± 3.2	7.5 ± 1.4
summer (Jun-Aug)	84 ± 28	122 ± 28	38 ± 7	55 ± 33	32.6 ± 9.8	18.0 ± 2.7	7.6 ± 2.7	13.7 ± 1.3	4.9 ± 1.6	7.2 ± 0.8
autumn (Sep-Nov)	168 ± 50	200 ± 51	32 ± 3	41 ± 30	16.9 ± 5.0	12.5 ± 2.9	3.5 ± 3.0	11.0 ± 1.8	4.3 ± 2.6	7.9 ± 1.3
annual mean	177 ± 107	218 ± 113	41 ± 12	46 ± 30	22.9 ± 11.2	13.6 ± 4.6	4.1 ± 3.7	11.3 ± 2.6	5.4 ± 2.4	7.8 ± 1.2
Max	422	458	75	127	56.6	22.5	12.1	15.8	11.5	10.0
Min	33	75	22	11	7.9	6.4	0.2	6.5	0.8	5.9

Table 4.2: Annual and seasonal mean concentrations and mean isotope values of nitrate, TDN, DON+NH_4^+ and PN, and annual and seasonal mean DON+NH_4^+ ratio in the Elbe River at the weir of Geesthacht, June 2005 - December 2007

4.3.4 Particulate nitrogen

Concentrations of PN had no clear seasonal trend, and δ^{15}PN fluctuated in a small range of 6 to 10‰ (Fig. 4.2c). In general, higher concentrations and lower δ^{15}PN values were measured in spring and summer seasons (55.0 – 65.6 µM, 7.2 – 7.5‰), while lower concentrations (20.2 – 41.4 µM) and higher δ^{15}PN values (7.9 – 9.1‰) were detected in samples from winter and autumn seasons. PN thus shows a similar seasonal cycle as nitrate, but varies in a considerably smaller range. In contrast to nitrate, no significant anti-correlation between δ^{15}PN and PN concentration was evident ($r^2 = 0.14$, $r = -0.30$, $\alpha > 0.05$, Table 4.1). δ^{15}PN is higher than but closely tracks δ^{15}DON+NH_4^+ (Fig. 4.4), with a correlation of $r^2 = 0.53$ ($r = 0.73$, $\alpha \leq 0.01$) between these two parameters (Fig. 4.5).

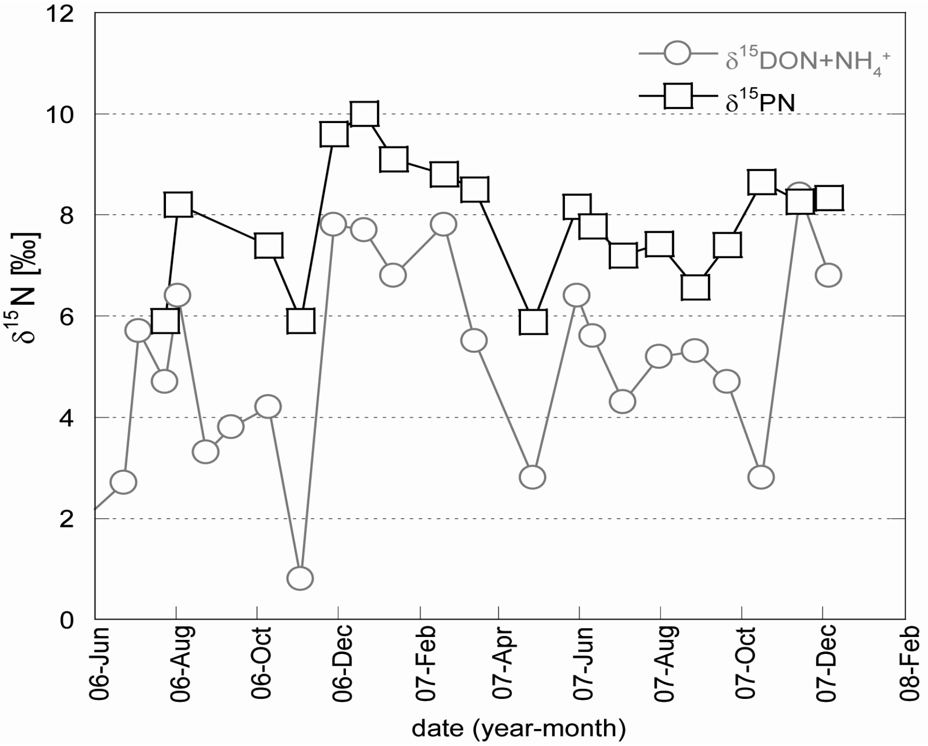


Figure 4.4: δ^{15}DON+NH$_4^+$and δ^{15}PN in the Elbe River at the weir of Geesthacht, June 2006 – December 2007

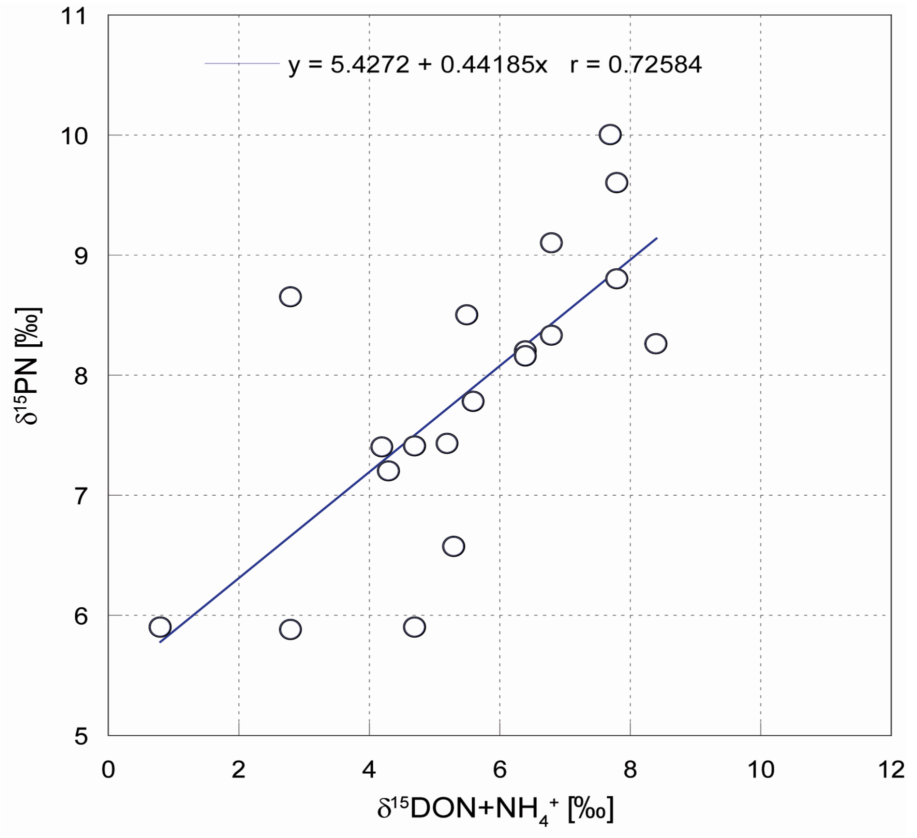


Figure 4.5: Relationship between δ^{15}DON+NH$_4^+$ and δ^{15}PN in the Elbe River at the weir of Geesthacht, June 2006 – December 2007

4.3.5 Loads and annual isotopic values

The annual loads transported in the Elbe River and discharged into the downstream estuary at the weir of Geesthacht are listed in Table 4.3. The annual TDN loads were 107 kt in 2006 and 72 kt in 2007. An unusual flood in April 2006 caused high discharge at high concentrations, and created the nearly 50 % difference in total dissolved nitrogen loads between the two years. Excluding the anomalous flood data, a clear seasonal pattern of higher loads in winter seasons (October to March) emerges: In the case of nitrate, the winter load is more than three times the summer load, and for DON+NH$_4^+$ the winter load is twice as high. In contrast, the PN load is relatively constant throughout the year.

Load	summer '05 (Jun - Sep)	winter '05/'06 (Oct - Mar)	summer '06 (Apr - Sep)	winter '06/'07 (Oct - Mar)	summer '07 (Apr - Sep)	winter '07 (Oct - Dec)	annual load '06	annual load '07
TDN [kt]	12.02	58.73	63.26	39.59	14.84	52.18	107.11	72.03
NO$_3^-$ [kt]	8.67	50.35	53.55	32.97	10.97	45.44	91.19	59.16
DON+NH$_4^+$ [kt]	3.35	8.38	9.71	6.62	3.87	6.74	15.92	12.87
PN [kt]	n.d.	n.d.	5.27	4.09	5.00	5.01	8.20	10.09

Table 4.3: Annual and seasonal loads (in kt) of nitrate, TDN, DON+NH$_4^+$ and PN in the Elbe River at the weir of Geesthacht, June 2005 – December 2007 (n.d. = not determined)

Table 4.4 lists the results of load-weighted annual isotope values for DON+NH$_4^+$, TDN, nitrate and PN in addition to seasonal load-weighted isotope values. In the case of nitrate, δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ values were higher in summer (δ^{15}N-NO$_3^-$: 11.7‰ – 18.7‰; δ^{18}O-NO$_3^-$: 1.6‰ – 5.6‰) than in winter (δ^{15}N-NO$_3^-$: 8.8‰ – 9.5‰; δ^{18}O-NO$_3^-$: 0.7‰ - 1.1‰). For the particulate loads, δ^{15}PN also showed lower values in summer seasons (4.8‰ – 7.1‰) than in winter seasons (8.2‰ – 8.4‰).

	summer '05 (Jun - Sep)	winter '05/'06 (Oct - Mar)	summer '06 (Apr - Sep)	winter '06/'07 (Oct - Mar)	summer '07 (Apr - Sep)	winter '07 (Oct - Dec)	annual load '06	annual load '07
δ^{15}TDN wml	14.5	8.6	7.7	8.8	12.4	9.1	8.3	9.4
δ^{15}N-NO$_3^-$ wml	18.7	8.8	8.1	9.3	15.0	9.5	8.6	10.2
δ^{18}O-NO$_3^-$ wml	n.d.	0.7	1.6	1.1	5.6	1.0	1.3	1.8
δ^{15}DON+NH$_4^+$ wml	5.6	7.5	5.9	6.5	5.0	6.3	6.5	6.0
δ^{15}PN wml	n.d.	n.d.	4.8	8.2	7.1	8.4	6.0	7.1

Table 4.4: Load weighted annual and seasonal mean isotope values of nitrate, TDN, DON+NH$_4^+$ and PN in the Elbe River at the weir of Geesthacht, June 2005 – December 2007 (n.d. = not determined)

Combined DON+NH_4^+ showed an opposite trend with lower δ^{15}DON+NH_4^+ values in summer (5.0‰ - 5.9‰) than in winter (6.3‰ – 7.5‰), so that isotopic differences between summer and winter seasons were much smaller than for nitrate and PN. Because of the greater share of nitrate in TDN, δ^{15}TDN followed the same trend as δ^{15}N-NO_3^- with the higher values in summer seasons (10.3‰ – 14.5‰) compared to winter seasons (8.6‰ – 9.1‰).

4.4 Discussion

Our results show the composition of total nitrogen and the isotopic composition of different reactive N sources in the Elbe River over a time period of more than 2 years from June 2005 to December 2007. In the next section we will discuss the data for nitrate, combined DON+NH_4^+ and PN under the aspects of seasonality and correlations between the measured parameters. We were interested if not only biological processes but also external factors affect seasonal patterns. Furthermore we wanted investigate the correlations of the different N pools like nitrate, DON+NH_4^+ and PN, since both DON and PN may be products of phytoplankton assimilation of the nitrate load; DON+NH_4^+ may also originate from dissimilation of PN within the river.

4.4.1 Nitrate

Nitrate concentrations were high in winter seasons and low in summer seasons and the isotopic composition of δ^{15}N-NO_3^- and δ^{18}O-NO_3^-, had maxima during summer seasons and minima during winter seasons (Fig. 4.2a). The seasonal variability is essentially due to seasonal changes in biological activity, which causes isotopic fractionation (Kendall, 1998). The first process is assimilation of nitrate: Phytoplankton preferentially incorporates light isotopes (^{14}N, ^{16}O) and discriminates slightly against nitrate with heavy isotopes. At higher temperatures in summer, increased phytoplankton productivity leads to an enrichment of heavy isotopes (^{15}N, respectively ^{18}O) in the residual nitrate (Johannsen et al., 2008; Kendall, 1998). A second process potentially raising δ^{15}N-NO_3^- and δ^{18}O-NO_3^- is water column denitrification, which strongly discriminates against the heavy isotopes. Available field and experimental data in seawater suggest equal permil fractionation factors $^{15}\varepsilon$ and $^{18}\varepsilon$ ($^{15}\varepsilon = (^{14}k/^{15}k - 1) * 1000$, where ^{14}k and ^{15}k are the rate coefficients of the reactions for the ^{14}N- and ^{15}N-bearing forms of nitrate, respectively $^{18}\varepsilon$ for oxygen) for nitrate assimilation

(Casciotti et al., 2002; Granger et al., 2004) and denitrification (Granger et al., 2004; Sigman et al., 2003), but little is known about the fractionation factors associated with nitrate assimilation in fresh water; in the case of denitrification, $^{18}\varepsilon : {}^{15}\varepsilon$ ratios of ~0.5-0.6 have been reported (Bottcher et al., 1990; Lehmann et al., 2003; Mengis et al. 1999). However, water column denitrification is unlikely given the oxygen concentrations in the study area, so we expect that coupled enrichment of oxygen and nitrogen isotopes will be mainly associated to assimilation. In Fig. 4.3 we plotted δ^{15}N-NO_3^- versus δ^{18}O-NO_3^- and obtain a slope of 0.81. This slope represents the ratio of the fractionation factors $^{15}\varepsilon$ and $^{18}\varepsilon$ as described by Granger et al. (2004) in their experiments of coupled nitrogen and oxygen isotope fractionation and indicates a major influence of nitrate assimilation, because both isotopes have almost the same fractionation factor (Granger et al., 2004). In July 2005 Deutsch et al. (2009) measured a ratio of 1.12 in the Elbe River. In combination with increased concentrations of chlorophyll *a* and particulate organic carbon they demonstrated that nitrate assimilation by phytoplankton plays a major role in nitrogen transformation processes in the Elbe River (Deutsch et al., 2009).

4.4.2 Particulate nitrogen

Because of the relative short sampling period and the lack of seasonality in both concentration and δ^{15}PN, it is difficult to establish the role of PN in the nitrogen cycle in the Elbe River, which is further complicated by its heterogeneous composition: PN consists both of detritus and newly produced phytoplankton, with presumably large differences in δ^{15}PN.
The higher mean concentrations in spring and summer (65.6 µM and 55.0 µM, Table 4.2) are accompanied by low δ^{15}PN values (7.5‰ in spring, 7.2‰ in summer) and decreasing DIN concentration. This pattern is consistent with PN originating from internal phytoplankton production (Raabe et al., 2004), and fits well with data from July 2005, when a PN concentration of 61 µM and a δ^{15}PN value of ~7‰ was determined (Deutsch et al., 2009). Assimilation of low-δ^{15}N ammonium in the beginning of the phytoplankton bloom in spring could be the reason for the decreasing δ^{15}PN, since ammonium is the preferred N source when abundant (Hadas et al., 2009). After ammonium is exhausted, ^{15}N-enriched DON and nitrate were assimilated, leading to increasing δ^{15}PN. Resuspension of particulate matter from surface sediments should result in an increase in both concentration and δ^{15}PN, thus explaining our observations in summer 2006, when both δ^{15}PN and PN concentration reach a maximum value.

The increase in δ^{15}PN and decrease in concentration in autumn and winter indicates consumption during this period, coupled to resuspension of low-N sedimentary organic matter due to increased flow rates. This suspended matter can be degraded due to biological activity in the sediments and have low N content, but high δ^{15}PN values. The similar curve progression of δ^{15}PN and δ^{15}DON+NH_4^+ indicates a relationship between the dissolved and the particulate matter fraction (Fig. 4.4), mirrored in the correlation coefficient of $r^2 = 0.53$ ($\alpha \leq 0.01$) (Fig. 4.5). The lower δ^{15}DON+NH_4^+ compared to δ^{15}PN suggest that particulate matter is, at any time of year, a significant source for DON and/or ammonium, so DON and/or ammonium are produced by release of small soluble fractions of PN (see next section).

4.4.3 DON+NH_4^+

The combined DON+NH_4^+ load of the Elbe River at the weir of Geesthacht apparently is fed by both external and internal sources. The abiotic external sources of DON+NH_4^+ include terrestrial runoff, such as DON and/or ammonium input by surface runoff, tributaries, groundwater (Valiela et al., 1990; Tobias et al., 2001) and from the atmosphere (Cornell et al., 1995). These external sources are often dominated by discharge of sewage treatment plants, and elution of slurry and liquid manure from farmland.

During our observation period, we found seasonal differences in both DON+NH_4^+ concentration and δ^{15}DON+NH_4^+ (Fig. 4.2b). In contrast to nitrate dynamics, the annual DON+NH_4^+ cycle appears to be more differentiated and can be separated into four seasonal phases: DON+NH_4^++ concentrations and δ^{15}DON+NH_4^+ decreasing in concert (spring), a coupled increase of both parameters (summer), decreasing DON+NH_4^+ concentration and isotope values in autumn and then another increase of both in winter.

4.4.3.1 Spring

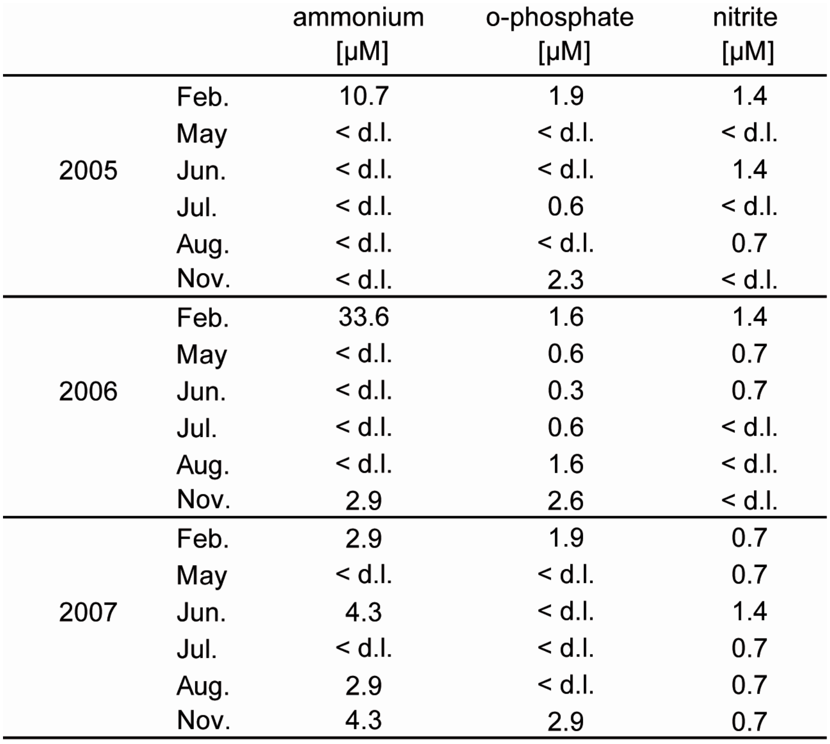
During spring, decreasing DON+NH_4^+ concentration may be explained by heterotrophic and autotrophic uptake of reactive low-molecular-weight DON (LMW DON) (Bronk et al., 2007) and ammonium. Only small fractions of the heterogeneous DON pool in river water are bioavailable (Bronk et al., 2007, Seitzinger and Sanders, 1997, Seitzinger et al., 2002). The proportion of DON that is utilizable by phytoplankton varies by source and land use pattern in the catchment: up to 59% of DON from urban/suburban stormwater runoff and 30% from agriculture sources can be bioavailable (Seitzinger et al., 2002). Incubation experiments by Berman et al. (1999) showed that LMW DON, composed mainly urea, is easily degraded by indigenous bacteria and/or free dissolved enzymes. The portion of this labile fraction is variable: In a study about fifty rivers draining a major part of the Baltic Sea watershed (Stepanauskas et al., 2002) total nitrogen was composed by 48% dissolved inorganic nitrogen (DIN), 41% DON and 11% particulate nitrogen (PN). The labile fraction of DON was composed of urea and dissolved combined amino acids (DCAA) with 4–20% of DON each, and <3% dissolved free amino acids (DFAA).

Besides land use pattern, the bioavailability of DON appears to depend also on seasonal influences that may determine the type of DON. Highest uptake rates of DON originating from urban/suburban stormwater runoffs and agriculture sources were observed in spring times (Seitzinger et al., 2002). The decreasing δ^{15}DON+NH_4^+ values observed in spring apparently reflect that uptake of the bioavailable fraction is complete to the point that we have no apparent isotope effect, and the low δ^{15}DON+NH_4^+ value measured in the remaining pool is due to recalcitrant DON that remains in the water column.

4.4.3.2 Summer

In the second distinct phase in DON+NH_4^+ seasonal cycling (June to August) both concentration and δ^{15}DON+NH_4^+ increase. In 2005 Deutsch et al. (2009) measured low δ^{15}N-NH_4^+ values of 2-3‰ in the Elbe River, so this increase in δ^{15}DON+NH_4^+ should mainly be due to increasing δ^{15}DON. In summer, elution of organic fertilisers in the form of slurry and liquid manure dispersed on farmland during the first main fertilisation period in spring leads to an increase in DON concentration and δ^{15}DON (Heaton, 1996), in accord with our data.

The data from monitoring at the weir also indicate a limitation of biological production in the river by ammonium and phosphate during summer (Table 4.5). This seasonal lack of nutrients apparently leads to high rates of DON release by phytoplankton: When phytoplankton cells are stressed by nutrient limitation, they react by high release rates of organic matter (Carlson et al., 1994; Larsson and Hagström, 1979). Furthermore, such nutrient limitation induces an uptake of DON as an alternative N-source (Jackson and Williams, 1985). Kaushal and Lewis Jr. (2005) examined two streams in Colorado and found highest uptake rates of bioavailable DON, when concentration of DIN in stream water was lowest. In incubation experiments they showed that 40% of the DON could be consumed by microbes in stream sediment. This suggested that DON has the potential to be used biotical at a high rate in nitrogen poor rivers, and may be generated by heterotrophic bacteria when DIN concentration dropped and labile DOM with low relative nitrogen content prevails. We assume that if ammonium is limited, the uptake of DON is an almost effective alternative to the uptake of nitrate. Stepanauskas et al. (1999b) postulate that DON may be even the dominant input of bioavailable nitrogen to coastal seas during summer, when nitrate concentrations in rivers decrease.



		ammonium [µM]	o-phosphate [µM]	nitrite [µM]
2005	Feb.	10.7	1.9	1.4
	May	< d.l.	< d.l.	< d.l.
	Jun.	< d.l.	< d.l.	1.4
	Jul.	< d.l.	0.6	< d.l.
	Aug.	< d.l.	< d.l.	0.7
	Nov.	< d.l.	2.3	< d.l.
2006	Feb.	33.6	1.6	1.4
	May	< d.l.	0.6	0.7
	Jun.	< d.l.	0.3	0.7
	Jul.	< d.l.	0.6	< d.l.
	Aug.	< d.l.	1.6	< d.l.
	Nov.	2.9	2.6	< d.l.
2007	Feb.	2.9	1.9	0.7
	May	< d.l.	< d.l.	0.7
	Jun.	4.3	< d.l.	1.4
	Jul.	< d.l.	< d.l.	0.7
	Aug.	2.9	< d.l.	0.7
	Nov.	4.3	2.9	0.7

Table 4.5: selected nutrient concentrations in the Elbe River at the weir of Geesthacht in the years 2005 – 2007, measured by the ARGE-Elbe (ARGE 2005, 2007a, 2007b) (d.l. = detection limit)

We interpret our data from summer, when both concentration and δ^{15}DON+NH_4^+ decrease, as a reflection of a dynamic equilibrium of uptake and release of DON: The elution of organic fertilisers and the uptake of DON by phytoplankton cause an increase in δ^{15}DON+NH_4^+, while the DON released due to nutrient limitation should cause a decrease in δ^{15}DON+NH_4^+. This is supported by the close correlation of δ^{15}DON+NH_4^+ and δ^{15}PN (Fig. 4.5), which suggests that ^{15}N depleted DON is released from particulate N. Phytoplankton, which is highly abundant at this time of year, is a likely source of this DON, as has been observed in Lake Kinneret, Israel, where Hadas et al. (2009) found a similar relation between particulate organic matter (POM) and DON, and conclude that algal production is a major source for DON.

4.4.3.3 Autumn

In autumn, from September to October, we again observed a decrease in DON+NH_4^+ concentration and δ^{15}DON+NH_4^+, followed by parallel increases in winter months to higher values in both concentration and δ^{15}DON+NH_4^+ than in summer.

In autumn, at the end of the biological production period, there is still a lack of phosphate in the river water, so that the release of ^{15}N-depleted DON is still in progress. However, due to sinking algal production, DON+NH_4^+ concentration is decreasing. The remaining DON is isotopically depleted. We infer that sedimentation is also a major sink of DON as an explanation for decreasing DON+NH_4^+ concentration and δ^{15}DON+NH_4^+. In the Colne River (which has a TDN composition comparable to the Elbe River), Agedah et al. (2009) observed a similar decrease when ^{15}N enriched DON is removed from the water column. The low PN concentrations further support this assumption.

4.4.3.4 Winter

The increase of DON+NH_4^+ concentration and δ^{15}DON+NH_4^+ in winter is due to the elution of organic fertilisers of the second main fertilisation period in autumn after the last harvest in October before the blocking period starts (from beginning of November until the end of January, DüV, 2009). Recent measurements of δ^{15}N-NH_4^+ in January 2010 showed low values of 0 – 1‰ at concentrations of ~13 µM (Schlarbaum et al., unpublished data), so DON must be highly enriched in ^{15}N to obtain measured δ^{15}DON+NH_4^+ values. The elevated concentration in comparison to summer is due to high ammonium concentrations in winter (10 – 30 µM, Table 4.5).

4.5 Summary and conclusions

In our study about different forms of nitrogen in the Elbe River at the weir of Geesthacht we measured both concentration and stable isotope signatures of nitrate, combined DON+NH_4^+ and PN. On an annual basis, nearly 23 % of TDN is in the form of DON+NH_4^+.

For nitrate the seasonal pattern has two periods with a contrasting development of concentration and dual nitrate isotopes, due to biological processes. We attribute this to nitrate assimilation during biological activity, as is supported by the co-variance of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$.

In contrast, the seasonal cycling of combined $DON+NH_4^+$ is more complex and is influenced by many different factors, both biotic and abiotic. The annual $DON+NH_4^+$ cycle can be separated into four periods, with an increase in both concentration and $\delta^{15}DON+NH_4^+$ in summer and winter, and a decrease in spring and autumn. As the main abiotic source, we assume the elution of ^{15}N-enriched organic fertiliser, after the main fertilisation periods in spring and after the harvest in autumn, to have an important influence on DON in the Elbe River. In summer, this is accompanied by DON release by phytoplankton due to nutrient limitation, indicated by the similar, almost parallel progression of $\delta^{15}DON+NH_4^+$ and $\delta^{15}PN$. The decrease in spring and autumn is on the one hand due to autotrophic and heterotrophic uptake (springtime) and on the other hand due to lower biological production in autumn. Our measurements suggest that the recalcitrant high-molecular-weight DON fraction in the Elbe River is isotopically depleted in ^{15}N, compared to the reactive low-molecular-weight DON.

5. Isotope ratios of nitrate, dissolved reduced and particulate nitrogen in the eastern Mediterranean Sea trace external N-inputs and internal N-cycling

Abstract

The eastern Mediterranean Sea is an unusually nutrient-poor ocean basin where the $^{15}N/^{14}N$ isotope ratios in many compartments of reactive nitrogen are lower than in comparable oceanic settings. To elucidate possible reasons, we determined stable isotope ratios in nitrate, suspended particulate and total dissolved reduced nitrogen for stations across the eastern Mediterranean Sea occupied in January and February 2007; sinking PN was collected at one of the stations in the period from February to September 2007. The $\delta^{15}N$ levels of all reactive N compartments in waters of the basin is very low (grand average 2.6 ‰) compared to other oceanic environments. Deep-water nitrate below 500 m water depth ($\delta^{15}N$=2.2±0.3‰) was more depleted in ^{15}N than nitrate generally found in deep water nitrate pools of other oceans ($\delta^{15}N$ ranges from 4.7-5.4 ‰), whereas ^{15}N was enriched in suspended particulate N ($\delta^{15}N$ = 7.3±0.8‰) and reduced dissolved N ($\delta^{15}N$ =5.7±3.8‰) compared to nitrate and sinking particulate N intercepted in sediment traps ($\delta^{15}N$= 0.9±0.8‰). We infer that extensive mineralisation is the cause of the isotopic makeup of reactive N in deep water, in concert with the lack of water column denitrification. Nitrogen and oxygen isotope ratios in nitrate of the mixed layer suggest an external source of nitrate depleted in ^{15}N, probably anthropogenic NO_x rather than fixed nitrogen. To explain the observed isotope anomaly in the mixed layer, either the ammonium formed by the breakdown of organic matter must be predominantly nitrified, or atmospheric NO_x characteristically enriched in ^{18}O was present.

5.1 Introduction

The modern eastern Mediterranean Sea is a highly oligotrophic oceanic environment (Antoine et al., 1995; Béthoux, 1989) where primary production is approximately half that observed in the Sargasso Sea or the Northeast Pacific (Krom et al., 2003). This low productivity is caused by the anti-estuarine circulation in the basin: Modified Atlantic Water flows in through the Strait of Sicily at the surface, sinks in the eastern part of the basin and feeds the Levantine Intermediate Water, a high temperature, high salinity water mass at depths between 200 and 500 m that leaves the basin as a westward current through the Sicily Strait. This intermediate water mass collects and exports the bulk of mineralisation products sinking out of the mixed layer. Below (>500 m) this surface circulation cell is the eastern Mediterranean Deep Water (Malanotte-Rizzolli and Bergamasco, 1989; Wüst, 1961) which forms when the intermediate water mass is entrained into surface waters of the two northern sub-basins (Aegean or Adriatic Seas) and gains sufficient density after winter cooling (Lascaratos et al., 1999).

The export of nutrients with outflowing intermediate water causes the deep waters of the eastern Mediterranean Sea to be nutrient depleted relative to the deep water in all other parts of the global ocean: Nitrate concentrations in deep water are 4-6 μM compared to 8 μM in deep water of the adjacent Western Mediterranean Sea, and 20 μM at similar depths in the Atlantic Ocean. The eastern Mediterranean Sea is also unusual in that it has a high nitrate:phosphate ratio (28:1) in the deep water and is phosphate-starved (Krom et al., 2005b). As a result, primary production during the winter phytoplankton bloom is limited by phosphate rather than by nitrate (Krom et al., 1991). In winter, the phosphate in the surface water is entirely depleted while excess nitrate remains (Kress and Herut, 2001). However, soon after the seasonal thermocline develops in spring, both nitrate and phosphate become depleted in the photic zone (Kress and Herut, 2001; Krom et al., 2005a) and phytoplankton productivity becomes N- and P- co-limited (Thingstad et al., 2005; Zohary and Robarts, 1998). The reason is that all P respired is available for assimilation, whereas part of the N respired enters the pool of dissolved organic nitrogen and subsequently is not available for assimilation (Thingstad et al., 2005; Zohary and Robarts, 1998).

Another unusual feature of the eastern Mediterranean Sea is the ^{15}N depletion of deep-water nitrate, suspended matter, and surface sediments in the basin (Coban-Yildiz et al., 2006; Pantoja et al., 2002; Struck et al., 2001) compared to values in other open-ocean environments. The isotope ratio is commonly expressed as δ^{15}N, which is the ratio in the abundance of ^{15}N and

^{14}N in relation to that ratio (R) in a standard: $\delta^{15}N_{sample} = (R_{sample}/R_{standard}-1)*1000$ in ‰; the international standard being $\delta^{15}N$ of air N_2 = 0 ‰.

The low abundance of ^{15}N in reactive nitrogen of the eastern Mediterranean Sea is unusual, and reflects a different balance between external sources of N and internal sinks here than in the global ocean, where a balance of N_2-fixation and water-column denitrification sets the average $\delta^{15}N$-NO_3^- to approximately 5‰ (Brandes and Devol, 2002; Sigman et al., 2009). A decisive difference of the eastern Mediterranean Sea is the abundance of oxygen even in mid-water oxygen minimum zones (rarely lower than 70% saturation) and thus the absence of water-column denitrification in the basin, which elsewhere raises the $\delta^{15}N$ of nitrate. Exchange of deep water with the Atlantic Ocean is impeded by sills at Gibraltar and Sicily, so that the Mediterranean Sea deep water does not communicate with global ocean deep water. In consequence, only the regional sources of reactive N and their $\delta^{15}N$ determine the general level of $\delta^{15}N$ in the basin.

Fig. 5.1 schematically illustrates possible nitrate sources and the processes that govern the $\delta^{15}N$ and $\delta^{18}O$ of nitrate in the surface and intermediate water masses of the eastern Mediterranean Sea and in other oligotrophic regions. One of the possible external sources that has been invoked to explain atypical ^{15}N depletion (and high N:P ratios) in the eastern Mediterranean Sea is significant levels of diazotrophic N_2 fixation (Pantoja et al., 2002; Sachs and Repeta, 1999), because newly fixed nitrogen is ^{15}N depleted ($\delta^{15}N$ = -2 to 0‰) (Minagawa and Wada, 1986; Montoya et al., 2002). This interpretation is in line with other authors´ inference from N:P ratios that N_2 fixation is the most important source of new nitrogen to the eastern Mediterranean Sea (Béthoux and Copin-Montegut, 1986; Gruber and Sarmiento, 1997; Mahaffey et al., 2005). At present, however, observational evidence for N_2-fixation is ambiguous (Berman-Frank et al., 2007; Ibello et al., 2010; Rees et al., 2006), and annual input estimates range from nil (Krom et al., 2004) to $120*10^9$ mol/a (Béthoux and Copin-Montegut, 1986), the latter estimate being ~⅔ of the total nitrate exported through the Straits of Sicily.

A second source depleted in ^{15}N is significant atmospheric NO_x input to the eastern Mediterranean Sea that is bordered by heavily industrialised regions to the North and is a busy shipping route. This basin is distinct from other oligotrophic settings of the world ocean (Duce et al., 2008) in that atmospheric deposition of NO_x presently dominates the external nitrogen inputs (Krom et al., 2004; Mara et al., 2009). Furthermore, regional atmospheric loads have increased dramatically over the last decades (Fagerli et al., 2007; Kouvarakis et al., 2001; Preunkert S. et al., 2003), and the input of nitrate from the atmosphere to the surface water mass of the eastern

Mediterranean Sea presently accounts for almost 60% of reactive N inputs (Krom et al., 2004; Mara et al., 2009). The atmospheric source (NO_3^- in dry and wet deposition) has an annually averaged (flux-weighted) $\delta^{15}N$-NO_3^- of -3.1‰ (Mara et al., 2009) and thus is a strong candidate for depressing the $\delta^{15}N$-NO_3^- levels in all water masses of the eastern Mediterranean Sea over time. Both N_2 fixation and atmospheric inputs have similar $\delta^{15}N$ signatures and cannot be separated based on $\delta^{15}N$. But whereas fixed N_2 has to be oxidised to nitrate in the mixed layer, atmospheric NO_x is a preformed input of nitrate, and is known to be significantly ^{18}O-enriched over nitrate originating from nitrification or anthropogenic effluents (Hastings et al., 2003; Kendall, 1998; Wankel et al., 2009).

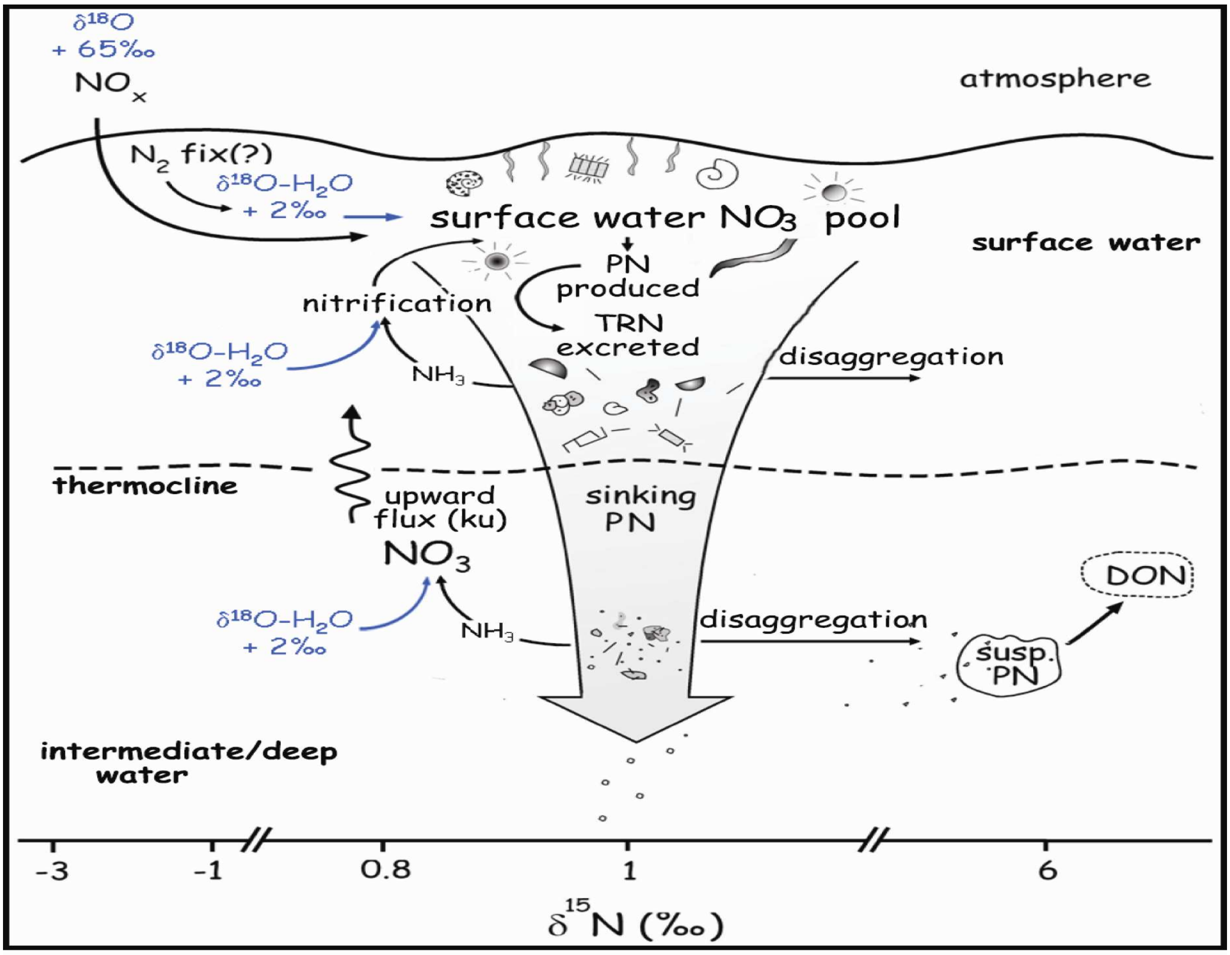


Figure 5.1: Conceptual diagram of the processes that determine the $\delta^{15}N$ and $\delta^{18}O$ of nitrate in the surface and intermediate water masses of the eastern Mediterranean Sea. Abbreviations are PN for particulate nitrogen, TRN for total reduced nitrogen, and DON for dissolved organic nitrogen as explained in the methods section. This diagram also serves as a schematic illustration of the model used in the discussion to calculate the nitrate isotope anomaly Δ(15,18) under assumptions of different sources as explained in the text (see also auxiliary material).

A previous study in the Mediterranean Sea (Struck et al., 2001) also inferred incomplete nitrate utilisation (Altabet and Francois, 1994) as the main reason for ^{15}N depletion in suspended matter and sediments: Because most biological processes discriminate against ^{15}N in NO_3^-, incomplete nitrate uptake by phytoplankton (limited by phosphate) would result in products (such as particulate nitrogen or dissolved organic nitrogen) depleted in ^{15}N, while unprocessed residual nitrate would be enriched in ^{15}N over the original nitrate. Together, residue and products have the original nitrate signature, and thus incomplete utilisation of typical marine nitrate alone cannot account for the overall ^{15}N depletion in all reactive N compartments.

The denitrifier method (Casciotti et al., 2002; Sigman et al., 2001) to simultaneously determine the $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$, has provided the analytical tool to investigate global (Sigman et al., 2009) and regional (Casciotti et al., 2008; Knapp et al., 2008; Lehmann et al., 2005; Sigman et al., 2005; Wankel et al., 2006) N-cycles that involve internal oceanic sources and sinks. Establishing the relationships between mixed layer $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ helps to decide, if the supply by deep winter mixing and subsequent nitrate utilisation governs isotopic compositions entirely, or alternatively to identify situations where other sources besides thermocline nitrate contribute to the isotopic ratio of nitrate in the mixed layer. The largest source of nitrate to the mixed layer is thermocline nitrate. But in oligotrophic oceanic settings, nitrate regenerated from particulate nitrogen (PN) and possibly from dissolved organic nitrogen (DON) via ammonium and nitrite oxidation can contribute significantly to primary production (Yool et al., 2007). Nitrate from recycling of particulate N is thought to be particularly important in the oligotrophic Mediterranean Sea (Diaz and Raimbault, 2000). Nitrification imparts a characteristic isotopic signature on the $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$ of regenerated nitrate (Casciotti et al., 2003; Wankel et al., 2006), which can be used to quantify its contribution (Sigman et al., 2009). Re-oxidation of assimilated N to nitrate via ammonium and nitrite transfers the $\delta^{15}N$ of sinking particulate N into the recycled nitrate; this applies also to the nitrification of newly fixed N. In contrast, the regeneration replaces nitrate previously enriched in ^{18}O in the course of nitrate assimilation with nitrate having a $\delta^{18}O$ characteristic of nitrification, and thus decoupling the two isotope pairs (see Fig. 5.1). Regenerated nitrate can either derive from particulate of dissolved organic N (Bronk, 2002; Knapp et al., 2008). Should dissolved total reduced nitrogen (TRN, dissolved organic nitrogen and ammonium) indeed be a key component in the recycling of reactive N in the eastern Mediterranean Sea (Thingstad et al., 2005), its isotopic composition must reflect active participation in the N-cycle (Knapp et al., 2005).

In this study, we determined the stable isotope ratios in nitrate ($\delta^{18}O$-NO_3^- and $\delta^{15}N$-NO_3^-), in dissolved total reduced nitrogen ($\delta^{15}N$-TRN, composed of DON+NH_4^+) and in particulate N of suspended particles ($\delta^{15}N$-PN_{susp}) obtained from filtration from the mixed-layer and deep-water pools for a set of 17 stations occupied in the eastern Mediterranean Sea in 2007 (Fig. 5.2). Furthermore, mass flux and $\delta^{15}N$ data of sinking particles ($\delta^{15}N$-SPN) intercepted by a sediment trap mooring in several periods from 1999 to 2007 at one of the stations in the Ierapetra Gyre south of Crete are reported and discussed. Aims of this study are 1) to provide a comprehensive data set of $\delta^{15}N$ in several relevant N-pools of the water column, covering pelagic and more nearshore provinces of the eastern Mediterranean Sea and different water masses, and 2) to analyse to what extent the interplay between nitrate origin (internal recycling versus external inputs) and biological processes (assimilation, mineralisation, N_2-fixation) in the euphotic zone of the eastern Mediterranean Sea may explain the observed isotope anomalies. We specifically were interested in evidence that external inputs (N_2 fixation or NO_x inputs) are a significant source of mixed-layer nitrate.

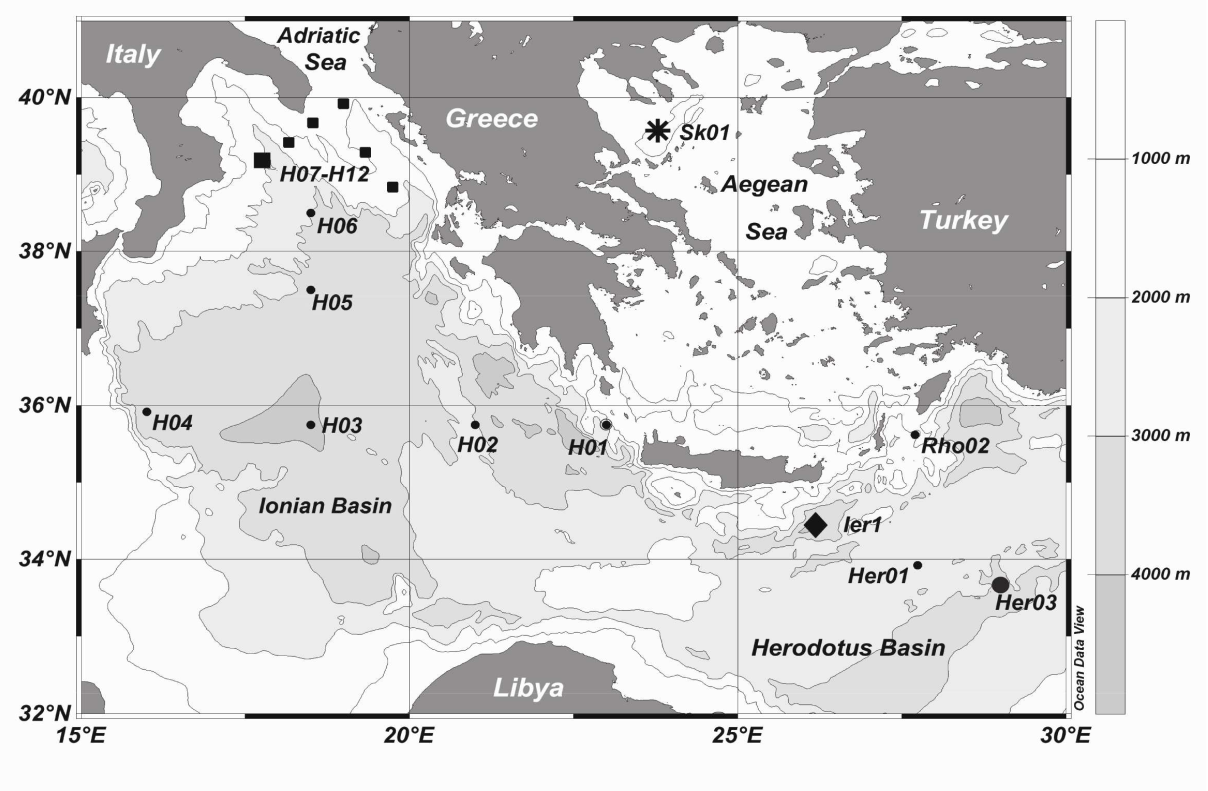


Figure 5.2: Map of stations occupied in the eastern Mediterranean Sea during r/v Meteor cruise 71-3 (January-February, 2007). Northern Ionian Sea (NIS) stations referred to in the text are stations H07 to H12 (black squares), pelagic stations are marked with black dots, the sole station in the northern Aegean Sea (Sk01) is marked by a black star. The black diamond SE of Crete (station Ier1) marks the location of the sediment trap deployment (MID). Stations H07 (large black square) and Her03 (large black dot) referred to in Fig 5.3 are marked.

5.2 Materials and Methods

Samples were obtained during an expedition with r/v METEOR in 2007, which visited 17 stations in the eastern Mediterranean Sea in January/February 2007; one cluster of stations was in the northern Ionian Sea (NIS), other stations sampled pelagic environments in the Ionian and Herodotus basins, and one station was located in the northern Aegean Sea (Fig. 5.2). Water samples were taken with a rosette sampler equipped with a Seabird 911 CTD; an aliquot of the bottom water at the sediment-water interface was also taken from multicorer deployments. Water samples were immediately filtered through pre-combusted and rinsed GF/C filters, and were transferred into PE bottles for shore based analyses of nutrient concentrations, total dissolved nitrogen content (TDN, after oxidation of reduced N) and its N-isotopic signature (δ^{15}N-TDN), and δ^{15}N/δ^{18}O of nitrate. Samples for nitrate isotope analysis were frozen on board ship for further analysis onshore, samples for TDN analysis were oxidised immediately after filtration on the ship with persulfate and then stored frozen in brown glass bottles until further analysis in the shore-based laboratory. Samples for nutrient analyses were poisoned with 3.5% mercury chloride solution and stored at room temperature. Nutrient and TDN concentrations were analysed immediately after the expedition using a Bran+Luebbe Autoanalyzer 2 in the home laboratory and standard colorimetric techniques (Grasshoff et al., 1999).

Large water samples (10-50 L) were filtered through pre-combusted and tared GF/F filters for analyses of total nitrogen concentrations in suspended solids (particulate nitrogen, PN_{susp}), as well as for δ^{15}N of PN_{susp} (δ^{15}N-PN_{susp}). Filters were frozen on board, lyophilised in the home laboratory and weighed before further analysis.

On January 29, 2007 a mooring system MID-03 (Mediterranean Ierapetra Deep) was deployed in the Ierapetra Deep off Crete (34°26.63' N, 026°11.58' E, bottom depth 3620 m) (Fig. 5.2). The system consisted of one sediment trap McLane PARFLUX MARK 7G-21 at 1508 m water depth (MID-03 Shallow) and one Kiel Sediment Trap K/MT 234 at 2689 m water depth (MID-03 Deep). The cups were filled with filtered (GF/F, combusted) sea water from the respective depths. In addition, 35 g l^{-1} NaCl and 3.3 g l^{-1} $HgCl_2$ were added in order to avoid diffusion and bacterial decomposition during the deployment. Sampling started on 30 January 2007 and ended on September 03, 2007. The particle flux was sampled at intervals of 12 days and the mooring was recovered in September 2007. Subsequent to trap recovery, trapped materials were sieved into >1 (including any large zooplankton trapped) and <1 mm fractions, the wet sample material was filtered onto pre-weighed nucleopore filters and dried at 40°C. The dry weights of the <1 mm fraction are used for calculating the total fluxes, and the filter cake was homogenised with an agate mortar prior to analysis. The same site has seen intermittent sediment trap deployments at 2700 m water depth in 1999 (30 January to 13 April, 1999) and in 2001/2002 (5 November 2001 to 1 April, 2002) (Warnken, 2003), and we use data from these earlier deployments to complement data obtained from the last trapping period.

The filtered and frozen water samples for determinations of δ^{15}N-NO_3^- and δ^{18}O-NO_3^- and δ^{15}N-TDN were thawed in the shore-based laboratory, and nitrate isotopic composition was determined with the denitrifier method (Casciotti et al., 2002; Dähnke et al., 2008; Sigman et al., 2001). The untreated filtered water samples or persulfate-digested TDN samples were injected into a suspension of *Pseudomonas aureofaciens* (ATCC#13985) for combined analysis of δ^{15}N and δ^{18}O, or *Ps. chlororaphis* for δ^{15}N analysis of TDN only. The resulting N_2O gas was flushed by purging the sample vials with helium, concentrated and purified on a GasBench II (ThermoFinnigan), and analyzed on a Delta Plus XP mass spectrometer (ThermoFinnigan). To avoid concentration-dependent fractionation effects, sample size in deep and thermocline water samples was adjusted to achieve a final gas amount of 10 nmol, the same amount of standard was used. Many mixed-layer samples, where nitrate concentrations were low, yielded smaller N_2O amounts. For each sample, replicate measurements were performed, and an international standard (IAEA-N3) was measured with each batch of samples; we used a δ^{15}N value of 4.7‰ and a δ^{18}O value of 25.6‰ for IAEA-N3 referenced to Standard Mean Ocean Water (δ^{18}O SMOW=0‰) (Böhlke et al., 2003; Lehmann et al., 2003; Sigman et al., 2005). To correct for exchange with oxygen from H_2O during the conversion to N_2O we used an ^{18}O-enriched water standard and applied the correction proposed by Casciotti et al. (2002). This correction has recently been revised (Sigman et al., 2009), and we applied an additional offset of -0.6‰. The contribution of nitrite was always below one per cent and will therefore not be considered further in our calculations, because the effect on δ^{18}O values is negligible (Casciotti and Mcilvin, 2007). The standard deviation for IAEA-N3 was better than 0.2‰ ($n = 5$) for δ^{15}N-NO_3^- and better than 0.4‰ for δ^{18}O-NO_3^-. For further quality assurance of the results, we used an in-house potassium nitrate standard that was measured with each batch of samples. The standard deviation for the in-house standard was within the same specification for both δ^{15}N and δ^{18}O as IAEA-N3. The duplicate analyses suggest an overall average standard deviation for δ^{15}N-NO_3^- of 0.2‰ and for δ^{18}O-NO_3^- of 0.4‰. The standard deviations of duplicate analyses of δ^{18}O-NO_3^- increased to 0.5‰ in samples with low nitrate concentrations in the mixed layer and upper thermocline. The majority of

85 samples analysed from the mixed layer gave unacceptable standard deviations and are not reported.

For calculations of the $\delta^{15}N$ of total reduced nitrogen (TRN, the sum of DON and ammonium) mass balance calculations were made using the measured concentrations (*c*) of nitrate and TRN, and $\delta^{15}N$-TDN values of the oxidised sample, the reagent blank and the $\delta^{15}N$-NO_3^- of the original (not oxidised) sample (Knapp et al., 2005):

$$\delta^{15}N - TRN = \frac{\delta^{15}TDN * c(TDN)}{c(TRN)} - \left[\frac{\left(\delta^{15}N - NO_3^- * c(NO_3^-)\right)}{c(TRN)} + \frac{\left(\delta^{15}Blank * c(Blank)\right)}{c(TRN)}\right] \quad \text{(Eq. 5.1)}$$

The pH of the digested samples for TRN analysis was around 6, so that no pH adjustment was necessary (Schlarbaum et al., 2010). Yield of the persulfate digestion procedure has been tested with different standards and the oxidation efficiency was between 98-105% (Schlarbaum et al., 2010). A urea standard (in solid form analysed by combustion in an elemental analyser) was used for quality control in $\delta^{15}N$-TRN measurements ($\delta^{15}N$ of solid was 0.5‰ ± 0.2‰; $\delta^{15}N$ after persulfate digestion and conversion to N_2O by the denitrifyer method was 0.4‰ ± 0.2‰). Because of low concentrations and error propagation, the calculated standard deviation of $\delta^{15}N$-TRN analyses ranged from 0.2 to 2.0‰ with a mean value of 0.9‰, and the calculated mean standard deviation for TRN concentration was 1.7 µM. The mean standard deviation for $\delta^{15}N$-TRN (0.9‰) was calculated with standard deviations of actual measurements of $\delta^{15}N$-NO_3^- and $\delta^{15}N$-TDN (after digestion), which were in part better than the 0.2‰ estimated as the overall standard deviation for the $\delta^{15}N$-NO_3^- measurements. If we recalculate using a standard deviation of 0.2‰, the standard deviation for $\delta^{15}N$-TRN rises to 1.3‰. Note that $\delta^{15}N$-TRN was not corrected for any contribution by ammonium. However, the only published data on ammonium from the eastern Mediterranean sea found values in the range of 40-80 nM with no major trend with depth (Krom et al., 2005b). In the same profiles the DON concentration was ~2-4 µM in deeper water, similar to the values measured in this study. It is thus likely that most of the isotopic signal is due to DON.

Total particulate nitrogen concentrations in suspended solids (PN_{susp}) and in sinking material captured by the sediment trap (SPN) were analysed after high-temperature flash combustion in a Carlo Erba NA-2500 elemental analyzer at 1100°C (Rixen et al., 2000). $\delta^{15}N$

values were determined using a Finnigan MAT 252 gas isotope mass spectrometer coupled to an elemental analyzer. Pure tank N_2 calibrated against the reference standards International Atomic Energy Agency (IAEA)-N-1 and IAEA-N-2 was used as a working standard. The within-lab standard-deviation was found to be <0.2 ‰ based on a set of replicate measurements of 6 sediment samples.

5.3 Results

5.3.1 Concentrations of N-bearing compounds

The study took place in winter when the water column at most stations was well mixed with a thermocline situated at depths of around 100m (NIS stations H07 to H12 in the northern Ionian Basin, and station Sk01 in the northern Aegean Sea; see Fig. 5.2) to 250 m (pelagic stations in the deep Ionian Basin, Ierapetra and Herodotus Basin) water depth. Fluorescence profiles (Fig. 5.3) show elevated values (uncalibrated chlorophyll concentrations) in the surface mixed layer typical of the winter phytoplankton bloom observed in the eastern Mediterranean Sea (Krom et al., 2003). Concentration versus depth plots of nitrate, TRN and PN_{susp} for all stations (Fig. 5.4) showed that the thermocline (coincident with the base of the fluorescence increase) in all cases coincided with the nitracline. Based on the criterion of nitrate concentrations, we distinguished between samples above the nitracline, in the nitracline, and deep-water samples below the nitracline for each station set.

Nitrate concentrations were low but measurable in the mixed layers of both the northern Ionian Sea and the pelagic stations (Fig. 5.4a), while phosphate concentrations were below the effective detection limit of the nutrient procedures being used (estimated to 0.05 μmol L^{-1}) (Li et al., 2008). TRN concentrations varied around averages of 1.6 μmol L^{-1} in the mixed layer of NIS and pelagic stations, and 3.3 μmol L^{-1} in the northern Aegean (Fig. 5.4b). Suspended PN concentrations in the mixed layer of the northern Ionian Sea were on average 0.5 μmol N L^{-1} (0.4 μ mol N L^{-1} in the mixed layer of the pelagic stations, and 0.5 μ mol N L^{-1} in the northern Aegean) (Fig. 5.4c).

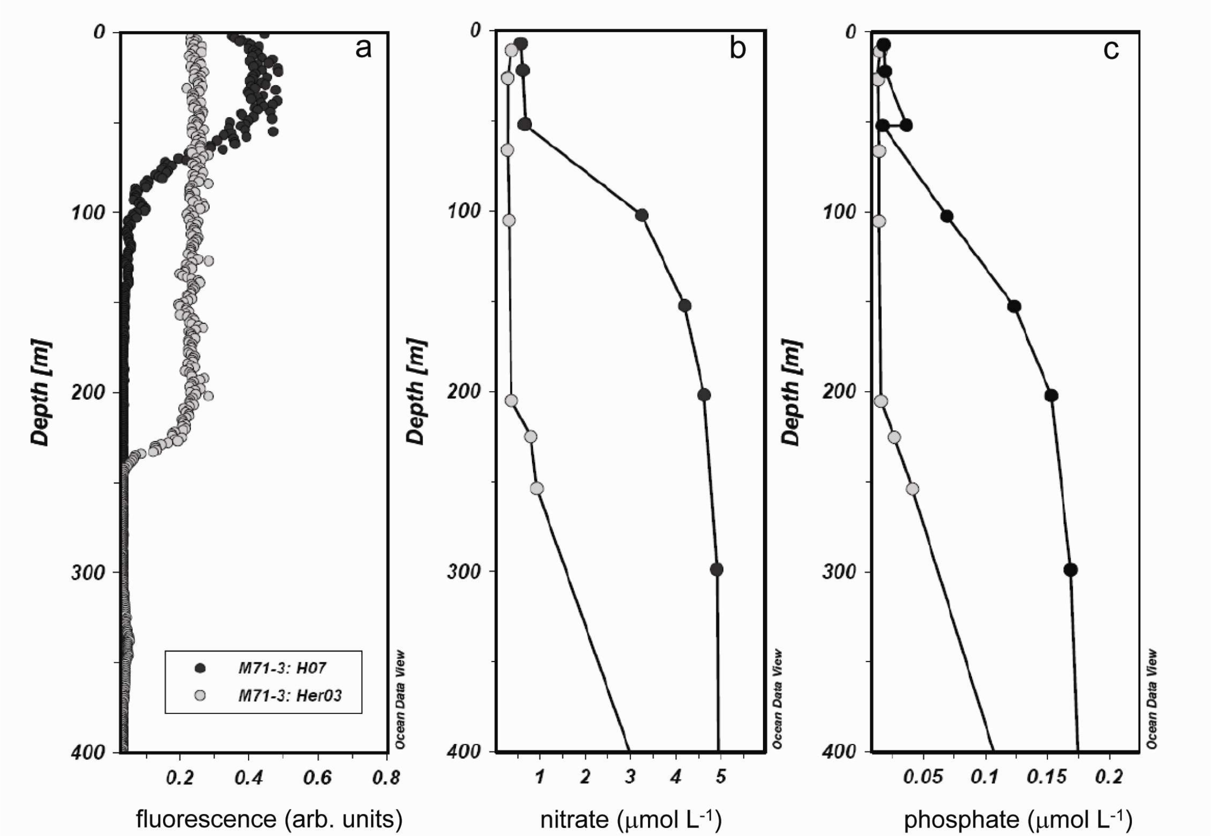


Figure 5.3: Profiles of fluorescence (a; arbitrary units), nitrate (b) and phosphate (b) concentrations in the upper 400 m at 2 stations representative of NIS (H07) and pelagic stations (Her03) show stratification between 80 and 230 m water depth and indicate the biologically active mixed layer. An ongoing phytoplankton bloom in the northern Ionian Sea (at station H07) is sustained by nitrate and phosphate provided from ongoing regional thermocline deepening, whereas station Her03 illustrates the mature and thick mixed layer with very low nutrient concentrations at pelagic sites.

The intermediate water mass had average nitrate concentrations around 3.5 µmol L^{-1} and average phosphate concentrations between 0.10 and 0.12 µmol L^{-1}; the mean nitrate:phosphate ratio in the intermediate water was around 30 (Fig. 5.4). TRN concentrations were around 1.5 µmol N L^{-1} in intermediate waters at NIS and pelagic stations, and 3.6 µmol N L^{-1} in the Aegean station. The TRN concentrations in the intermediate water mass depth interval scatter widely due to measurement uncertainties at the low concentration levels measured. Suspended PN (not determined in the intermediate water mass of NIS stations) averaged 0.2 µmol N L^{-1} at both pelagic stations and the single northern Aegean station.

Concentrations of most dissolved and particulate constituents in samples below the nitracline were more uniform than in the biologically active surface (Fig. 5.4): Average nitrate (4.5 to 4.8 µmol L^{-1}) and phosphate (0.16 to 0.23 µmol L^{-1}) concentrations over all station sets resulted in an average nitrate:phosphate ratio of >27, similar to previous measurements across the eastern Mediterranean Sea (Kress and Herut, 2001). Average TRN concentrations varied around 1.3 µmol N L^{-1} in deep water at northern Ionian Sea and pelagic stations, but were elevated (average 3.6 µmol N L^{-1}) in the northern Aegean. Average suspended PN concentration was below 0.2 µmol N L^{-1} in all waters below the nitracline to total depth.

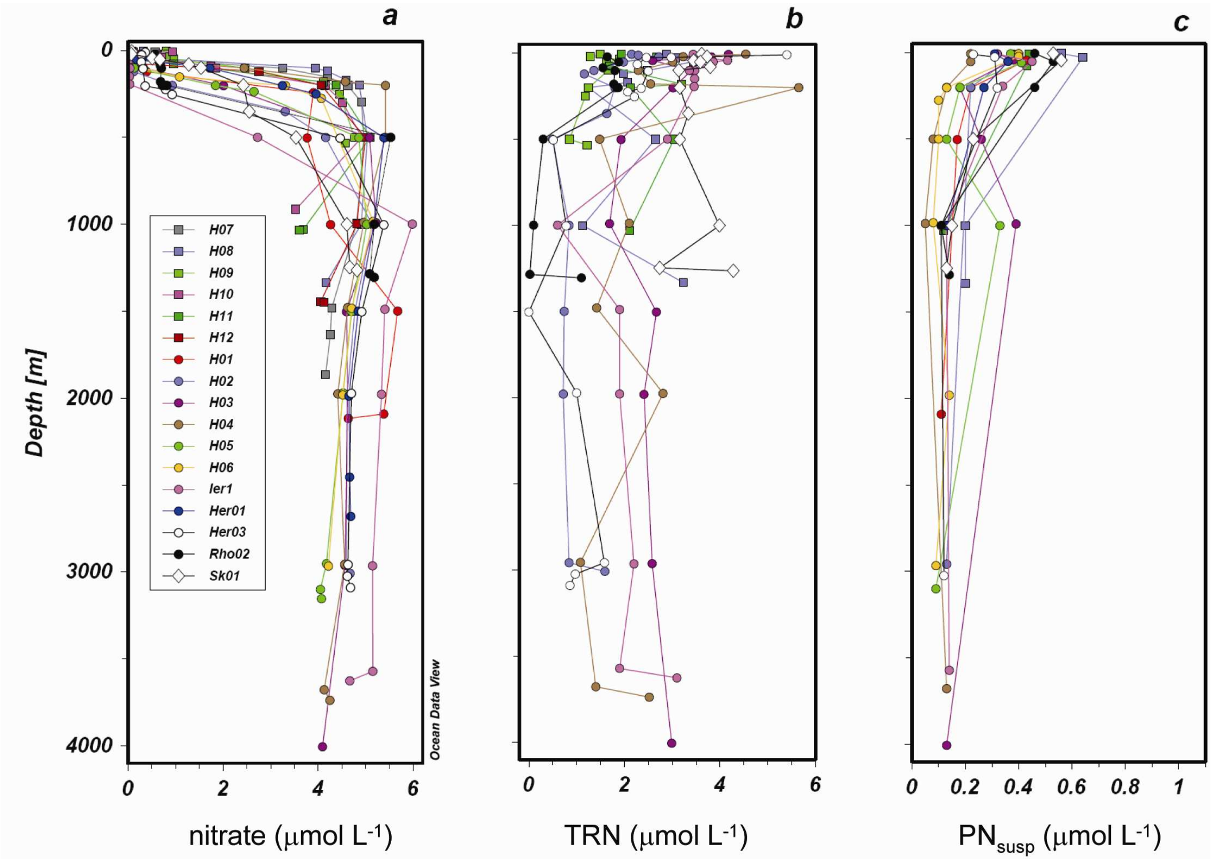


Figure 5.4: Concentrations of nitrate (a), TRN (b), and particulate N in total suspended solids (c) plotted against water depth for all stations.

The two sediment traps deployed over 216 days from February to September 2007 at station Ier1 (Fig. 5.2) monitored a total sinking particulate N (SPN) flux in the shallow trap (at 1500 m) of 5.7 mmol N m^{-2} (or 26.3 µmol N m^{-2} d^{-1}) over that period, while the deep trap (at 2700 m) collected 1.4 mmol N m^{-2} (6.5 µmol N m^{-2} d^{-1}) of SPN over the same period. Earlier sediment trap deployments at the MID location (Warnken, 2003) reported similar N fluxes in the deep traps (MID-01, February to April 1999, 4.5 µmol N m^{-2} d^{-1}; and MID-02, November 2001 to March 2002, 6.0 µmol N m^{-2} d^{-1}); unfortunately, sampling in the shallower trap failed during these earlier deployments.

5.3.2 Isotopic composition of N-bearing compounds

At all stations, the isotopic composition of N-bearing species differed not only between the mixed layer, and the intermediate and deep water masses (Fig. 5.5), but also among station sets. Mixed-layer nitrate had average δ^{15}N-NO_3^- of 5.6‰, 2.3‰, and 4.1‰ at NIS, pelagic, and northern Aegean stations, respectively; δ^{18}O-NO_3^- was 10.7‰ at NIS stations, and 5.2‰ at pelagic stations; no sample yielded a reliable value in the northern Aegean. δ^{15}N of suspended PN in the mixed layer was low (δ^{15}N-PN_{susp} average of all stations = 1.3±0.7‰), and TRN in the surface layer (average δ^{15}N-TRN of all stations = 0.0±1.1‰) was roughly similar to δ^{15}N-PN_{susp} .

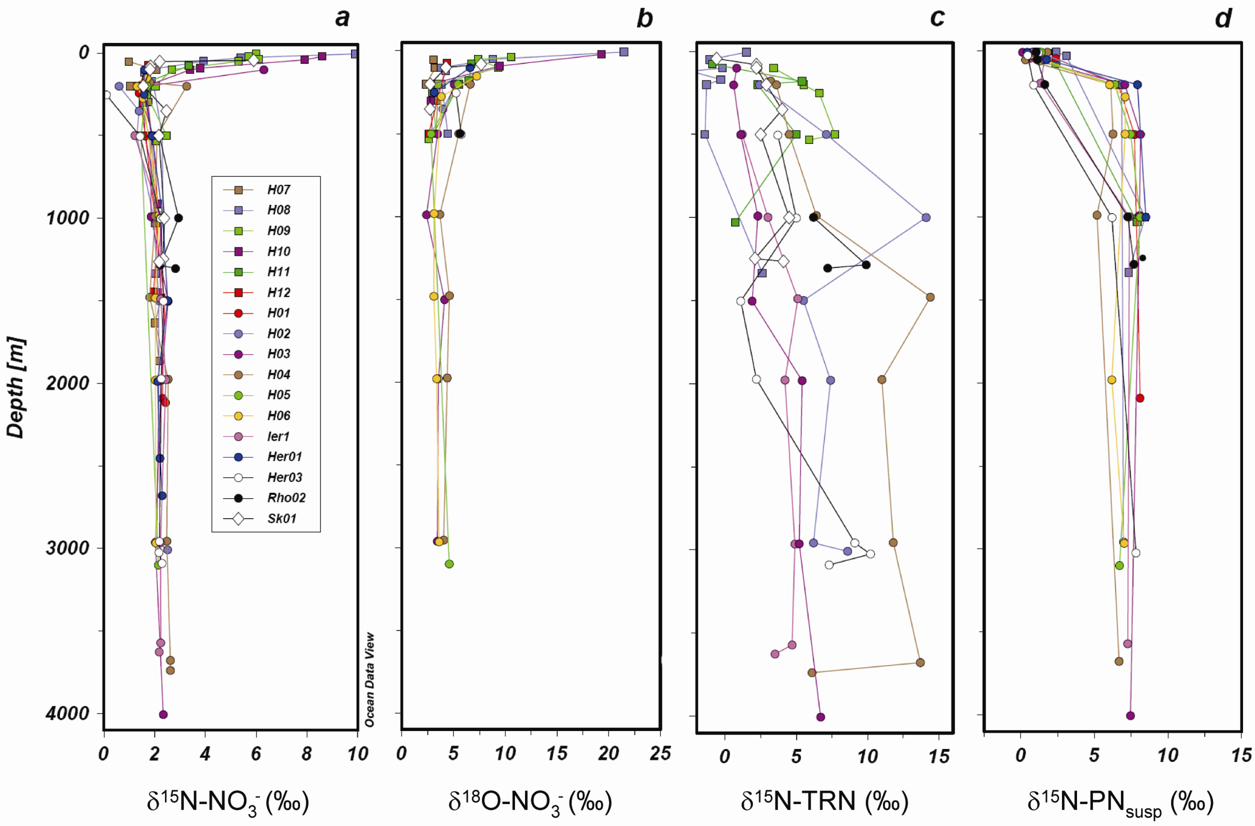


Figure 5.5: Isotopic composition δ^{15}N-NO_3^- (a), δ^{18}O-NO_3^- (b), δ^{15}N-TRN (c), and δ^{15} of suspended PN (d) plotted against water depth for all stations.

The average isotopic composition of nitrate in samples from the nitracline was similar in all station sets: δ^{15}N-NO_3^- averaged 1.6 and 2.1‰ in samples below the mixed layers of the pelagic and NIS stations, respectively, and 2.0‰ in the thick nitracline in the northern Aegean. δ^{18}O-NO_3^-

of samples from the nitracline had averages of 5.1‰ (NIS stations), 5.0‰ (pelagic stations), and 4.4‰ in the northern Aegean nitracline. Values of δ^{15}N-TRN and δ^{15}N-PN$_{susp}$ were higher in the thermocline samples than in the mixed layer.

In deep waters, δ^{15}N-NO$_3^-$ averaged between 2.0‰ and 2.3‰ in the three stations sets, and δ^{18}O-NO$_3^-$ was on average between 3.2‰ (NIS stations) and 3.8‰ for pelagic sites, respectively, and these deep water values were not statistically different. Suspended matter in deep waters at all sites had high δ^{15}N-PN$_{susp}$ averages of 7.2‰ to 8.1‰, while δ^{15}N-TRN measurements averaged between 2.4‰ and 6.7‰ over all sites. We attribute part of the high variability in δ^{15}N-TRN to measurement artefacts at the low concentrations encountered. Samples of deep waters below the nitracline at pelagic sites, where a relatively large sample pool was measured, had an average δ^{15}N-TRN of 6.7±3.5‰.

The δ^{15}N of sinking material captured by the two sediment traps during the deployment in 2007 (MID-03) differed significantly from δ^{15}N of suspended matter collected by filtration in deep water, and was essentially the same as δ^{15}N-PN$_{susp}$ found in the mixed layer during the expedition: The upper trap had an average δ^{15}N-SPN of 0.9±0.8‰ within the 216 days sampling period (1.0‰ flux weighted), whereas the lower trap average δ^{15}N-SPN was 0.8±1.0‰. This is somewhat lower than δ^{15}N-SPN for the 1999 deployment (MID-01; δ^{15}SPN =2.2±0.4‰), but is in agreement with data from trap MID-02 (deployment 2001/2002) that collected sinking particles with a mean δ^{15}N-SPN of 1.2±0.6 ‰ (Warnken, 2003). Together the three deployments cover a period from November to October, and thus the biologically active season for which we can expect the bulk of particle transport out of the mixed layer to occur. In a composite annual cycle constructed from all three deployments at 2700 m water depth (Fig. 5.6), SPN- flux peaks in March, whereas highest values of δ^{15}N-SPN (1.8‰) are indicated for the months of November and December. In general, seasonality in δ^{15}N-SPN is subdued and varies around an annual average of 1.1‰ with low values from April to May (0.3-0.5‰) and higher values (1.2–1.8‰) later in the year from June to December. A data synopsis is given in the auxiliary material.

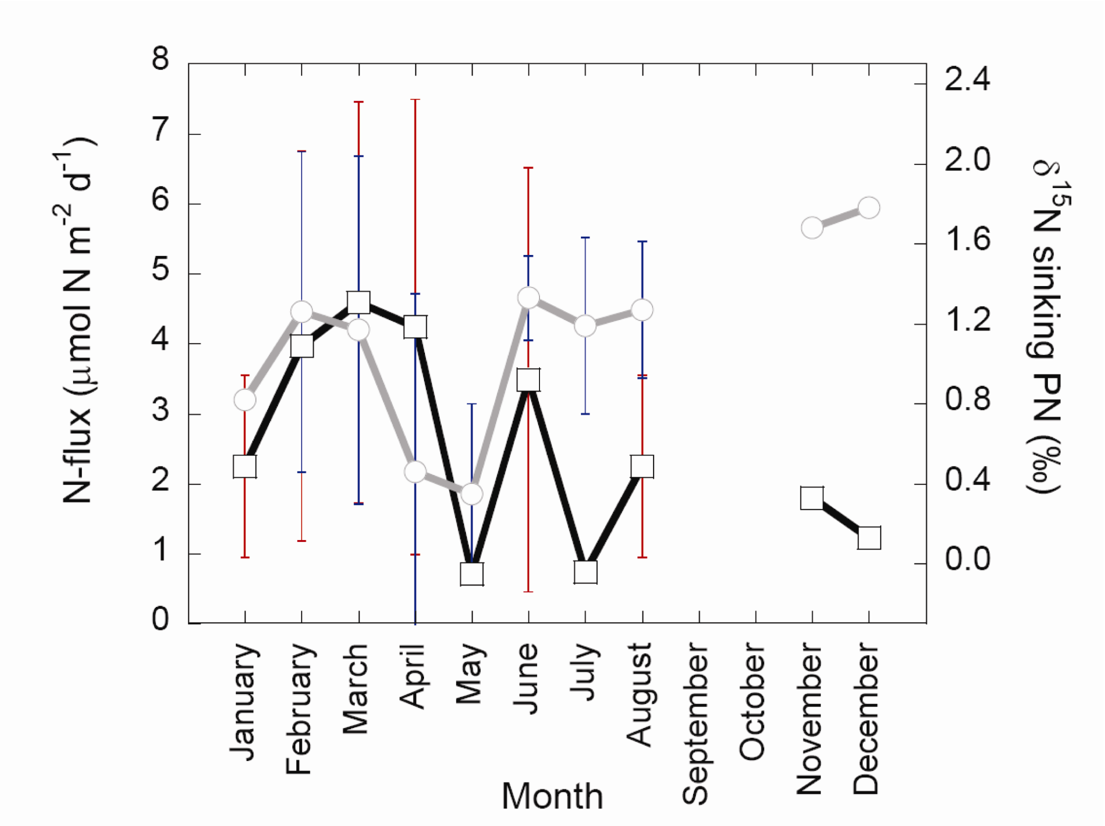


Figure 5.6: Composite seasonal diagram of sinking PN fluxes (squares) and δ^{15} of sinking PN (circles) and their standard deviations at 2600 m water depth at station Ierapetra (34°26´N, 26°11´E, water depth 3750 m). Fluxes (squares, black line and s.d. in red) and δ^{15}N-SPN (circles, grey line, s.d. in blue) for three deployment periods (MID-1: 01/30/1999 to 04/13/1999), MID-2: 11/05/2001 to 04/01/2002 and MID-3: 01/30/2007 to 09/05/2007) at 2700 m water depth have been assembled in a surrogate annual cycle.

5.4 Discussion

Unusual nutrient ratios and isotopic composition of reactive N (particulate and dissolved) in the Mediterranean Sea have fuelled disputes on the roles of natural processes and anthropogenic inputs in causing these anomalies. Using diagnostics of excess nitrate (Gruber and Sarmiento, 1997) and N-isotope compositions (Pantoja et al., 2002), most previous studies inferred substantial input of reactive N from N_2-fixation as the underling reason for both anomalies. More recent work has identified anthropogenic sources as a cause of both unbalanced N:P ratios and ^{15}N-depleted reactive nitrogen pools (Krom et al., 2010; Krom et al., 2004; Mara et al., 2009). But data on nitrate isotope composition in the basin are scarce, and that of the substantial pool of reduced dissolved nitrogen is unknown. Furthermore, it is yet unclear how a relatively recent contribution to the N-cycle in the basin, namely ^{15}N-depleted atmospheric NO_x inputs (Mara et al., 2009), may have been able to invade deep waters of the eastern Mediterranean Sea. The first aim of this study thus was to raise data of δ^{15}N from all water masses and in several relevant N-pools of the water column. The second aim was to evaluate if the N-isotope patterns of major dissolved and particulate phases of reactive N indeed bear an imprint of either N_2 fixation or the atmospheric NO_x input, and whether nitrate isotopic composition – using both N and O isotopes – clarifies the mechanisms by which the anthropogenic nitrate signal is entrained into the deep-water pool. In the following, we first establish average isotopic compositions of various N-pools and compare them with those of other environments. We then go on to evaluate previous hypotheses on the unusual composition of nitrate in light of our data, and finally use a simple model to constrain candidate sources of nitrate to the mixed layer of the eastern Mediterranean Sea.

5.4.1 Isotopic composition of reactive nitrogen in sub-nitracline and deep water masses

Table 5.1 is a compilation of inventories and δ^{15}N of different water masses in the eastern Mediterranean Sea and in different compartments of reactive N based on our data, according to which the mass-weighted and depth-integrated δ^{15}N of the basin is 2.8‰. Judging from this value, the inventory of reactive N in the eastern Mediterranean Sea is fundamentally different from the inventories of other oceans or regional marine systems studied so far. The most obvious difference is in the isotopic composition of reactive nitrogen in the depth interval below the nitracline and in the deep-water pool, which integrates the isotopic signal of all internal and external nitrate sources over the deep-water residence time of roughly 100 years (Roether et al., 1996). Differing from the pool in surface waters (see below), this deep nitrate pool is isotopically homogeneous in the eastern Mediterranean Sea. There are three previous data sets of widely differing δ^{15}N-NO_3^- in these deep and intermediate water masses: One group (Sachs and Repeta, 1999) determined δ^{15}N-NO_3^- as -0.7±0.1‰ in two samples of deep water (depths not given), analysed using the ammonium diffusion method (Sigman et al., 1997). Another group (Struck et al., 2001), also by the ammonium diffusion method, determined a mean of 7.3±2.8‰ in 6 samples of waters from between 200 m and 400 m water depth. A third group (Pantoja et al., 2002), who used a similar denitrifier method to that used in this study and show 6 data points below 500 m, report an average δ^{15}N-NO_3^- of 2.5±0.1‰ for only 4 samples in the text of that publication, and the mean is given as 2.4‰.

water depth interval (m)	water depth interval volume (km³)	mass nitrate (Gmol)	δ^{15}N-NO_3^- (‰)	mass suspended PN (Gmol)	δ^{15}N-PN_{susp} (‰)	mass TRN (Gmol)	δ^{15}N-TRN (‰)	sum reactive N (Gmol)	mass weighted δ^{15}N of reactive N (‰)
0-200	306200	357	2.4	112	1.6	876	1.1	1345	1.5
200-500	365300	1411	1.8	59	6.4	700	6.2	2171	3.3
>500	1719000	7949	2.2	249	7.2	3510	5.2	11709	3.2
all depths	2390500	9717	2.1	421	5.6	5086	4.6	15224	3.0

Table 5.1: Estimate of reactive N-inventories of the eastern Mediterranean Sea in different water depth intervals (corresponding to mixed layer, intermediate, and deep water masses) and mass-weighted δ^{15}N of the different components (nitrate, particulate nitrogen PN, and total reduced nitrogen, TRN). We calculated inventories (given in gigamol N) based on interval water volumes for the eastern Mediterranean Sea without Adriatic and Aegean sub-basins (R. Grimm, pers. comm., 2009) and weighted δ^{15}N values by average concentrations found during our cruise. The last column is the integrated and mass-weighted δ^{15}N over all components of reactive N for each interval, the last line are the integrated inventories and δ^{15}N over the entire water column.

Our data (Table 5.1) confirm that δ^{15}N-NO_3^- of nitrate in the deep water of the eastern Mediterranean Sea (average of samples below 500 m) is indeed low (2.2±0.3‰; n=68) compared to the narrow range of 4.7 to 5.4‰ for global ocean deep-water nitrate (Sigman et al., 2009). Our data set further shows that nitrate in the intermediate water mass from the base of nitracline to 500 m is even more ^{15}N-depleted (1.8±0.4‰; n=29) (Table 5.1). Deep water nitrate in the western Mediterranean Sea has a δ^{15}N-NO_3^- of 3.0 ±0.1‰ below 1500 m water depth (Pantoja et al., 2002), while δ^{15}N-NO_3^- is around 5‰ in waters >800 m deep in the adjacent NE subtropical Atlantic Ocean (Bourbonnais et al., 2009). The residence time of deep-water nitrate in the eastern Mediterranean sea has been estimated to be 125 years (Mara et al., 2009), and the pool is not directly linked to deep waters of the western Mediterranean or beyond. It is fed mainly by mineralisation/nitrification of particles sinking from the mixed layer and by preformed nitrate downwelled during deep-water formation which is not subsequently modified by denitrification. The main reactive N sink is the LIW water mass that exports nitrate and reduced nitrogen (mainly in the form of DON) to the western Mediterranean Sea at depths between 150 and 500 m (Ribeira d´Alcala et al., 2003). Plausible external sources of reactive N are N_2-fixation, atmospheric N-deposition, river runoff and the relatively small amount advected into the basin with surface water inflow from the western Mediterranean and the Black Sea.

The comparatively small size of the deep-water nitrate pool as a result of its relatively low concentrations (5-6 µmol L^{-1}), its relatively short residence time compared to that of other oceans, and the ^{15}N depleted external nitrate sources (runoff and atmospheric deposition without N_2 fixation have an annual load-weighted $\delta^{15}NO_3^-$ estimated at 0.5 to 1.5‰) (Mara et al., 2009) conspire with the lack of mid-water denitrification to depress the overall level of $\delta^{15}N$ in the eastern Mediterranean Sea. Depleted inputs imply that over one residence time (~125 yrs) the deep-water $\delta^{15}N$-NO_3^- should be depressed to approach the $\delta^{15}N$ of inputs (Mara et al., 2009). The only data of $\delta^{15}N$-NO_3^- available (also determined by the denitrifier method and with the same internal standard deviation of the method) to gauge possible systematic time-dependent changes are those for 4 samples taken in 1999 in the eastern Mediterranean Sea (Pantoja et al., 2002). These data had an average of 2.5±0.1‰ below 500 m depth. Average $\delta^{15}N$-NO_3^- in our samples taken from below 500 m water depth 8 years later is 2.2±0.3‰ (n=68). While the difference in isotopic ratio between the two data sets is in accord with a gradual decrease of $\delta^{15}N$-NO_3^- over time, the magnitude of the change is not large enough to confirm the hypothesis. We point out, however, that the interval-integrated and mass-weighted $\delta^{15}N$ of reactive N in the water column (Table 5.1) is lowest in the surface layer, which has a much shorter residence time than deep water (on the order of years), is within the $\delta^{15}N$ range of estimated external N inputs (Mara et al., 2009). Furthermore, the intermediate water which is being exported from the basin has a nitrate $\delta^{15}N$ value (1.8±0.4‰) close to the external supply. It is thus suggested that the $\delta^{15}N$-NO_3^- in the deeper waters of this basin reflect the ^{15}N depleted (anthropogenically dominated) external inputs, particularly since it has been concluded that there is no regionally significant N_2 fixation in the eastern Mediterranean basin (Krom et al., 2010). As stated above, the residence time of nitrate in deep water is on the order of 125 years and ^{15}N depleted inputs from anthropogenic sources may be assumed to data back only 4 or 5 decades (Preunkert et al., 2003). We infer that the isotopic signature of anthropogenic nitrate inputs to the surface layer has since then been imprinted on the deep water pool more rapidly than by mere water mass mixing and convection via the shortcut of assimilation, rapid downward particle transport (SPN), and mineralisation of reduced N to nitrate in the deep water mass. This N-shuttle will be discussed below.

The deep-water (>500 m) nitrate pool is further unusual in that it is ^{18}O richer ($\delta^{18}O$ = 3.7±0.9‰, n=25) than other deep-water pools in the world ocean (Sigman et al., 2009). In Table 2 of that publication, available data for the $\delta^{18}O$ of deep nitrate from a variety of other deep oceanic environments are reported, and all are in the range of 1.8 to 2.8 ‰ (except in the water depth interval from 300 to 1500 m eastern tropical Pacific with a $\delta^{18}O$ of 7.0 ‰) than the deep nitrate pool of the eastern Mediterranean. One previous reported value for the $\delta^{18}O$-NO_3^- of deep water in the westernmost Mediterranean Sea (Sigman et al., 2009) was 2.6‰ for the depth range of 1500 m to the seafloor, and 3.1±0.1 ‰ for a composite value of in parallel measurements of 4 samples in deep water.

One possible reason for more positive δ^{18}O-NO_3^- in eastern Mediterranean Sea deep water than in other deep ocean pools may be that it is a concentration basin where evaporation exceeds precipitation and river runoff (E/P+R ratio of 1.2 in winter and 1.83 in summer) (Gat et al., 1996). The average δ^{18}O-H_2O in water samples deeper than 500 m is 1.43±0.18‰ and 1.44±0.16‰ in the 0-500 m depth range (Pierre, 1999), and thus is around 1.4‰ heavier than in other ocean basins (LeGrande and Schmidt, 2006). Based on experiments (Granger et al., 2004), it has been suggested that the nitrate produced by nitrification does not permanently retain oxygen from dissolved O_2 – required for the electron transfer to oxidise NH_4^+ to NO_2^- and subsequently to NO_3^- –, but that this oxygen is rapidly exchanged with ambient water. It is thus possible that the δ^{18}O-NO_3^- is overprinted by the δ^{18}O of ambient water with a positive offset of approximately 2‰. The offset observed in deep waters of the global ocean is thought to result from nitrification (in low latitudes with essentially complete nitrate assimilation in surface waters) with a small positive shift in δ^{18}O, and an added positive shift from admixture of preformed nitrate with elevated δ^{18}O-NO_3^- due to partial assimilation in high latitudes (Sigman et al., 2009). An analogous situation may be at play in the eastern Mediterranean: Deep water formation in the northern basins (Aegean and Adriatic Seas), where surface water cools and sinks during winter months, exports preformed and ^{18}O enriched nitrate (from partial assimilation) from the sea surface to the deep eastern Mediterranean Sea, where it mixes with nitrate from nitrification.

Concentrations of TRN (predominantly DON) in deep and sub-nitracline intermediate waters are also very low compared with other environments (Berman and Bronk, 2003) and match low suspended PN concentrations. To our knowledge, no data exist on δ^{15}N-TRN from deep waters of the global ocean, but TRN in the deep (>500m) eastern Mediterranean Sea is clearly more enriched in ^{15}N (δ^{15}N-TRN = 6.0±3.7‰, n=39) than in surface waters. It is also more enriched than in the subtropical (3.9±0.4‰) and equatorial NW Atlantic (δ^{15}N-TRN = 4.1±0.6‰), and the subtropical NE Atlantic (2.6±0.4‰), but in the range of values reported from shallower depths of the subtropical North Pacific (5.4±0.8‰) (Bourbonnais et al., 2009; Knapp et al., 2005; Meador et al., 2007).

The ^{15}N-enriched TRN in deep water of the eastern Mediterranean Sea coexists with low concentrations of even more ^{15}N enriched suspended PN, whereas our sediment trap data suggest that the δ^{15}N of material sinking rapidly from the mixed layer is <2‰ over the entire year and – differing from other observations (Altabet, 1988; Gaye-Haake et al., 2005) – is apparently not enriched in ^{15}N during its passage through the water column. We did measure a significant decrease in the flux of sinking PN with depth, with the flux in the upper sediment trap being 5.7 mmol N m^{-2} over the period of 216 days, decreasing to 1.4 mmol N m^{-2} at 2700 m water depth (second trap). This implies a loss 75% of particulate N flux to disintegration (and partial remineralisation) over an 1100 m water column. Because both the concentrations of suspended PN and TRN in ambient water are low, and also decrease with depth (or are invariant), most of the loss must be to ammonification and rapid nitrification to nitrate. In consequence, both TRN and suspended PN in deep water are very likely enriched residues of mineralisation, from which some lighter product originated (Fig. 5.1). Because at the same time all meso-zooplankton size classes in deep waters (Koppelmann et al., 2009) and surface sediments (Struck et al., 2001) of the eastern Mediterranean Sea are also enriched over the sinking PN flux, the product that is depleted in ^{15}N is likely to be nitrate. Thus the efficient cycling of labile sinking PN in this ultra-oligotrophic system results in ^{15}N depleted nitrate and enriched TRN and suspended PN.

5.4.2 Nitrate in the surface layer: Testing the model of incomplete nitrate utilisation

The intermediate water nitrate pool provides the bulk of nitrate available for assimilation in the euphotic zone of the eastern Mediterranean Sea. The phytoplankton bloom takes place over the winter period (October-March) soon after the seasonal thermocline breaks down in autumn (October) and nutrients are mixed into the surface layer. Nutrients are removed from the mixed layer, until all of the phosphate has been taken up and excess nitrate remains, together with biomass and TRN produced; sinking particles exit the mixed layer across the pycnocline.

At all stations sampled during M71-3, there was excess nitrate remaining in the surface mixed layer. The average nitrate remaining in the euphotic zone was between 0.24 and 0.48 µM, and the actual amount of residual nitrate varied with location and stage of thermocline evolution. These concentrations are similar to those found previously for the average winter residual nitrate across the Southern Levantine basin (0.6±0.5 µM) (Kress and Herut, 2001). At all stations, phosphate was depleted in surface waters, with all values being below detection limits (<20 nM).

If the concept of incomplete nitrate utilisation (Struck et al., 2001) is correct and no other sources are involved, it requires that residual nitrate evolves isotopically from the nitrate provided by mixing (Mariotti et al., 1981). The initial mixed-layer nitrate pool in a closed system is progressively assimilated and will become enriched in ^{15}N (and ^{18}O) in the course of assimilation, in analogy to the Rayleigh distillation process. The enrichment can be approximated by (Mariotti et al., 1981):

$$\delta^{15}NO^-_{3\,residual} = \delta^{15}NO^-_{3\,initial} + {}^{15}\varepsilon \times \ln(f) \qquad \text{(Eq. 5.2)}$$

with $f = [NO_3^-]_{residual}/[NO_3^-]_{initial}$, and $^{15}\varepsilon$ expressing the fractionation factor (in ‰) between product and substrate. The range of $^{15}\varepsilon$ reported in the literature is large and differs for different primary producers. A recent compilation (York et al., 2007) reports $^{15}\varepsilon$ from -16 to 6‰ (negative values meaning that ^{14}N is preferentially assimilated), but is commonly assumed to be -5‰. Available field and experimental data suggest equal separation factors $^{15}\varepsilon$ and $^{18}\varepsilon$ for nitrate assimilation (Casciotti et al., 2002; Granger et al., 2004; Lehmann et al., 2005).

Simplifying the mixed layer to being a closed system and using the Rayleigh closed-system approach, we assume an initial nitrate concentration in the mixed layer of 1.8 μmol L^{-1} at the onset of the phytoplankton bloom (Krom et al., 2003), and at the nitrate isotope signature of water below the thermocline before the onset of phytoplankton assimilation. The Rayleigh model predicts that by the time that 70% of the initial nitrate in the NIS mixed layer has been assimilated into PN, SPN and TRN (such as is the case in the average profile over all stations there), the residual nitrate should have a $\delta^{15}N\text{-}NO_3^-$ of 8.6‰, which is higher than the average of 5.6‰ found in our samples. Surface layer nitrate concentrations at the pelagic stations were only 15% of the assumed initial nitrate and should have a $\delta^{15}N\text{-}NO_3^-$ of 11.5‰, whereas we found 2.3‰. The $\delta^{15}N\text{-}NO_3^-$ of nitrate remaining in the mixed layers found in both environments suggests either a much lower fractionation factor for nitrate assimilation than -5‰, or an additional source of ^{15}N-depleted nitrate.

5.4.3 Constraints from differences in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$

At all stations (NIS and pelagic), a second and related indicator for a deviation from simple enrichment due to assimilation of thermocline nitrate is the decoupling of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O$-

NO_3^- in residual nitrate in the mixed layer. This is seen in Figures 5.5a and 5.5b, where δ^{18}O-NO_3^- in surface layer samples is significantly higher than corresponding δ^{15}N-NO_3^-. This could be caused by unequal separation factors for δ^{15}N-NO_3^- and δ^{18}O-NO_3^- during nitrate assimilation by phytoplankton, but this would run counter to available evidence from culture studies and field observations (Casciotti et al., 2002; Granger et al., 2004; Lehmann et al., 2005). Accepting this, the two isotopes of nitrate should evolve in parallel from the isotopic composition of nitrate at the base of the thermocline, if only one nitrate source were assimilated. This is not the case.

Any deviation of δ^{15}N and δ^{18}O from the 1:1 enrichment line during assimilation and originating from the nitrate source may expressed as a nitrate isotope anomaly Δ(15,18) (Sigman et al., 2005):

$$\Delta(15{,}18) = \left(\delta^{15}N_{measured} - \delta^{15}N_{source}\right) - {}^{15}\varepsilon/{}^{18}\varepsilon \times \left(\delta^{18}O_{measured} - \delta^{18}O_{source}\right) \qquad \text{(Eq. 5.3)}$$

Fig. 5.7 is a depth plot of Δ(15,18) for different station sets over the top 500 m and is calculated by using the average δ^{15}N-NO_3^- and δ^{18}O-NO_3^- of deep water as $\delta^{15}N_{source}$ and $\delta^{18}O_{source}$, respectively. Our data points are scarce in the nitrate-depleted mixed layers, but suggest an average Δ(15,18) of mixed layer nitrate of around -3‰, and both station sets suggest a decrease towards the sea surface. The negative values imply that nitrate in the surface layer is not solely a residue of an initial nitrate pool or nitrate provided by cross-thermocline transport; in this case, Δ(15,18) should be 0‰, because $\delta^{15}N_{source}$ and $\delta^{18}O_{source}$ would evolve in parallel. Instead, the negative deviation indicates an additional nitrate source depleted in ^{15}N (or enriched in ^{18}O) over the residual thermocline nitrate, and suggests either nitrate generation via nitrification while nitrate in the mixed layer is being assimilated, or external input of nitrate with a different isotopic makeup than the thermocline nitrate (Sigman et al., 2005). These alternatives are discussed below.

Nitrification of fixed N or recycled N

Nitrification in the presence of nitrate assimilation, via ammonification of particulate N and/or DON, is the standard mechanism to cause a negative Δ(15,18) in eupthotic zones. In the few oligotrophic environments studied so far, observed negative Δ(15,18) have been attributed to nitrification of comparatively ^{15}N-depleted PN produced from N_2-fixation and possibly of DON (Bourbonnais et al., 2009; Casciotti et al., 2008; Knapp et al., 2008). In these cases, the isotopic composition of recycled nitrate is set by the $\delta^{15}N$ of fixed N (~ -1‰) on the one hand, and by the $\delta^{18}O$ of ambient seawater with a positive offset of 2‰. Thus nitrate deriving from N_2 fixation is added to the mixed-layer nitrate pool with a $\delta^{15}N\text{-}NO_3^-$ of ~ -1‰ and $\delta^{18}O\text{-}NO_3^-$ of ~ 2‰ (Bourbonnais et al., 2009). In the eastern Mediterranean Sea with its higher $\delta^{18}O$ of seawater, we would expect the recycled nitrate from fixed N_2 to have a $\delta^{15}N\text{-}NO_3^-$ of ~ -1‰ and a $\delta^{18}O\text{-}NO_3^-$ of ~ 3.4‰.

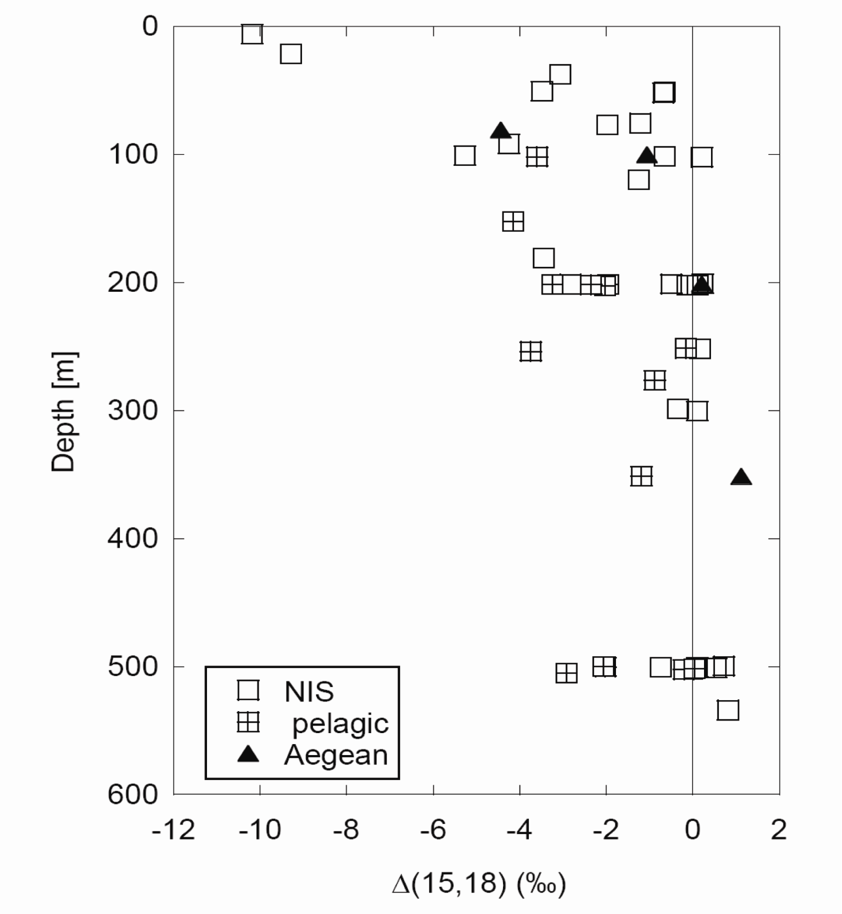


Figure 5.7: Depth plot of Δ(15,18) of nitrate for different station sets in the upper 500 m of the water column.

Partitioning of ammonium released from PN to either nitrification or ammonium uptake in the mixed layer has also been shown to cause a range of Δ(15,18), because both processes are associated with different $^{15}\varepsilon$ (Sigman et al., 2005; Wankel et al., 2006). Negative Δ(15,18) in residual nitrate may ensue, because nitrate returned from PN mineralisation via ammonium oxidation is relatively more depleted in ^{15}N than the residual nitrate pool, whereas its $\delta^{18}O$ is pegged to ambient water (Fig. 5.1). Because ammonium assimilation by phytoplankton has a higher preference for ^{15}N than nitrification (Casciotti et al., 2003; Cifuentes et al., 1989), significant ratios of ammonium assimilation versus nitrification will cause biomass and thus PN to be relatively enriched in ^{15}N compared with the recycled nitrate. Because we find no indication for enriched SPN or PN in the mixed layers, and the sediment trap data suggest that SPN is on annual average even more depleted in ^{15}N than thermocline nitrate, we believe that assimilation of mineralised ammonium is unlikely to play a major role as a substrate for phytoplankton growth in the mixed layer, and as a cause for negative $\Delta(15,18)$. But the isotopic data cannot completely rule out assimilation of remineralized ammonium: If relatively little nitrification takes place, particulate N could be remineralized and ammonium completely re-assimilated without involving isotope fractionation.

Input of NO_x

Aside from an internal source of nitrate, there must be a second source that causes negative $\Delta(15,18)$ in the mixed layer of the eastern Mediterranean Sea. We have evidence to suggest that NO_x inputs play a relatively larger role in the eastern Mediterranean Sea than elsewhere. Two previous studies (Bourbonnais et al., 2009; Knapp et al., 2008) acknowledge (but dismiss) the possibility that the negative $\Delta(15,18)$ observed in mixed layer nitrate of the oligotrophic Sargasso Sea and subtropical NE Atlantic Ocean indicate atmospheric NO_x inputs, which differ strongly from thermocline nitrate in $\delta^{18}O\text{-}NO_3^-$ (>60‰) at $\delta^{15}N\text{-}NO_3^-$ ranging from -15 to 15‰ (Kendall et al., 2007). By contrast, such an input to the eastern Mediterranean Sea surface layer would not be immediately assimilated due to phosphate limitation, and thus would introduce nitrate with low $\delta^{15}N\text{-}NO_3^-$ and high $\delta^{18}O\text{-}NO_3^-$ into the surface mixed layer. Model estimates of annual total NO_x inputs in wet and dry deposition to the eastern Mediterranean Sea surface range between 200 and 400 mg N m^{-2} a^{-1}; reduced N (mainly ammonium) deposition is lower (200 - 20 mg N m^{-2} a^{-1}) (http://www.emep.int). Both inputs have a pronounced N-S gradient, with reduced N having a steeper land-sea gradient. An estimated 14-29 mmol NO_x m^{-2} a^{-1} of industrial origin are thus supplied annually to the eastern Mediterranean sea surface, plus a smaller contribution of reduced N.

The atmospheric deposition on the island of Crete in winter months of 2006/2007 had a mean $\delta^{15}N\text{-}NO_3^-$ of -2.0‰ (Mara et al., 2009), and likely had a high $\delta^{18}O\text{-}NO_3^-$: 13 samples of dry atmospheric deposition (no rain events recorded) collected on the Island of Crete during June-September 2007 had an average $\delta^{18}O\text{-}NO_3^-$ of 67.5+4.2‰ (Mara, unpubl. data). Although this data does not cover the time of predominantly wet deposition in winter, the values are within in the range reported from other environments (Kendall et al., 2007) and from the nearby northern Red Sea (Wankel et al., 2009). There, $\delta^{15}N$ values of water-soluble nitrate in aerosol samples ranged from −6.9‰ to +1.9‰ and $\delta^{18}O$ was found to range from 65.1‰ to 84.9‰ with highest $\delta^{18}O$ values in the winter. That nitrate was deposited from air masses deriving from the Mediterranean Sea and western Europe (Wankel et al., 2009). Thus, although only <10% of nitrate remaining in the mixed layer of the eastern Mediterranean Sea may have originated from the atmospheric source over 4 months, the effect on average $\delta^{18}O\text{-}NO_3^-$ and $\Delta(15,18)$ is large due to the small amount of nitrate in the mixed layer of the eastern Mediterranean Sea and the high $\delta^{18}O\text{-}NO_3^-$ of atmospheric inputs. Of all oligotrophic ocean regions, the eastern Mediterranean surface mixed layer is arguably the most sensitive place in the world ocean to register that input. The nitrate pool here is very small and the system is phosphate limited. Most other upper ocean waters that receive a sizeable NO_x input (Duce et al., 2008) are N-limited, so that any nitrate entering the surface ocean there should be immediately assimilated. This assimilation (and subsequent recycling to nitrate) would eradicate the $\delta^{18}O$ signal diagnostic of atmospheric NO_x and would make it impossible to trace.

We can use the isotopic signatures of putative internal (recycling) and external (N_2 fixation and NO_x) inputs together with an estimate of N export flux in a conceptual steady-state mass and isotope balance model to explore the effects of externally and internally supplied N on $\Delta(15,18)$. The model is the same as that used in the subtropical SE Atlantic (Bourbonnais et al., 2009), and we refer to that publication for more details. Fig. 5.1 sketched possible sources, sinks and transformations in the N-cycle of the eastern Mediterranean Sea that is suitable to illustrate our reasoning and the modelling approach: The nitrate pool in surface water is fed by external (NO_x, N_2 fixed, and nitrate from cross-thermocline mixing, *ku*) and internal (mineralisation and nitrification of PN in the surface mixed layer) sources. The nitrate sink for the surface layer is assimilation into particulate N (with TRN/DON as a by-product) and export of sinking particulate nitrogen across the thermocline.

The nitrate below the thermocline is a mixture of a standing stock of nitrate in deep/intermediate water and addition from mineralisation of sinking particulate nitrogen. In our interpretation, the difference between deep-water suspended matter and TRN on the one hand and sinking PN and nitrate on the other hand arise, when small particles shed from the sinking PN are ammonified into ^{15}N-depleted ammonium that is then completely oxidised to nitrate. The small residual particles (suspended PN) are thus enriched in ^{15}N. A by-product of feeding on this suspended particulate N is that TRN/DON is also enriched in ^{15}N as compared to the sinking material. Nitrification of ammonium liberated from sinking PN adds nitrate with a δ^{18}O (marked in blue in Fig. 5.1) approximately 2‰ higher than ambient seawater – in the case of the eastern Mediterranean sea, this nitrate is expected to have a δ^{18}O-NO_3^- of 3.4‰. As stated above, we consider ammonium assimilation to be of minor importance.

We point out that nitrate added from the mineralisation of fixed N_2 or from sinking PN produced from assimilation of thermocline nitrate both acquire the same δ^{18}O after nitrification (through exchange with ambient water), but have slightly different δ^{15}N. Both these additions cause negative excursions in Δ(15,18), because this term expresses the deviation in the dual isotopes of nitrate from the isotopic composition of nitrate provided by the thermocline flux *ku* and enriched during assimilation with a 1:1 slope. If only the upward flux across the thermocline were the source of nitrate in the surface, Δ(15,18) would remain 0‰, regardless of the extent of nitrate assimilated,. The other possible source of nitrate (NO_x from the atmosphere) has a δ^{18}O of 65‰ at a δ^{15}N of -2‰. If not assimilated immediately, this atmospheric input lowers the δ^{15}N-NO_3^- while raising the δ^{18}O-NO_3^- of the surface nitrate pool, and would also cause a negative Δ(15,18) for a different reason.

We adapted the model to calculate Δ(15,18) (Bourbonnais et al., 2009) to conditions in the eastern Mediterranean Sea (see auxiliary material). We chose appropriate boundary conditions for the thermocline nitrate isotopic composition, the δ^{18}O of seawater, and the observed N-export production of 56 mmol per year (see auxiliary material). That flux is fixed; added inputs from N_2 fixation or NO_x reduce *ku* by the equivalent amount. If nitrification of particulate N occurs in the surface layer, this requires that the amount of nitrate assimilated in the surface layer is higher by the amount nitrified to maintain the export flux, but it does not affect *ku*. All N sources have a specific δ^{15}N signature, and the nitrate mixture in the surface layer integrates these isotopic contributions. That pool of nitrate is assimilated into biomass, and the residual nitrate pool in the surface layer is isotopically enriched during phytoplankton assimilation with equal fractionation factors $^{15}\varepsilon = {}^{18}\varepsilon = -5$‰: At any stage of assimilation, the δ^{15}N of sinking PN is 5‰ more depleted than the residual nitrate pool, while the δ^{18}O signal of the assimilated nitrate is lost. In the model, recycled nitrate (nitrification) inherits the δ^{15}N of sinking PN (that has or not a contribution from N_2 fixation) and the δ^{18}O of seawater (with an offset of +2‰).

We calculated hypothetical Δ(15,18) for a range of input conditions from 0-40 mmol N from N_2 fixation and 0-40 mmol N of recycled nitrate, both of which acquire a $\delta^{18}O\text{-}NO_3^-$ of 3.4‰. Nitrate input from N_2-fixation needs to be 40 mmol m^{-2} a^{-1} (>70% of the 56 mmol m^{-2} a^{-1} PN export flux) to create a Δ(15,18) of around –2‰; input of 40 mmol m^{-2} a^{-1} of recycled nitrate yielded a Δ(15,18) of –3.6‰, which is close to the roughly -3‰ observed. For an assumed unassimilated NO_x input of only 5 mmol m^{-2}, which is well within the known atmospheric NO_x flux to the eastern Mediterranean, the model calculates a Δ(15,18) of –5.8‰ (see auxiliary material).

These calculations suggest a number of possible combinations of external and internal sources that can theoretically result in the nitrate isotope anomaly observed in the mixed layer. Taken alone they are inconclusive because the Δ(15,18), which is well suited to diagnose nitrate sources in other areas of the oligotrophic surface ocean, is somewhat blunt here due the similarities in isotopic composition of all external and internal sources. However, our preferred interpretation of the data is that they represent a mixture of regenerated nitrate and NO_x input, because both are known to be inputs to the mixed layer in the necessary magnitudes and isotopic ranges to fully describe the changes observed. We consider significant input from N_2 fixation unlikely for the time of our expedition, both because we sampled a winter P limited situation, and because N_2 fixation measurements across the region sampled here were very low when sampled in June 2007 (Ibello et al., 2010). Furthermore nutrient budgets of the basin suggest there is no significant nitrogen fixation (Krom et al., 2010). By contrast there is ample evidence that primary production in the eastern Mediterranean Sea is supported by regenerated nutrients that are entrained into a microbial loop operating in surface waters (Thingstad et al., 2005; Zohary and Robarts, 1998). Nutrient budgets (Ribera d´Alcala et al., 2003) and experimental work (Thingstad and Rassoulzadegan, 1999; Thingstad et al., 2005) both imply that surface productivity is to a significant extent supported by regenerated nitrate. In addition, recent genetic investigations suggest that ammonium-oxidizing *Archaea* in mesopelagic waters of the eastern basin may have a central role in nitrification of ammonium liberated from particulate N (De Corte et al., 2009). Our data underline the importance of recycled nitrate, but also stress that atmospheric NO_x inputs to the basin must be taken into account in mass-based (Krom et al., 2004) and isotope-based (Mara et al., 2009) budgets of the N-cycle in the eastern Mediterranean Sea.

5.4.4 Is TRN involved in N-cycling?

TRN is the largest pool or reactive N in the mixed layer and after nitrate the second largest in the entire water column of the eastern Mediterranean Sea (Table 5.1). Because ammonium levels are low, the major constituent of TRN in our samples is likely to be dissolved organic nitrogen, which is a by-product of N_2-fixation or of grazing in the mixed layer (Berman and Bronk, 2003). It has been discussed as a possible substrate for phytoplankton assimilation or ammonification and nitrification processes (Bronk et al., 2007), and its isotopic composition should reflect TRN cycling (Knapp et al., 2005). In a previous study from the Sargasso Sea, the small concentration and δ^{15}N-DON differences between the surface and subsurface waters suggested that DON there is recalcitrant and the data indicated only limited DON turnover in that area (Knapp et al., 2005). Although our data is somewhat limited, we see a trend of decreasing concentrations and increasing δ^{15}N-TRN between surface and deep-water samples that imply active participation of TRN in reactive N turnover. Our interpretation of the similar trends in δ^{15}N composition of PN_{susp} and TRN (except in the intermediate water mass, where our data are scarce) is that they are closely coupled, and that both reflect ^{15}N enrichment in the course of mineralisation to produce ^{15}N depleted nitrate in the deep water mass, and most likely even more intensely in the intermediate water mass.

The low δ^{15}N-TRN found in the surface layer of the eastern Mediterranean Sea is in accord with rapid grazing and recycling of nutrients during the winter bloom. At pelagic sites, the stable seasonal thermocline had developed prior to our expedition, and had caused the winter phytoplankton bloom to cease, a condition typically reached at northern Ionian Sea sites in March/April. By May, phytoplankton in the surface layers of the eastern Mediterranean Sea are commonly N- and P-co-limited (Zohary and Robarts, 1998). According to our current understanding, active grazing of small phytoplankton populations persisting on recycled nitrate continues in the mixed layer (Krom et al., 2005b; Thingstad et al., 2005), with the result that over time the residual nitrate is progressively converted into DON. No isotopic measurements have been made of suspended PN and TRN/DON in summer, but we would predict that the both should be isotopically enriched compared to values obtained in this study of a winter situation.

5.5 Conclusions

The total pool of reactive nitrogen (nitrate, dissolved total reduced nitrogen, and particulate nitrogen) in the eastern Mediterranean Sea is unusually depleted in ^{15}N compared to the global ocean. This must to a large extent be due to the isolation and anti-estuarine circulation of the silled basin that prevents communication with the global deep-water nitrate pool that is very homogeneous at $\delta^{15}N$-NO_3^- ~ 5‰/ $\delta^{18}O$-NO_3^- ~2‰ (Sigman et al., 2009). The low values of $\delta^{15}N$ in this ocean basin reflect the dominance of isotopically depleted N-sources, because processes that enrich ^{15}N in nitrate of other oceans (such as mid-water denitrication) are not acting here. The ^{15}N depleted nitrate in the deep water contrasts with ^{15}N-enriched TRN (mainly DON) and suspended PN in the same water mass. It is inferred that this is due to extensive and efficient mineralisation of the rapidly sinking fresh PN (sampled in sediment traps) in the ultra-oligotrophic system. We hypothesize that the $\delta^{15}N$ value of nitrate in the deep water may be decreasing with time in response to the depleted (mainly anthropogenic) external supply of NO_x, although the data is too sparse as yet to be conclusive. The data in this study was collected in the winter of 2006/2007, which coincided with a typical winter bloom at the stations in the northern Ionian basin and with an (unusually early) mature thermocline at maximum depth in the remaining pelagic stations. Although they are only a snapshot of a seasonal cycle in the mixed layer, the data from two situations (deepening thermocline and mature thermocline) in that cycle permit an initial assessment of different nitrate sources to the mixed layer.

The $\delta^{15}N$ of nitrate, TRN and PN cannot only result from partial N uptake caused by the extant P-limited phytoplankton bloom. It requires in addition a source of isotopically distinct nitrate, which may be internal (recycled nitrate) or external (N_2 fixation or NO_x input). Possible ranges for the nitrate isotope anomaly $\Delta(15,18)$ in the mixed layer calculated by a simple model point towards nitrification and/or a relatively small (and realistic) contribution of atmospheric nitrate with diagnostic $\delta^{15}N$ and $\delta^{18}O$ as the most likely sources of additional nitrate. It is known that the eastern Mediterranean Sea receives a significant supply of anthropogenic NO_x, which together with known recycling processes adequately describes the isotope distribution. These patterns could also be caused by extensive N_2 fixation, but that would require very high rates of diazotrophy in winter, when there are insignificantly low levels measured in the region even in summer.

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Auxiliary material

Δ(15,18) model boundary conditions and simulation results

The model is analogous to a published model [*Bourbonnais et al.*, 2009] with boundary conditions adapted to the EMS; processes are schematically shown in Fig. 5.1.

At steady state (run 0) with no contribution of fixed N_2, NO_x or nitrification, the modelled mixed layer nitrate pool in the eastern Mediterranean Sea is solely fed by sub-thermocline nitrate. This new nitrate is again exported as SPN and the upward nitrate flux balances export flux. The N-export monitored in our sediment traps is used to give an estimate of the SPN export and thus the upward nitrate flux. SPN flux in the ocean is exponentially linked to water depth [*Pace et al.*, 1987], and from the fluxes at the two trap depths of the Ierapetra mooring, we estimate the PN flux across base of the mixed layer (set at 200 m water depth) to 33 mmol N m^{-2} in the 216 days of the MID-3 deployment, or 56 mmol N m^{-2} prorated for an entire year, which agrees well with export fluxes reported from other sediment trap experiments in the area [*Boldrin et al.*, 2002]. This SPN export flux should not increase from external N additions, because it is essentially limited by phosphate. Any external addition of N (by fixation or NO_x input) would decrease the upward flux *ku* in steady state [*Bourbonnais et al.*, 2009], while internal nitrate generation/assimilation from nitrification of SPN in the surface layer does not affect the upward flux or the export flux. The $\delta^{15}N$ of SPN is determined by the $\delta^{15}N\text{-}NO_3^-$ of the surface box and the fractionation factor $^{15}\varepsilon$. We set $^{15}e = {}^{18}e = -5‰$ for the permil fractionation factor of mixed layer nitrate assimilation. We then calculate the $\delta^{15}N$ and $\delta^{18}O$ of the isotopic mixture of nitrate remaining in the mixed layer under steady-state conditions, and the Δ(15,18) of that nitrate mixture under varying inputs. Assumed inputs to mixed layer nitrate are 1) only thermocline nitrate (control run 0), 2) varying contributions of nitrate recycled from SPN assimilated from deep-water nitrate and recycled nitrate with a $\delta^{18}O\text{-}NO_3^-$ of 3.4‰ (runs 1-4), 3) varying contributions of nitrate recycled from SPN deriving from deep-water nitrate and nitrification of fixed N_2 (runs 5-8), and 4) input of 5 mmol a^{-1} of NO_x (run 9).

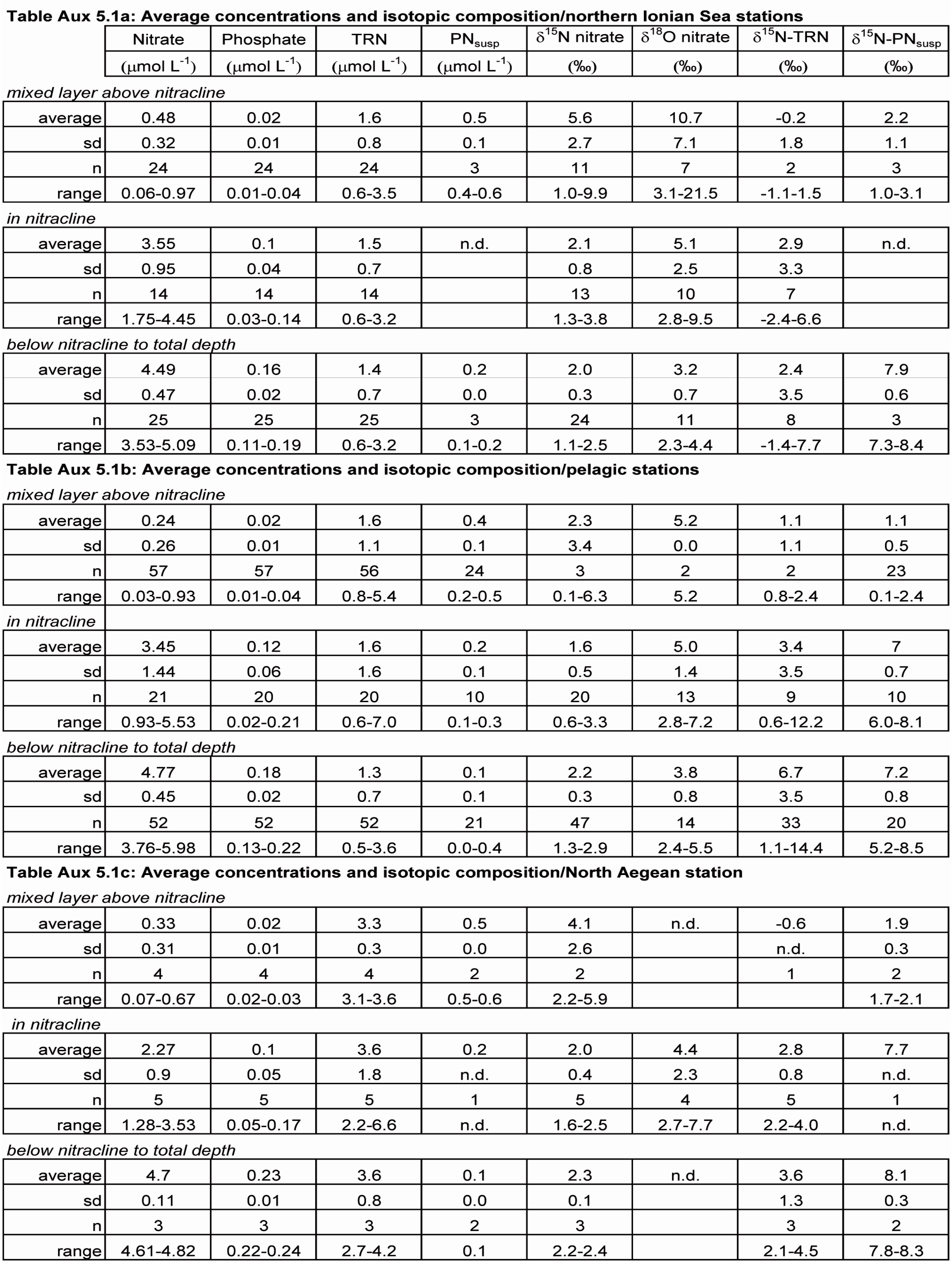


Table Aux 5.1a: Average concentrations and isotopic composition/northern Ionian Sea stations

	Nitrate (μmol L^{-1})	Phosphate (μmol L^{-1})	TRN (μmol L^{-1})	PN_{susp} (μmol L^{-1})	δ^{15}N nitrate (‰)	δ^{18}O nitrate (‰)	δ^{15}N-TRN (‰)	δ^{15}N-PN_{susp} (‰)
mixed layer above nitracline								
average	0.48	0.02	1.6	0.5	5.6	10.7	-0.2	2.2
sd	0.32	0.01	0.8	0.1	2.7	7.1	1.8	1.1
n	24	24	24	3	11	7	2	3
range	0.06-0.97	0.01-0.04	0.6-3.5	0.4-0.6	1.0-9.9	3.1-21.5	-1.1-1.5	1.0-3.1
in nitracline								
average	3.55	0.1	1.5	n.d.	2.1	5.1	2.9	n.d.
sd	0.95	0.04	0.7		0.8	2.5	3.3	
n	14	14	14		13	10	7	
range	1.75-4.45	0.03-0.14	0.6-3.2		1.3-3.8	2.8-9.5	-2.4-6.6	
below nitracline to total depth								
average	4.49	0.16	1.4	0.2	2.0	3.2	2.4	7.9
sd	0.47	0.02	0.7	0.0	0.3	0.7	3.5	0.6
n	25	25	25	3	24	11	8	3
range	3.53-5.09	0.11-0.19	0.6-3.2	0.1-0.2	1.1-2.5	2.3-4.4	-1.4-7.7	7.3-8.4

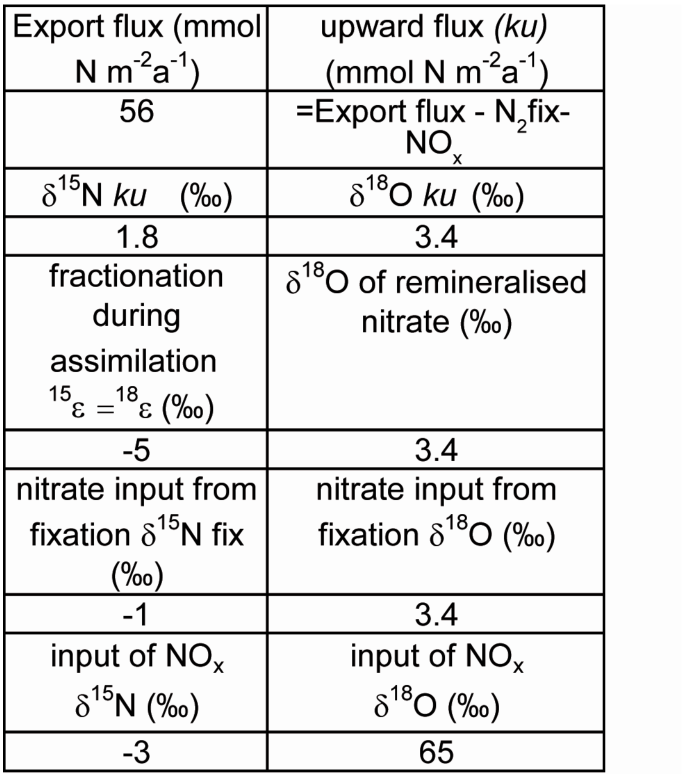
Table Aux 5.1b: Average concentrations and isotopic composition/pelagic stations

	Nitrate (μmol L^{-1})	Phosphate (μmol L^{-1})	TRN (μmol L^{-1})	PN_{susp} (μmol L^{-1})	δ^{15}N nitrate (‰)	δ^{18}O nitrate (‰)	δ^{15}N-TRN (‰)	δ^{15}N-PN_{susp} (‰)
mixed layer above nitracline								
average	0.24	0.02	1.6	0.4	2.3	5.2	1.1	1.1
sd	0.26	0.01	1.1	0.1	3.4	0.0	1.1	0.5
n	57	57	56	24	3	2	2	23
range	0.03-0.93	0.01-0.04	0.8-5.4	0.2-0.5	0.1-6.3	5.2	0.8-2.4	0.1-2.4
in nitracline								
average	3.45	0.12	1.6	0.2	1.6	5.0	3.4	7
sd	1.44	0.06	1.6	0.1	0.5	1.4	3.5	0.7
n	21	20	20	10	20	13	9	10
range	0.93-5.53	0.02-0.21	0.6-7.0	0.1-0.3	0.6-3.3	2.8-7.2	0.6-12.2	6.0-8.1
below nitracline to total depth								
average	4.77	0.18	1.3	0.1	2.2	3.8	6.7	7.2
sd	0.45	0.02	0.7	0.1	0.3	0.8	3.5	0.8
n	52	52	52	21	47	14	33	20
range	3.76-5.98	0.13-0.22	0.5-3.6	0.0-0.4	1.3-2.9	2.4-5.5	1.1-14.4	5.2-8.5

Table Aux 5.1c: Average concentrations and isotopic composition/North Aegean station

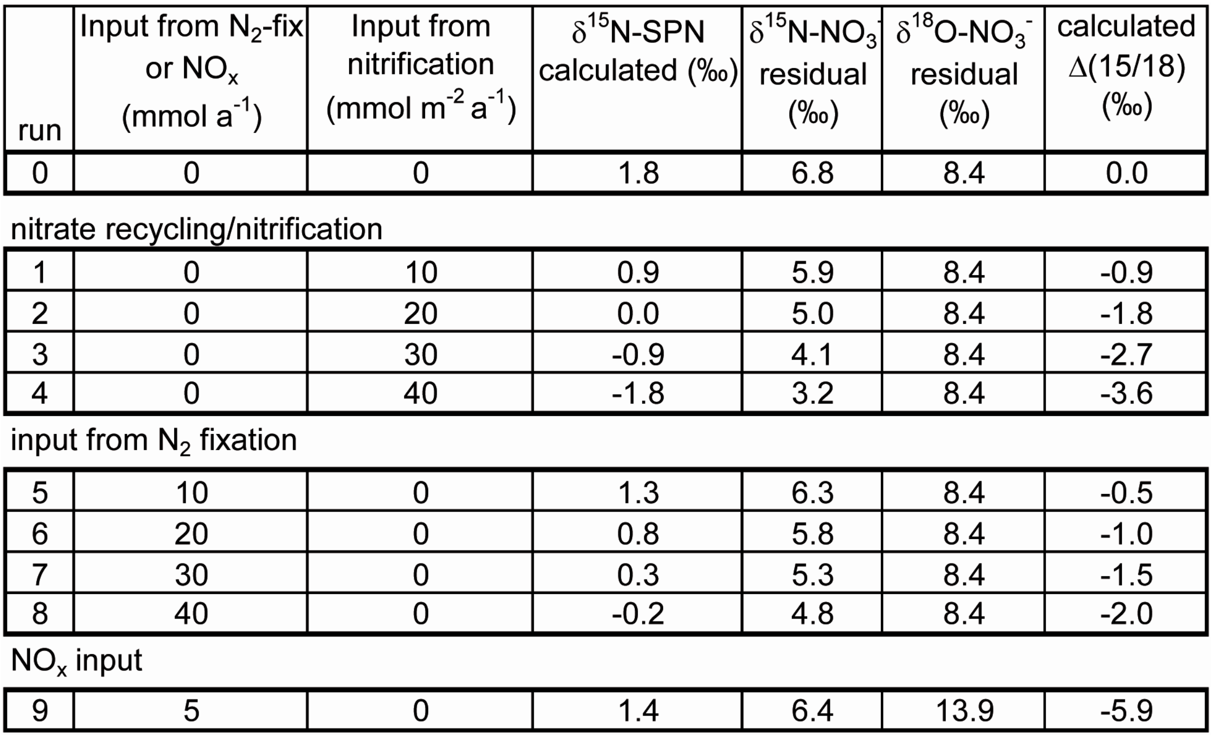
	Nitrate (μmol L^{-1})	Phosphate (μmol L^{-1})	TRN (μmol L^{-1})	PN_{susp} (μmol L^{-1})	δ^{15}N nitrate (‰)	δ^{18}O nitrate (‰)	δ^{15}N-TRN (‰)	δ^{15}N-PN_{susp} (‰)
mixed layer above nitracline								
average	0.33	0.02	3.3	0.5	4.1	n.d.	-0.6	1.9
sd	0.31	0.01	0.3	0.0	2.6		n.d.	0.3
n	4	4	4	2	2		1	2
range	0.07-0.67	0.02-0.03	3.1-3.6	0.5-0.6	2.2-5.9			1.7-2.1
in nitracline								
average	2.27	0.1	3.6	0.2	2.0	4.4	2.8	7.7
sd	0.9	0.05	1.8	n.d.	0.4	2.3	0.8	n.d.
n	5	5	5	1	5	4	5	1
range	1.28-3.53	0.05-0.17	2.2-6.6	n.d.	1.6-2.5	2.7-7.7	2.2-4.0	n.d.
below nitracline to total depth								
average	4.7	0.23	3.6	0.1	2.3	n.d.	3.6	8.1
sd	0.11	0.01	0.8	0.0	0.1		1.3	0.3
n	3	3	3	2	3		3	2
range	4.61-4.82	0.22-0.24	2.7-4.2	0.1	2.2-2.4		2.1-4.5	7.8-8.3

Table Aux 5.1: Comparison of averages in water above, in and below the nitracline for samples taken in stations of the Northern Ionian Sea (6 stations, Table 1a), at pelagic stations (10 stations, Table 1b) and at one station in the northern Aegean Sea (Table 1c) in the eastern Mediterranean Sea in January/February 2007 during Meteor expedition 71-3.



Export flux (mmol N $m^{-2}a^{-1}$)	upward flux *(ku)* (mmol N $m^{-2}a^{-1}$)
56	=Export flux - N_2fix-NO_x
$\delta^{15}N$ *ku* (‰)	$\delta^{18}O$ *ku* (‰)
1.8	3.4
fractionation during assimilation $^{15}\varepsilon = ^{18}\varepsilon$ (‰)	$\delta^{18}O$ of remineralised nitrate (‰)
-5	3.4
nitrate input from fixation $\delta^{15}N$ fix (‰)	nitrate input from fixation $\delta^{18}O$ (‰)
-1	3.4
input of NO_x $\delta^{15}N$ (‰)	input of NO_x $\delta^{18}O$ (‰)
-3	65

Table Aux 5.2: Input parameters (isotopic composition from Table Aux 5.1) to the model.



run	Input from N_2-fix or NO_x (mmol a^{-1})	Input from nitrification (mmol m^{-2} a^{-1})	$\delta^{15}N$-SPN calculated (‰)	$\delta^{15}N$-NO_3^- residual (‰)	$\delta^{18}O$-NO_3^- residual (‰)	calculated Δ(15/18) (‰)
0	0	0	1.8	6.8	8.4	0.0
nitrate recycling/nitrification						
1	0	10	0.9	5.9	8.4	-0.9
2	0	20	0.0	5.0	8.4	-1.8
3	0	30	-0.9	4.1	8.4	-2.7
4	0	40	-1.8	3.2	8.4	-3.6
input from N_2 fixation						
5	10	0	1.3	6.3	8.4	-0.5
6	20	0	0.8	5.8	8.4	-1.0
7	30	0	0.3	5.3	8.4	-1.5
8	40	0	-0.2	4.8	8.4	-2.0
NO_x input						
9	5	0	1.4	6.4	13.9	-5.9

Table Aux 5.3: Simulation results to explore the effects on Δ(15,18) of possible nitrate inputs (N_2 fixation, nitrification, NO_x input) in addition to upward flux of deep nitrate (*ku*) into the mixed layer of the eastern Mediterranean Sea.

6 Conclusions and outlook

6.1 Conclusions

Until some years ago, DON was assumed to be a recalcitrant inert pool of nitrogen without any relevant function as an alternative nitrogen source for microorganisms and phytoplankton (William and Druffel, 1987). Many studies in recent years led to a reassessment of the role of DON, both as an active compartment of reactive N, and as an alternative source of N when inorganic supplies are exausted (Stepanauskas et al., 1999a, b; Bronk, 2002; Bronk et al., 2007).

In this thesis I investigated dissolved organic nitrogen (DON) in two different aquatic ecosystems: the Elbe River as an example for a eutrophic aquatic ecosystem, and the eastern Mediterranean Sea as an example for an oligotrophic marine environment. By using stable isotope analyses I assessed the role of DON in the aquatic nitrogen cycle and the relationships between DON and other forms of reactive nitrogen, i.e. nitrate and particular nitrogen. To my knowledge, I presented the first dataset on estuarine mixing patterns of δ^{15}DON in seasonal resolution. Combined with the two year study of the non-tidal part of the Elbe River, I provided an excellent insight into the role of DON in this ecosystem. A weakness of my study is that the data always are combined signals of DON and ammonium, due to analytical difficulties. The day is saved by the fact that the data represent essentially DON, since the ammonium contribution was less than 10%, and often ammonium could not be detected in most of the samples.

The two studies in the Elbe River and the Elbe estuary show that DON is significantly affected by abiotic processes. In the Elbe estuary I observed no seasonal trends in the nitrate, DON and PN cycling, so turnovers due to biological processes can be excluded. However, DON is not mixed conservatively in the salinity gradient and thus differs from nitrate concentrations. Non-conservative behaviour of DON is in my interpretation caused only by abiotic processes. The city of Hamburg and its port are distinct sources of DON, due to input from direct discharge, local biogenic sources and/or sewage treatment plants. Adsorption and desorption processes are likely the reason for a decrease in DON concentration at the freshwater head of the estuarine turbidity maximum zone (TMZ), and an increase at the salty tail of the TMZ. Compared to older data the DON load in the estuary has not changed since the 1980’s: Then and today DON concentrations range from 20 to 60 µM. This is a remarkable fact, since the ammonium and phosphate concentrations decreased significantly in the last 25 years, and also nitrate concentrations have a decreasing trend since the early 1990’s (FGG-Elbe, 2010). So, sinking nutrient concentrations do not cause automatically decreasing DON concentrations.

In the non-tidal Elbe River, which I studied over a period of two years, the main abiotic source of DON is the elution of ^{15}N-enriched organic fertiliser after the main periods of spreading manure, which are regulated by policy measures (DüV, 2009), in spring and after the harvest in autumn. This δ^{15}N enriched precursor material increases both the concentration of DON and δ^{15}DON in summer and winter river loads. In addition to this abiotic process, biotic processes such as seasonally varying primary production, autotrophic and heterotrophic uptake during springtime, and DON release due to nutrient limitation in summer create a seasonal rhythm in both concentrations and isotopic composition of DON. Human impact imposes an external abiotic rhythm on the annual cycle of DON in the Elbe River, making it more complex than the cycling of nitrate: DON concentrations and isotopic composition have four distinct annual phases, whereas nitrate concentrations and isotopic composition have only two periods. Decreasing nitrate concentrations in spring and summer accompanied by an increase in δ^{15}N- and δ^{18}O-NO_3^- were due to nitrate assimilation during primary production. Changing biological activity in autumn and winter caused the opposite trends (Johannsen et al., 2008; Kendall, 1998).

DON in the Elbe River represents 23% of TDN (in annual mean, summer contribution is higher than in the other seasons); in the outer estuary this contribution increases to 50%. No significant changes in the DON load in the last 25 years could be observed. DON is subject to many processes, but most of these processes are abiotic, especially in the tidal estuary. Main sources of DON are input from direct discharge, local biogenic sources and/or sewage treatment plants and elution of organic fertilisers, but also release from PN. The similar trends in the δ^{15}N composition of DON and PN indicate a close coupling of these nitrogen species in the water column.

In the study of reactive N in the eastern Mediterranean Sea, the ammonium concentrations were so low that ammonium could be neglected, so measured TRN data can be interpreted as DON.

Next to nitrate, the DON fraction is the second largest fraction of total reactive nitrogen in the eastern Mediterranean Sea. In the mixed layer, DON concentrations exceed even nitrate concentrations. Thus, DON is an important factor that should not be neglected.

Lower DON concentrations accompanied by increasing $\delta^{15}N$ values in deep sea samples as compared to surface water samples of the eastern Mediterranean Sea are evidence for DON taking part in nitrogen turnover in this environment. DON is strongly connected with PN, since both show similar trends in $\delta^{15}N$. The low $\delta^{15}N$ values in surface samples are caused by rapid grazing and recycling of nutrients during winter bloom. The enrichment in ^{15}N of both DON and PN in deeper water masses is due to the production of ^{15}N depleted nitrate. The rapidly sinking fresh PN is subject to extensive and efficient mineralisation processes, and DON is presumably a by-product of heterotrophic PN recycling.

The low $\delta^{15}N$ of nitrate, TRN and PN result not only from partial nitrogen uptake caused by the phytoplankton bloom. Nitrification and/or a relatively small input of anthropogenic NO_x are the most likely sources of additional isotopically distinct nitrate in the eastern Mediterranean Sea.

So it is obvious that DON affected by different processes both in eutrophic and oligotrophic aquatic ecosystems. While in eutrophic ecosystems abiotic processes play a major role in DON cycling and main DON sources are human caused, DON is a relevant nitrogen source for heterotrophs and has an active part in reactive nitrogen turnover in oligotrophic ecosystems.

6.2 Outlook

This thesis shows that the study of DON is an interesting field of research with many different aspects to investigate, and that the utilisation of stable isotope analyses is a suitable tool to detect transformation processes.

However, the analysis of DON is still the main problem, since so far no adequate method to measure DON concentration directly has been developed, and also the measurement of δ^{15}DON is possible only via measurement of δ^{15}TDN and recalculation.

As often mentioned in this thesis, the method chosen here does not separate DON and ammonium, so this is a part of the method that has to be improved. One possibility is to drive off ammonium as ammonia by changing the pH-value, but here the risk of hydrolysis of amino groups may be a problem. The measurement of $\delta^{15}NH_4^+$ and inclusion of these data in the calculation of δ^{15}DON is an opportunity, but will probably increase the calculated standard deviation for δ^{15}DON. So in environments with low DON content or small, but significant changes analytical errors may exceed these values.

Besides these analytical challenges the diversity of the DON composition is a challenge for itself. Different DON forms with different isotopic signatures interfere with each other, resulting in a bulk δ^{15}DON which is difficult to interpret. The amount of interesting labile DON compounds with small turnover times is often low; the quantitative dominant DON pool is often recalcitrant. The separation via ultracentrifugation in LMW and HMW DON with subsequent stable isotope analysis may be a first step, but other more differentiated isolation techniques are necessary and have to be developed.

To come back to the areas studied in this thesis, the TMZ in the Elbe estuary clearly is a zone of high N turnover, with interesting and relevant processes to be studied. A more detailed sampling campaign, with a combination of δ^{15}DON, $\delta^{15}NH_4^+$, and δ^{15}PN analyses, coupled with a separation in LMW and HMW DON may result in more precise data about the mechanisms proceeding in the TMZ. Also the port of Hamburg is an interesting place for further DON research studies to investigate the nature of the mainly anthropogenic source of DON.

To my knowledge, the δ^{15}N-TRN data presented in this thesis are the first isotopic dataset of DON in the eastern Mediterranean Sea. Although the dataset is somewhat limited, it supports the interpretation of nitrogen turnover processes. But for better comparison more data are needed, especially from intermediate and deep water masses. Also other seasons may be of interest. However, due to the low concentrations in this oligotrophic ecosystem the current method for DON analyses is at its limit, so further DON research studies should be made when the analytical difficulties mentioned above have been solved.

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Finally, I thank my family and my friends, my parents for supporting and encouraging me all the time, and last but not least a special thank to Nicky for always believing in me.

Figure Captions

(Kerner and Spitzy, 2001); October 2005 to December 2006 data (indexed d) are from this study.

Figure 3.11: Box-and-whisker plots of calculated δ^{15}DON values in the Elbe estuary in comparison with measured δ^{15}DON+NH_4^+ values. For the calculation of mean NH_4^+ concentrations and isotopic contribution, we used data from the ARGE Elbe (ARGE Elbe, 2001, 2002, 2003, 2004, 2005a, 2005b, 2007a, 2007b) and $\delta^{15}NH_4^+$ values of the Tyne estuary (1) and the Tweed estuary (2) (Ahad et al. 2006)

Figure 3.12: Box-and-whisker plots of data on combined DON+NH_4^+ concentrations (a) and mean ammonium and DON concentration (b) in the Elbe estuary. Data sources as in Figure 3.10.

Figure 3.13: Box-and-whisker plots of calculated DIN/DON ratios in the Elbe estuary over the last two decades. Data sources as in Figure 3.10.

Figure 4.1: Sample station weir of Geesthacht, Northern Germany, NW Europe

Figure 4.2: concentration and isotopic values in the Elbe River at the weir of Geesthacht, June 2005 – December 2007, bars represent the river discharge. Note the different scales of the y-axes. a) Nitrate concentration, δ^{15}N-NO_3^- and δ^{18}O-NO_3^- in the Elbe River. Data from 2006 have been published in Johannsen et al. (2008). b) combined DON+NH_4^+ concentration and δ^{15}DON+NH_4^+. c) PN concentration and δ^{15}PN

Figure 4.3: Relationship between δ^{15}N-NO_3^- and δ^{18}O-NO_3^- to examine the fractionation factor ratio $\varepsilon^{15}/\varepsilon^{18}$, dashed line represents a 1:1 ratio

Figure 4.4: δ^{15}DON+$NH_4^+$and δ^{15}PN in the Elbe River at the weir of Geesthacht, June 2006 - December 2007

Figure 4.5: Relationship between δ^{15}DON+NH_4^+ and δ^{15}PN in the Elbe River at the weir of Geesthacht, June 2006 – December 2007

Figure 5.1: Conceptual diagram of the processes that determine the δ^{15}N and δ^{18}O of nitrate in the surface and intermediate water masses of the eastern Mediterranean Sea. Abbreviations are PN for particulate nitrogen, TRN for total reduced nitrogen, and DON for dissolved organic nitrogen as explained in the methods section. This diagram also serves as a schematic illustration of the model used in the discussion to calculate the nitrate isotope anomaly $\Delta(15,18)$ under assumptions of different sources as explained in the text (see also auxiliary material).

Figure 5.2: Map of stations occupied in the Eastern Mediterranean Sea during r/v Meteor cruise 71-3 (January-February, 2007). Northern Ionian Sea (NIS) stations referred to in the text are stations H07 to H12 (black squares), pelagic stations are marked with black dots, the sole station in the northern Aegean Sea (Sk01) is marked by a black star. The black diamond SE of Crete (station Ier1) marks the location of the sediment trap deployment (MID). Stations H07 (large black square) and Her03 (large black dot) referred to in Fig. 5.3 are marked.

Figure 5.3: Profiles of fluorescence (a; arbitrary units), nitrate (b) and phosphate (b) concentrations in the upper 400 m at 2 stations representative of NIS (H07) and pelagic stations (Her03) show stratification between 80 and 230 m water depth and indicate the biologically active mixed layer. An ongoing phytoplankton bloom in the northern Ionian Sea (at station H07) is sustained by nitrate and phosphate provided from ongoing regional thermocline deepening, whereas station Her03 illustrates the mature and thick mixed layer with very low nutrient concentrations at pelagic sites.

Figure 5.4: Concentrations of nitrate (a), TRN (b), and particulate N in total suspended solids (c) plotted against water depth for all stations.

Figure 5.5: Isotopic composition δ^{15}N-NO_3^- (a), δ^{18}O-NO_3^- (b), δ^{15}N-TRN (c), and δ^{15} of suspended PN (d) plotted against water depth for all stations.

Figure 5.6: Composite seasonal diagram of sinking PN fluxes (squares) and δ^{15} of sinking PN (circles) and their standard deviations at 2600 m water depth at station Ierapetra (34°26´N, 26°11´E, water depth 3750 m). Fluxes (squares, black line and s.d. in red) and δ^{15}SPN (circles, grey line, s.d. in blue) for three deployment periods (MID-1: 01/30/1999 to 04/13/1999), MID-2: 11/05/2001 to 04/01/2002 and MID-3: 01/30/2007 to 09/05/2007) at 2700 m water depth have been assembled in a surrogate annual cycle.

Figure 5.7: Depth plot of $\Delta(15,18)$ of nitrate for different station sets in the upper 500 m of the water column.

Table Captions

Table 5.1: Estimate of reactive N-inventories of the EMS in different water depth intervals (corresponding to mixed layer, intermediate, and deep water masses) and mass-weighted $\delta^{15}N$ of the different components (nitrate, particulate nitrogen PN, and total reduced nitrogen, TRN). We calculated inventories (given in gigamol N) based on interval water volumes for the EMS without Adriatic and Aegean sub-basins (R. Grimm, pers. comm., 2009) and weighted $\delta^{15}N$ values by average concentrations found during our cruise. The last column is the integrated and mass-weighted $\delta^{15}N$ over all components of reactive N for each interval, the last line are the integrated inventories and $\delta^{15}N$ over the entire water column.

Table Aux 5.1: Comparison of averages in water above, in and below the nitracline for samples taken in stations of the Northern Ionian Sea (6 stations, Table Aux 5.1a), at pelagic stations (10 stations, Table Aux 5.1b) and at one station in the northern Aegean Sea (Table Aux 5.1c) in the EMS in January/February 2007 during Meteor expedition 71-3.

Table Aux 5.2: Input parameters (isotopic composition from Table Aux-1) to the model.

Table Aux 5.3: Simulation results to explore the effects on $\Delta(15,18)$ of possible nitrate inputs (N_2 fixation, nitrification, NO_x input) in addition to upward flux of deep nitrate (*ku*) into the mixed layer of the eastern Mediterranean Sea.

Table App 1: Salinity gradient in the Elbe estuary, October 2005 (cf. Chapter 3), sampled with RV *Ludwig Prandtl*

Table App 2: Salinity gradient in the Elbe estuary, June 2006 (cf. Chapter 3), sampled with RV *Ludwig Prandtl*

Table App 3: Salinity gradient in the Elbe estuary, December 2006 (cf. Chapter 3), sampled with RV *Ludwig Prandtl*. Water samples taken from the surface (1 m depth) with the ship’s membrane pump.

Table App 4: concentration data and c(DON) ratio at the Elbe weir at Geesthacht (June 2005 – December 2007, cf. Chapter 4)

Table App 5: isotope ratios and discharge data at the Elbe weir at Geesthacht (June 2005 – December 2007, cf. Chapter 4)

List of abbreviations

α - significance level
AAP - 4-aminoantipyrine
ACA - 6-amino caproic acid
ARGE - Arbeitsgemeinschaft Elbe
CDT - Canyon Diabolo Triolite
DCAA - dissolved combined amino acids
DFAA - dissolved free amino acids
DIN - dissolved inorganic nitrogen
DIW - deionised water
d.l. - detection limit
DOC - dissolved organic carbon
DOM - dissolved organic matter
DON - dissolved organic nitrogen
ε - fractionation factor
EDTA - ethylenediamine-tetraacetate
EMS - eastern Mediterranean Sea
HCl - hydrochloric acid
HMW - high molecular weight
kt - kilo tons
LMW - low molecular weight
N - nitrogen
NaOH - sodium hydroxide
n.d. - not determined
N/R - not reported
NW - North-West
PDB - Pee Dee Belemnite
PE - polyethylene
PN - particulate nitrogen
poH - port of Hamburg
POR - persulfate oxidising reagent
r - correlation coefficient
R/V - Research Vessel
SE - South-East
SMOW - standard mean ocean water
SPM - suspended particulate matter
SPN - sinking particulate nitrogen
TDN - total dissolved nitrogen
TMZ - turbidity maximum zone
VSMOW - Vienna standard mean ocean water

References

Agedah, E.C., Binalaiyifa, H.E., Ball, A.S., and Nedwell, D.B., 2009. Sources, turnover and bioavailability of dissolved organic nitrogen (DON) in the Colne estuary, UK. *Mar. Ecol. Prog. Ser.* **382**, 23-33

Ahad, J.M.E., Ganeshram, R.S., Spencer, R.G.M., Uher, G., Upstill-Goddard, R.C. and Cowie, G.L., 2006. Evaluating the sources and fate of anthropogenic dissolved inorganic nitrogen (DIN) in two contrasting North Sea estuaries. *Science of the Total Environment* **372**, 317-333

Altabet, M.A., 1988. Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particle transformation in the open ocean. *Deep-Sea Res.* **35**, 535-554

Altabet, M.A., and Francois, R., 1994. The use of nitrogen isotopic ratio for reconstruction of past changes in surface ocean nutrient utilization. In: R. Zahn et al. (Eds.) "Carbon Cycling in the Glacial Ocean: Constraints on the Ocean´s Role in Global Change", Springer-Verlag, Heidelberg

Antia, N.J., Harrison, P.J. and Oliveira, L., 1991. Phycological reviews: The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology, and ecology. *Phycologia* **30**, 1-89

Antoine, D., Morel, A., and André, J.-M., 1995. Algal pigment distribution and primary production in the eastern Mediterranean as derived from coastal zone colour scanner observations. *Journal of Geophysical Research* **100**(C8), 16193-16209

ARGE, 2000. Stoffkonzentrationen in mittels Hubschrauber entnommenen Elbewasserproben (1979 bis 1998) Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DBerichte.php

ARGE, 2001. Wassergütedaten der Elbe - Zahlentafel 1999, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2002. Wassergütedaten der Elbe - Zahlentafel 2000, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2003. Wassergütedaten der Elbe - Zahlentafel 2001, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2004. Wassergütedaten der Elbe - Zahlentafel 2002, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2005a. Wassergütedaten der Elbe - Zahlentafel 2003, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2005b. Wassergütedaten der Elbe - Zahlentafel 2004, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2007a. Wassergütedaten der Elbe - Zahlentafel 2005, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2007b. Wassergütedaten der Elbe - Zahlentafel 2006, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DDaten.php

ARGE, 2008. Gewässergütebericht der Elbe 2006, Arbeitsgemeinschaft für die Reinhaltung der Elbe, Hamburg, http://www.arge-elbe.de/wge/Download/DBerichte.php

Armstrong, F.A.J., Williams, P.M., and Strickland, J.D.H., 1966. Photo-oxidation of organic matter in sea water by ultra-violet radiadtion, analytical and other applications. *Nature* **211**, 481-483

Badr, E.S.A., Tappin, A.D., and Achterberg, E.P., 2008. Distributions and seasonal variability of dissolved organic nitrogen in two estuaries in SW England. *Mar. Chem.* **110**, 153-164

Behrendt, H., Bach, M., Opitz, D., and Pagenkopf, W.G., 2004. Maßgebliche anthropogene Einflüsse auf die Gewässerqualität. In: A. Becker and W. Lahmer (Eds.), "Wasser- und Nährstoffhaushalt im Elbegebiet und Möglichkeiten zur Stoffeintragsminderung", Weissensee Verlag, Berlin, 42-58

Benner, R., Biddanda, B., Black, B., and McCarthy, M., 1997. Abundance, size distributions, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Mar. Chem.* **57**, 243-263

Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I., and Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **255**, 1561-1564

Berg, G.M., Glibert, P.M., Lomas, M.W., and Burford, M.A., 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.* **129**, 377-387

Bergemann, M., 1995. Die Lage der oberen Brackwassergrenze im Elbeaestuar. *Deutsche Gewaesserkundliche Mitteilungen* **39**(4/5), 134-137

Berman, T. and Bronk, D.A., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat. Microb. Ecol.* **31**, 279-305

Berman, T., Béchemin, C. and Maestrini, S.Y., 1999. Release of ammonium and urea from dissolved organic nitrogen in aquatic ecosystems. *Aquat. Microb. Ecol.* **16**, 295-302

Berman, T., Chava, S., Kaplan, B. and Wynne, D., 1991. Dissolved organic substrates as phosphorus and nitrogen sources for axenic batch cultures of freshwater green algae. *Phycologia* **30**, 339-345

Berman-Frank, I., Yogev, T., Aharonovich, D., and Beja, O., 2007. Nitrogen fixation in the Eastern Mediterranean Sea, paper presented at IUGG XXIV General Assembly, Perugia, Italy.

Bertilsson, S., Stepanauskas, R., Cuadros-Hansson, R., Granéli, W., Wikner, J., and Tranvik, L., 1999. Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquat. Microb. Ecol.* **19**, 47-56

Béthoux, J.P., 1989. Oxygen consumption, new production, vertical advection and environmental evolution of the Mediterranean Sea. *Deep-Sea Research* **36**(5), 769-781

Béthoux, J.P., and Copin-Montegut, G., 1986. Biological fixation of atmospheric nitrogen in the Mediterranean Sea. *Limnol. Oceangr.* **31**(6), 1353-1358

Billen, G., 1991. Protein degradation in aquatic environments. In: J.D. Chrost (Ed.), "Microbial Enzymes in Aquatic Environments", Springer-Verlag, New York, 123-143

Böhlke, J.K., Mroczkowski, S.J., and Coplen, T.B., 2003. Oxygen isotopes in nitrate: new reference materials for ^{18}O:^{17}O:^{16}O measurements and observations on nitrate-water equilibration. *Rapid Comm. in Mass Spectrometry* **17**(16), 1835-1846

Bottcher, J., Strebel, O., Voerkerlius, S. and Schmidt, H.L.,1990. Using isotope fractionation of nitrate nitrogen and nitrate oxygen for evaluation of microbial denitrification in a sandy aquifer. *J.Hydrol.* **114**, 413-424

Bourbonnais, A., Lehmann, M.F., Waniek, J.J., and Schulz-Bull, D.E., 2009. Nitrate isotope anomalies reflect N_2 fixation in the Azores Front region (subtropical NE Atlantic), *JGR* **114**(C03003), 1-16

Branch, G.M., and Griffiths, C.L., 1988. The Benguela ecosystem part V. The coastal zone. *Oceanogr. Mar. Biol. Annu. Rev.* **26**, 395-486

Brandes, J.A., and Devol, A.H., 2002. A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling. *Global Biogeochemical Cycles* **16**(4), doi:10.1029/2001GB001856

Brandes, J.A., Devol, A.H., Yoshinari, T., Jayakumar, D.A., and Naqvi, S.W.A., 1998. Isotopic composition of nitrate in the central Arabian Sea and eastern tropical North Pacific: A tracer for mixing and nitrogen cycles. *Limnol. Oceanogr.* **43**(7), 1680-1689

Brockmann, U.H., Raabe, T., Hesse, K., Viehweger, K., Rick, S., Starke, A., Fabiszisky, B., TopÇu, and Heller, R., 1999. Seasonal budgets of the nutrient elements N and P at the surface of the German Bight during winter 1996, spring 1995, and summer 1994. *German Journal of Hydrography* **51**(2/3), 267-291

Brockmann, U.H., 1994. Organic Matter in the Elbe Estuary. *Neth. Journal of Aquatic Ecology* **28**(3-4), 371-381

Brockmann, U.H., and Pfeiffer, A., 1990. Seasonal changes of dissolved and particulate material in the turbidity zone of the River Elbe. In: W. Michaelis (Editor), "Estuarine Water Quality Management". Springer, Heidelberg, 327-334

Bronk, D.A., 2002. Dynamics of DON. In: D.A. Hansell and C.A. Carlson (Eds.), "Biogeochemistry of Marine Dissolved Organic Matter". Academic Press, New York, 153-247.

Bronk, D.A., Lomas, M.W., Gilbert, P.M., Schukert, K.J., and Sanderson, M.P., 2000. Total dissolved nitrogen analysis: Comparisons between the persulfate, UV, and high temperature oxidation methods. *Mar. Chem.* **69**, 163-178

Bronk, D.A., See, J.H., Bradley, P., and Killberg, L., 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences* **4**, 283-296

Burdige, D.J., 2002. Sediment pore waters. In: D.A. Hansell, C.A. Carlson (Eds.), "Biogeochemistry of Marine Dissolved Organic Matter", Academic press, San Diego, 611-663

Bushaw, K.L., Zepp, R.G., Tarr, M.A., Schulz-Jander, D., Bourbonniere, R.A., Hodson, R., Miller, W.L., Bronk, D.A., and Moran, M.A., 1996. Photochemical release of biologically labile nitrogen from dissolved organic matter. *Nature* **381**, 404-407

Butler, E.I., Knox, S., and Liddicoat, M.I., 1979. The relationship between inorganic and organic nutrients in seawater. *J. Mar. Biol. Assoc. UK* **59**, 239-250

Capone, D., Zehr, J., Pearl, H., Bergman, B., and Carpenter, E., 1994. Amino acid cycling in colonies of the planktonic marine cyanobacterium. *Science* **276**, 1221-1229

Carlson, C.A., 2002. Production and removal processes. In: D.A. Hansell, C.A. Carlson (Eds.), "Biogeochemistry of Marine Dissolved Organic Matter", Academic press, San Diego, 91-151

Carlson, C.A., Ducklow, H.W., and Michaels, A.F., 1994. Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea. *Nature* **371**, 405-408

Carpenter, E.J., Harvey, H.R., Fry, B., and Capone, D.G., 1997. Biogeochemical tracers of the marine cyanobacterium *Trichodesmium*. *Deep Sea Res. I* **44**, 27-38

Casciotti, K.L., 2009. Inverse kinetic isotope fractionation during bacterial nitrite oxidation. *Geochim. Cosmochim. Acta* **73**(7), 2061-2076

Casciotti, K.L., and Mcilvin, M.R., 2007. Isotopic analyses of nitrate and nitrite from reference mixtures and application to Eastern Tropical North Pacific waters. *Mar. Chem.* **107**, 184–201

Casciotti, K.L., Sigman, D.M., and Ward, B.B., 2003. Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria. *Geomicrobiology Journal* **20**(4), 335-353

Casciotti, K.L., Sigman, D.M., Hastings, M.G., Bohlke, J.K., and Hilkert, A., 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Analytical Chemistry* **74**(19), 4905-4912

Casciotti, K.L., Trull, T.W., Glover, D.M., and Davies, D., 2008. Constraints on nitrogen cycling at the subtropical North Pacific Station ALOHA from isotopic measurements of nitrate and particulate nitrogen. *Deep-Sea Research II* **55**(14-15), 1661-1672

Cifuentes, L.A., Fogel, M.L., and Pennock, J.R., 1989. Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. *Geochim Cosmochim Acta* **53**, 2713–2721

Coban-Yildiz, Y., Altabet, M.A., Yilmaz, A. and Tugrul, S., 2006. Carbon and nitrogen isotopic ratios of suspended particulate organic matter (SPOM) in the Black Sea water column. *Deep Sea Research II* **53**, 1875-1892

Codispoti, L.A., Brandes, J.A., Christensen, J.P., Devol, A.H., Naqvi, S.W.A., Paerl, H.W., and Yoshinari, T., 2001. The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter anthropocene? *Sci. Mar.* **65**, 85-105

Cornell, S., Rendell, A., and Jickells, T., 1995. Atmospheric inputs of dissolved organic nitrogen to the oceans. *Nature* **376**, 243-246

Dagg, M.J., 1974. Loss of prey body contents during feeding by an aquatic predator. *Ecology* **55**, 903-906

Dähnke, K., Bahlmann, E., and Emeis, K.-C., 2008. A nitrate sink in estuaries? An assessment by means of stable nitrate isotopes in the Elbe estuary. *Limnol. Oceanogr.* **53**(4), 1504-1511

De Corte, D., Yokokawa, T., Varela, M,, Agogué, H., and Herndl, G.J., 2009. Spatial distribution of Bacteria and Archaea and amoA gene copy numbers throughout the water column of the Eastern Mediterranean Sea. *The ISME Journal* **3**(2), 147-158

Deutsch, B., Voss, M. and Fischer, H., 2009. Nitrogen transformation processes in the Elbe River: Distinguishing between assimilation and denitrification by means of stable isotope ratios in nitrate. *Aquat. Sci.* **71**, 228-237

Diaz, F., and Raimbault, P., 2000. Nitrogen regeneration and dissolved organic nitrogen release during spring in a NW Mediterranean coastal zone (Gulf of Lions): implications for the estimation of new production. *MEPS* **197**, 51-65

Duce, R.A., LaRoche, J., Altieri, K., Arrigo, K.R., Baker, A.R., Capone, D.G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R.S., Geider, R.J., Jickells, T., Kuypers, M.M., Langlois, R., Liss, P.S., Liu, S.M., Middelburg, J.J., Moore, C.M., Nickovic, S., Oschlies, A., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L.L., Uematsu, M., Ulloa, O., Voss, M., Ward, B., and Zamora, L., 2008. Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* **320**(893), 893-897

Düngeverordnung (DüV) in der Fassung der Bekanntmachung vom 27. Februar 2007 (BGBl. I S. 22), geändert durch Artikel 1 der Verordnung vom 6. Februar 2009 (BGBl. I S.153)"

Eisma, D., 1986. Flocculation and de-flocculation of suspended matter in estuaries. *Netherlands Journal of Sea Research* **20**(2/3), 183-199

Fagerli, H., Legrand, M., Preunkert, S., Vestreng, V., Simpson, D., and Cerqueira M., 2007. Modeling historical long-term trends of sulfate, ammonium, and elemental carbon over Europe: A comparison with ice core records in the Alps, *Journal of Geophysical Research-Atmospheres* **112**, doi:10.1029.2006JD008044

FGG-Elbe,FlussgemeinschaftElbe,2010. www.fgg-elbe.de/tl_fgg_neu/allgemein/statistik.html

Fogg, G.E., 1966. The extracellular products of algae. *Oceanogr. Mar. Biol. Ann. Rev.* **4**, 195-212

Fogg, G.E., 1983. The ecological significance of algal extracellular products of phytoplankton photosyntesis. *Bot. Mar.* **26**, 3-14

Fry, B., 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries* **25**(2), 264-271

Fuhrman, J.A., 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399**, 541-548

Fuhrman, J.A., 2000. Impact of viruses on bacterial processes. In: D.L. Kirchman (Ed.), "Microbial Ecology of the Oceans", Wiley-Liss, New York, 327-350

Gat, J.R., Shemesh, A., Tziperman, E., Hecht, A., Georgopoulos, D., and Basturk, O., 1996. The stable isotope composition of waters of the eastern Mediterranean Sea. *Journal of Geophysical Research* **101**(C3), 6441-6451

Gaye-Haake, B., Lahajnar, N., Emeis, K.C., Rixen, T., Unger, D., Schulz, H., Ramaswamy, V., Paropkari, A.L., Guptha, M.V.S., and Ittekkot, V., 2005. Stable nitrogen isotopic ratios of sinking particles and sediments from the northern Indian Ocean. *Marine Chemistry* **96**, 243-255

Glibert, P.M., and Bronk, D.A., 1994. Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria *Trichodesmium spp*. *Appl. Environ. Microbiol.* **36**, 3996-4000

Gobler, C.J., Hutchins, D.A., Fisher, N.S., Cosper, E.M., and Sañudo-Wilhelmy, S.A., 1997. Release and bioavailability of C, N, P, Se, and Fe following viarl lysis of a marine chrrysophyte. *Limnol. Oceanogr.* **42**, 1492-1504

Granger, J., Sigman, D.M., Needoba, J.A., and Harrison, P.J., 2004. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. *Limnol. Oceanogr.* **49**(5), 1763-1773

Grasshoff, K., Ehrhardt, M., Kremling, K., and Anderson, L.G. (Eds), 1999. Methods of Seawater Analysis. Verlag Chemie, Weinheim, 632 pp.

Gruber, N., and Sarmiento, J., 1997. Global patterns of nitrogen fixation and denitrification. *Global Biogeochemical Cycles* **11**, 235-266

Hadas, O., Altabet, M.A., and Agnihotri, R., 2009. Seasonally varying nitrogen isotope biogeochemistry of particulate organic matter in Lake Kinneret, Israel. *Limnol. Oceanogr.* **54**(1), 75-85

Hastings, M.G., Sigman, D.M., and Lipschultz, F., 2003. Isotopic evidence for source changes of nitrate in rain at Bermuda. *Journal of Geophysical Research-Atmospheres* **108**(D24), 4790, doi:10.1029/2003JD003789

Hayes, J.M., 1983. Practice and principles of isotopic measurements in organic geochemistry. In: W.G. Meinschein (Ed.), “Organic Geochemistry of Contemporaneous and Ancient Sediments”. Soc. Econ. Paleont. Minerol., 5-1 - 5-31

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology: Isotope Geoscience section* **59**, 87-102

Hebbel, H., and Steuer, D., 2006. Empirische Untersuchungen zur Berechnung von Frachten in Fließgewässern. In: Hauptmann, H. and Krumbholz, W. (Eds.), "Discussion Papers in Statistics and Quantitative Economics", Hamburg

Hedges, J.I., and Hare, P.E., 1987. Amino acid adsorption by clay minerals in distilled water. *Geochim. Cosmochim. Acta* **51**, 255-259

Hoch, M.P., Fogel, M.L., and Kirchman, D.L., 1992. Isotope Fractionation Associated with Ammonium Uptake by a Marine Bacterium. *Limnol. Oceanogr.* **37**(7), 1447-1459

Horrigan, L., Lawrence, R.S., and Walker, P., 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental health perspectives* **110** (5), 445–456, doi:10.1289/ehp.02110445. PMID 12003747

Howarth, R.W., 2004. Human acceleration of the nitrogen cycle: drivers, consequences, and steps toward solutions. *Water Sci Technol* **49**, 7-13

Ibello, V., Cantoni, C., Cozzi, S., and Civitarese, G., 2010. First basin-wide experimental results on N2 fixation in the open Mediterranean Sea. *Geophys. Res. Lett.* **37**(3), 1-5

IKSE, 2005. Die Elbe und ihr Einzugsgebiet, ein geographisch-hydrologischer und wasserwirtschaftlicher Überblick, IKSE, Magdeburg.

Ishiwatari, R., 1992. Macromolecular material (humic substances) in the watercolumn and sediments. *Mar. Chem.* **39**, 151-166

Johannsen, A., Dähnke, K. and Emeis, K., 2008. Isotopic composition of nitrate in five German rivers discharging into the North Sea. *Org. Geochem.* **39**, 1678-1689

Jumars, P.A., Penry, D.L., Baross, J.A., Perry, M.J., Frost, B.W., 1989. Closing the microbial loop: Dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion, and absorption in animals. *Deep-Sea Res.* **36**, 483-495

Kaushal, S.S., and Lewis Jr., W.M., 2005. Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. *Biogeochemistry* **74**, 303-321

Keil, R.G., and Kirchman, D.L., 1993. Dissolved combined amino acids: Chemical form and utilization by marine bacteria. *Limnol. Oceanogr.* **38**, 1256-1270

Kendall, C., 1998. Tracing nitrogen sources and cycling in catchments. In: Kendall, C., and McDonnell, J.J. (Eds.), "Isotope Tracers in Catchment Hydrology", Elsevier, 521-576

Kendall, C., Elliott, E.M., and Wankel, S.D., 2007. Tracing Anthropogenic Inputs of Nitrogen to Ecosystems. In: K. Lajtha and R. H. Michener (Eds.), "Stable Isotopes in Ecology and Environmental Science"9, Wiley, Hoboken, 375-449

Kerner, M., and Spitzy, A., 2001. Nitrate regeneration coupled to degradation of different size fractions of DON by the picoplankton in the Elbe estuary. *Microb. Ecol.* **41**, 69-81

Knapp, A., DiFiore, P.J., Deutsch, C., Sigman, D.M., and Lipschultz, F., 2008. Nitrate isotopic composition between Bermuda and Puerto Rico: Implications for N_2 fixation in the Atlantic Ocean. *Global Biogeochem. Cycles* **22**(GB3014), 1-14

Knapp, A.N., Sigman, D.M., and Lipschultz, F., 2005. N-isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-Series study site. *Global Biogeochem. Cycles* **19**(GB1018), 1-15

Koppelmann, R., Böttger-Schnack, R., Möbius, J., and Weikert, H., 2009. Trophic relationships of zooplankton in the eastern Mediterranean Sea based on stable isotope measurements. *Journ. of Plankton Res.* **31**(6), 669-686

Koroleff, F., 1976. Total and organic nitrogen. In: K. Grasshoff, M. Ehrhard and K. Kremling (Eds.), "Methods of Seawater Analysis". Verlag Chemie, Weinheim, pp. 125-139

Kouvarakis, G., Mihalopoulos, N., Tselepides, A., and Stavrakakis, S., 2001. On the importance of atmospheric inputs of inorganic nitrogen species on the propductivity of the eastern Mediterranean Sea. *Global Biogeochemical Cycles* **15**(4), 805-817

Kranck, K., 1984. Role of flocculation in the filtering of particulate matter in estuaries. In: V.S. Kennedy (Ed.), "The Estuary as a Filter". Academic Press, Orlando FL., New York, pp. 159-175

Kress, N., and Herut, B., 2001. Spatial and seasonal evolution of dissolved oxygen and nutrients in the Southern Levantine Basin (Eastern Mediterranean Sea): Chemical characterisation of the water masses and inferences on the N: P ratios. *Deep-Sea Res. Part I* **48**, 2347-2372

Krom, M., Emeis, K., and Van Cappellen, P., 2010. Why is the Eastern Mediterranean phosphorus limited? *Progress in Oceanography* **85**, 236-244

Krom, M.D., Groom, S., and Zohary, T., 2003. The Eastern Mediterranean. In: K.D. Black and G.B. Shimmield (Eds.), "The Biogeochemistry of Marine Systems", Blackwell Publishing, Oxford, pp. 91-122

Krom, M.D., Herut, B., and Mantoura R.F.C., 2004. Nutrient budget for the Eastern Mediterranean: Implications for phosphorus limitation. *Limnol. Oceanogr.* **49**(5), 1582-1592

Krom, M.D., Kress, N., Brenner, S., and Gordon, L.I., 1991. Phosphorus limitation of primary production in the eastern Mediterranean Sea. *Limnol. Oceanogr.* **36**, 424-432

Krom, M.D., Thingstad, T.F., Brenner, S., Carbo, P., Drakopoulos, P., Fileman, T.W., Flaten, G.A.F., Groom, S., Herut, B., Kitidis, V., Kress, N., Law, C.S., Liddicoat, M.I., Mantoura, R.F.C., Pasternak, A., Pitta, P., Polychronaki, T., Psarra, S., Rassoulzadegan, F., Skjoldal, E.F., Spyres, G., Tanaka, T., Tselepides, A., Wassmann, P., Wexels Riser, C., Woodward, E.M.S., Zodiatis, G., and Zohary, T., 2005a. Summary and overview of the CYCLOPS P addition Lagrangian experiment in the Eastern Mediterranean Sea. *Deep-Sea Research II* **52**, 3090-3108

Krom, M.D., Woodward, E.M.S., Herut, B., Kress, N., Carbo, P., Mantoura, R.F.C., Spyres, G., Thingstad, T.F., Wassmann, P., Wexels-Riser, C., Kitidis, V., Law, C.S., and

Zodiatis, G., 2005b. Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: Results from a phosphorus starved system. *Deep-Sea Research 2*, **52**, 2879-2896

Lampert, W., 1978. Release of dissolved organic carbon by grazing zooplankton. *Limnol. Oceanogr.* **23**, 831-834

Lara, R.J., Rachold, V., Kattner, G., Hubberten, H.W., Guggenberger, G., Skoog, A., and Thomas, D.N., 1998. Dissolved organic matter and nutrients in the Lena River, Siberia Arctic: Characteristics and distribution. *Marine Chemistry* **59**, 301-309

Larsson, U., and Hagström, Å., 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Mar. Biol.* **52**, 199-206

Lascaratos, A., Roether, W., Nittis, K., and Klein, B., 1999. Recent changes in deep water formation and spreading in the eastern Mediterranean Sea: a review. *Progress in Oceanography* **44**, 5–36

Lawton, L.A., Codd, G.A., 1991. Cyanobacterial (blue-green algae) toxins and their significance in UK and European waters. *Journal of Soil and Water Conservation* **40**, 87–97

LeGrande, N.A., and Schmidt, G.A., 2006. Global gridded data set of the oxygen isotopic composition in seawater. *Geophys. Res. Lett.* **33**(L12604), 1-5

Lehmann, M.F., Reichert, P., Bernasconi, S.M., Barbieri, A., and McKenzie, J.A. 2003. Modelling nitrogen and oxygen isotope fractionation during nitrate reduction in a hypolimnetic redox transition zone. *Geochim. Cosmochim. Acta* **67**, 2529-2542

Lehmann, M.F., Sigman, D.M., McCorkle, D.C., Brunelle, B.G., Hoffmann, S., Kienast, M., Cane, G., and Clement, J., 2005. The origin of the deep Bering Sea nitrate deficit - Constraints from the nitrogen and oxygen isotopic composition of water-column nitrate and benthic nitrate fluxes. *Global Biogeochemical Cycles* **19**(GB4005), 1-15

Li, Q. P., Hansell, D.A., and Zhang, J.Z., 2008. Underway monitoring of nanomolar nitrate plus nitrite and phosphate in oligotrophic seawater. *Limnol. Ocean. Methods* **6**, 319-326

Liss, P.S., 1976. Conservative and non-conservative behaviour of dissolved constituents during estuarine mixing. In: J.D. Burton and P.S. Liss (Eds.), "Estuarine Chemistry". Academic Press, New York, 93-130

Lomas, M.W., and Glibert, P.M., 1999. Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. *Limnol. Oceanogr.* **44**, 556-572

Lytle, C.R., and Perdue, E.M., 1981. Free, proteinaceous, and humic bound amino acids in river water containing high concentrations of aquatic humics. *Environ. Sci. Technol.* **15**, 224-228

Mahaffey, C., Michaels, A., and Capone D.G., 2005. The conundrum of marine nitrogen fixation. *American Journal of Science* **305**, 546–595

Malanotte-Rizzolli, P., and Bergamasco, A., 1989. The circulation of the eastern Mediterrean, Part 1. *Oceanol. Acta* **12**, 335-351

Mann, K.H., 1982. Ecology of Coastal Waters. A System Approach, Blackwell Scientific, Oxford, pp. 322

Mannino, A., and Harvey, H.R., 1999. Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: Sources and diagenetic patterns. *Geochim Cosmochim Acta* **63**(15), 2219-2235

Mara, P., Mihalopoulos, N., Gogou, A., Dähnke, K., Schlarbaum, T., Emeis, K.-C., and Krom, M., 2009. Isotopic composition of nitrate in wet and dry atmospheric deposition on Crete in the eastern Mediterranean Sea. *Global Biogeochem. Cycles* **23**(GB4002), 1-11

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant Soil* **62**, 413-430

Mariotti, A., Lancelot, A., and Billen, G., 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended matter in the Scheldt Estuary. *Geochimica and Cosmochimica Acta* **48**, 549-555

Martin, A., and Cooke, G.D., 1994. Health risks in eutrophic water supplies. *Lake Line* **14**, 24–26

McClelland, J.W., and Valiela, I., 1998. Linking nitrogen in estuarine producers to land-derived sources. *Limnol. Oceanogr.* **43**, 577-585

Meador, T.B., Aluwihare, L.I., and Mahaffey, C., 2007. Isotopic heterogeneity and cycling of organic nitrogen in the oligotrophic ocean. *Limnol. Oceanogr.* **52**(3), 934-947

Mengis, M., Schiff, S.L., Harris, M., English, M.C., Aravena, R., Elgood, R.J., and MacLean, A., 1999. Multiple geochemical and isotopic approaches for assessing ground water NO_3^- elimination in a riparian zone. *Ground Water* **37**, 448-457

Menzel, D.W., and Vaccaro, R.F., 1964. The measurement of dissolved organic and particulate carbon in seawater. *Limnol. Oceanogr.* **9**, 138-142

Meybeck, M., 1993. C, N, P and S in rivers: From sources to global inputs. In: Wollast, R., Mackenzie, F.T., and Chou, L. (Eds.), "Interactions of C, N, P, and S Biogeochemical Cycles and Global Change", Springer Verlag Berlin, 163-193

Middelburg, J.J., and Herman, P.M.J., 2007. Organic matter processing in tidal estuaries. *Mar. Chem.* **106**(1-2), 127-147

Middelburg, J.J., and Nieuwenhuize, J., 2001. Nitrogen Isotope Tracing of Dissolved Inorganic Nitrogen Behaviour in Tidal Estuaries. *Estuarine, Coastal and Shelf Science* **53**, 385-391

Miller, C.A., and Glibert, P.M., 1998. Nitrogen excretion by the calanoid copepod Acartia tonsa: Results from mesocosm experiments. *J. Plankton Res.* **20**, 1767-1780

Minagawa, M., and Wada, E., 1986. Nitrogen isotope ratios of red tide organisms in the East China Sea: A characterization of biological nitrogen fixation. *Mar. Chem.* **19**, 245-249

Montoya, J., Carpenter, E., and Capone, D., 2002. Nitrogen fixation and nitrogen isotope abundance in zooplankton of the oligotrophic North Atlantic. *Limnol. Oceanogr.* **47**, 1617– 1628

Münster, U., and De Haan, H., 1998. The role of microbial extracellular enzymes in the transformation of dissolved organic matter in humic waters. In: D.O. Hessen, T.J. Tranvik (Eds.), "Aquatic Humic Substances", Springer-Verlag, Berlin, 199-257

OSPAR, 2008. Second OSPAR Integrated Report on the Eutrophication Status of the OSPAR Maritime Area, OSPAR Commission.

Palenik, B., and Hensen, S.E., 1997. The use of amides and other organic nitrogen sources by the phytoplankton *Emiliana huxlleyi*. *Limnol. Oceanogr.* **42**, 1544-1551

Palenik, B., and Morel, F.M.M., 1990a. Amino acid utilization by marine phytoplankton: A novel mechanism. *Limnol. Oceanogr.* **35**, 260-269

Palenik, B., and Morel, F.M.M., 1990b. Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton. *Mar. Ecol. Prog. Ser.* **59**, 195-201

Palenik, B., and Morel, F.M.M., 1991. Amine oxidases of marine phytoplankton. *App. Environ. Microbiol.* **57**, 2440-2443

Pantoja, S., Repeta, D.J., Sachs, J.P., and Sigman, D.M., 2002. Stable isotope constraints on the nitrogen cycle of the Mediterranean Sea water column. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.* **49**(9), 1609-1621

Pierre, C., 1999. The oxygen and carbon isotope distribution in the Mediterranean water masses. *Mar. Geology* **153**, 41-55

Preunkert, S., Wagenbach, D., and Legrand, M., 2003. A seasonally resolved alpine ice core record of nitrate:Comparison with anthropogenic inventories and estimation of preindustrial emissions of NO in Europe. *Journal Geophysical Research* **108**(D21, 4681), 1-10

Raabe, T., Yu, Z., Zhang, J., Sun, J., Starke, A., Brockmann, U., and Hainbucher, D., 2004. Phase-transfer of nitrogen species in the water column of the Bohai Sea. *Journal of Marine Systems* **44**, 213-232

Radach, G., and Paetsch, J., 2007. Variability of continental riverine freshwater and nutrient inputs into the North Sea for the years 1977-2000 and its consequences for the assessment of eutrophication. *Estuaries and Coasts* **30**(1), 66-81

Rees, A.P., Law, C.S., and Woodward, E.M.S., 2006. High rates of nitrogen fixation during an in-situ phosphate release experiment in the Eastern Mediterranean Sea. *Geophysical Res. Lett.* **33**(L10607), 1-4

Ribera d´Alcala, M., Civitarese, G., Conversano, F., and Lavezza, R., 2003. Nutrient ratios and fluxes hint at overlooked processes in the Mediterranean Sea. *Journal of Geophysical Research* **108**(C9), 1-16

Rixen, T., Ittekkot, V., Haake-Gaye, B., and Schäfer, P., 2000. The influence of the SW monsoon on the deep-sea organic carbon cycle in the Holocene. *Deep Sea Research Part II* **47**(14), 2629-2651

Roether, W., Manca, B.B., Klein, B., Bregant, D., Georgopoulos, D., Beitzel, V., Kovacevic, V., and Luchetta, A., 1996. Recent changes in Eastern Mediterranean deep waters. *Science* **271**, 333-335

Sachs, J.P., and Repeta, D.J., 1999. Oligotrophy and nitrogen fixation during Eastern Mediterranean sapropel events. *Science* 286, 2485-2488

Schlarbaum, T., Dähnke, K., and Emeis, K., 2010. Dissolved organic nitrogen turnover in the Elbe estuary/NW Europe: results of nitrogen isotope investigations. *Mar. Chem.* **119**, 91-107

Seitzinger, S., Gardner, W.S., and Spratt, A.K., 1991. The effect of salinity on ammonia sorption in aquatic sediments: Implications for benthic nutrient recycling. *Estuaries* **2**, 167-174

Seitzinger, S.P., and Sanders, R.W., 1997. Contributions of dissolved organic nitrogen from rivers to estuarine eutrophication. *Mar. Ecol. Prog. Ser.* **159**, 1-12

Seitzinger, S.P., Sanders, R.W., and Styles, R., 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. *Limnol. Oceanogr.* **47**(2), 353-366

Sharp, J.H., 1973. Total organic carbon in seawater - comparison of measurements using persulfate oxidation and high temperature combustion. *Mar. Chem.* **1**, 211-229

Sharp, J.H., Rinker, K.R., Savidge, K.B., Abell, J., Benaim, J.Y., Bronk, D.A., Burdige, D.J., Cauwet, G., Chen, W., Doval, M.D., Hansell, D., Hopkinson, C., Kattner, G., Kaumeyer, N., McGlathery, K.J., Merriam, J., Morley, N., Nagel, K., Ogawa, H., Pollard, C., Pujo-Pay, M., Raimbault, P., Sambrotto, R., Seitzinger, S., Spyres, G., Tirendi, F., Walsh, T.W., and Wong, C.S., 2002. A preliminary methods comparison for measurement of dissolved organic nitrogen in seawater. *Mar. Chem.* **78**, 171-184

Sigman, D.M., Altabet, M.A., Michener, R., McCorkle, D.C., Fry, B., and Holmes, R.M., 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Mar. Chem.* **57**(3-4), 227-242

Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., and Böhlke, J.K., 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* **73**(17), 4145-4153

Sigman, D.M., DiFiore, P.J., Hain, M.P., Deutsch, C., Wang, Y., Karl, D.M., Knapp, A.N., Lehmann, M.F., and Pantoja, S., 2009. The dual isotopes of deep nitrate as a constraint on the cycle and budget of oceanic fixed nitrogen. *Deep-Sea Res. Part I* **56**(9), 1419-1439

Sigman, D.M., Granger, J., DiFiore, P.J., Lehmann, M.F., Ho, R., Cane, G., and van Geen, A., 2005. Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. *Global Biogeochemical Cycles* **19**(4 (GB4022)), 1-14

Sigman, D.M., Robinson, R., Knapp, A.N., van Geen, A., McCorkle, D.C., Brandes, J.A., and Thunell, R.C., 2003. Distinguishing between water column and sedimentary denitrification in the Santa Barbara Basin using stable isotopes of nitrate. *Geochem. Geophys. Geosyst.* **4**, 1040-1059

Smith, D.C., Simon, M., Alldredge, A.L., Azam, F., 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359**, 139-141

Solórzano, L., and Sharp, J.H., 1980. Determination of total dissolved nitrogen in natural waters. *Limnol. Oceanogr.* **25**(4), 751-754

Stanley, E.H., and Maxted, J.T., 2008. Changes in the dissolved nitrogen pool across land cover gradients in Wisconsin streams. *Ecological Applications* **18**(7), 1579-1590

Stepanauskas, R., Edling, H., and Tranvik, L.J., 1999a. Differential dissolved organic nitrogen availability and bacterial aminopeptidase activity in limnic and marine waters. *Microbiol. Ecology* **38**, 264-272

Stepanauskas, R., Jørgensen, N.O.G., Eigaard, O.R., Žvikas, A., Tranvik, L.J., and Leonardsen, L., 2002. Summer inputs of riverine nutrients to the Baltic Sea: bioavailability & eutrophication relevance. *Ecol. Monogr.* **72**(4), 579-597

Stepanauskas, R., Leonardson, L., and Tranvik, T.J., 1999b. Bioavailabilty of wetland-derived DON to freshwater and marine bacterioplankton. *Limnol. Oceanogr.* **44**(6), 1477-1485

Stevenson, F.J., 1994. "Humus Chemistry", Wiley, New York

Struck, U., Emeis, K.-C., Rau, G.H., Voss, M., and Krom, M., 2001. Biological productivity during sapropel S5 formation in the eastern Mediterranean Sea - Evidence from stable isotopes of nitrogen and carbon. *Geochimica et Cosmochimica Acta* **65**(19), 3241-3258

Thingstad, T.F., and Rassoulzadegan, F., 1999. Conceptual models for the biogeochemical role of the photic zone microbial food web, with particular reference to the Mediterranean Sea. *Progress in Oceanography* **44**, 271–286

Thingstad, T.F., Krom, M.D., Mantoura, R.F.C., Flaten, G.A.F., Groom, S., Herut, B., Kress, N., Law, C.S., Pasternak, A., Pitta, P., Psarra, S., Rassoulzadegan, F., Tanaka, T., Tselepides, A., Wassmann, P., Woodward, E.M.S., Wexels Riser, C., Zodiatis, G., and Zohary, T., 2005. Nature of Phosphorus limitation in the ultraoligotrophic Eastern Mediterranean. *Science* **309**, 1068-1071

Thurman, E.M., 1985. Organic Geochemistry of Natural Waters. Niyoff/Junk, Boston

Tobias, C.R., Harvey, J.W., and Anderson, I.C., 2001. Quantifying groundwater discharge through fringing wetlands to estuaries: Seasonal variability, methods comparison, and implications for wetland-estuary exchange. *Limnol. Oceanogr.* **46**, 604-615

Tupas, L., and Koike, I., 1990. Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. *Limnol. Oceanogr.* **35**, 1145-1155

Valiela, I., and Teal, J.M., 1979. The nitrogen budget of a salt marsh ecosystem. *Nature* **280**, 652-656

Valiela, I., Costa, J. Foreman, K., Teal, J.M., Howes, B., and Aubrey, D., 1990. Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. *Biogeochemistry* 10, 177-197

Veuger, B., Middelburg, J.J., Boschker, H.T.S., Nieuwenhuize, J., van Rijswijk, P., Rochelle-Newall, E.J., and Navarro, N., 2004. Microbial uptake of dissolved organic and inorganic nitrogen in Randers Fjord. *Estuarine Coastal and Shelf Science* **61**(3), 507-515

Voss, M., Nausch, G., and Montoya, J.P., 1997. Nitrogen stable isotope dynamics in the central Baltic Sea: influence of deep-water renewal on the N-cycle changes. *Mar. Ecol. Prog. Ser.* **158**, 11-21

Wankel, S.D., Chen, Y., Kendall, C., Post, A.F., and Paytan, A., 2009. Sources of aerosol nitrate to the Gulf of Aqaba: Evidence from $\delta^{15}N$ and $\delta^{18}O$ of nitrate and trace metal chemistry. *Mar. Chem.* **120**, 90-99

Wankel, S.D., Kendall, C., Francis, C.A., and Paytan, A., 2006. Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach. *Limnol. Oceanogr.* **51**(4), 1654-1664

Warnken, C., 2003. Biogeochemie von Schwebstoffen, Sinkstoffen und Sedimenten im Ierapetra-Tief (östliches Mittelmeer). M.Sc. Thesis, 80 pp, University of Hamburg, Hamburg.

Wiegner, T.N., Seitzinger, S.P., Glibert, P.M., and Bronk, D.A., 2006. Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. *Aquatic Microbial Ecology* **53**, 277-287

Williams, P.M., and Druffel, E.R.M., 1987. Radiocarbon in dissolved organic matter in the central North Pacific Ocean. *Nature* **330**, 246-248

Wüst, G., 1961. On the vertical circulation of the Mediterranean Sea. *Journal of Geophysical Research* **66**, 3261-3271

Yool, A., Martin, A.P., Fernández, C., and Clark, D.R., 2007. The significance of nitrification for oceanic new production. *Nature* **447**, 999-1002

York, J.K., Tomasky, G., Valiela, I., and Repeta, D.J., 2007. Stable isotopic detection of ammonium and nitrate assimilation by phytoplankton in the Waquoit Bay estuarine system. *Limnol. Oceanogr.* **52**, 144–155

Zohary, T., and Robarts, R.D., 1998. Experimental study of microbial P limitation in the eastern Mediterranean. *Limnol. Oceanogr.* **43**(3), 387-395

Data Appendix

station	no.	Lat (°N)	Lon (°E)	depth [m]	stream km	salinity [psu]	c(NO_3^-) [µM]	c(TDN) [µM]	c(DON) [µM]	c(PN) [µM]	δ^{15}N-NO_3^- [‰]	δ^{15}N-TDN [‰]	δ^{15}N-DON [‰]	δ^{15}N-PN [‰]	date
Tesperhude	2-2	53°24.025'	10°25.510'	4.0	579	0.46	184.82	218.27	33.45	n.d.	11.3	9.8	2.1	n.d.	24.10.2005
Zollenspieker	2-1	53°23.781'	10°10.716'	1.5	598	0.46	184.19	213.14	28.95	n.d.	11.4	10.5	4.5	n.d.	24.10.2005
Zollenspieker	2-3	53°23.781'	10°10.716'	5.0	598	0.46	185.09	212.59	27.50	n.d.	11.5	10.4	3.3	n.d.	24.10.2005
Köhlbrandhöft, Norderelbe	2-1	53°32.469'	9°56.184'	1.5	625.3	0.43	169.91	217.42	47.51	n.d.	11.4	10.0	4.7	8.4	24.10.2005
Köhlbrandhöft, Norderelbe	2-3	53°32.469'	9°56.184'	15.0	625.3	0.43	161.34	216.91	55.57	45.24	11.2	10.3	7.7	8.1	24.10.2005
Köhlbrandhöft, Südereleb	2-1	53°32.234'	9°56.075'	1.5	625.5	0.43	170.60	210.36	39.76	n.d.	11.4	10.1	4.2	n.d.	24.10.2005
Köhlbrandhöft, Südereleb	2-3	53°32.234'	9°56.075'	14.0	625.5	0.43	170.46	206.89	36.42	n.d.	11.3	10.5	6.6	n.d.	24.10.2005
Seemannhöft	2-1	53°32.528'	9°53.974'	1.5	628	0.43	172.43	217.92	45.49	n.d.	11.6	10.3	5.5	9.9	24.10.2005
Seemannhöft	2-3	53°32.528'	9°53.974'	15.0	628	0.43	172.66	216.08	43.42	38.26	11.3	10.2	5.6	8.8	24.10.2005
Pagensander Nebenelbe	2-1	53°42.002'	9°31.467'	1.5	660	0.56	185.30	222.81	37.51	n.d.	11.4	10.4	5.3	8.9	25.10.2005
Pagensander Nebenelbe	2-3	53°42.002'	9°31.467'	6.0	660	0.56	184.44	n.d.	n.d.	n.d.	11.6	n.d.	n.d.	9.9	25.10.2005
Grauerort	2-1	53°40.510'	9°30.552'	1.5	660.5	0.63	190.00	224.82	34.83	n.d.	11.4	10.2	3.7	9.8	25.10.2005
Grauerort	2-3	53°40.510'	9°30.552'	15.0	660.5	0.51	188.42	225.92	37.50	64.39	11.4	10.2	4.5	8.9	25.10.2005
Glückstadt	2-1	53°47.170'	9°23.088'	1.5	675.5	0.77	181.51	220.07	38.56	n.d.	11.8	10.2	2.8	n.d.	25.10.2005
Glückstadt	2-2	53°47.170'	9°23.088'	7.5	675.5	0.85	187.02	214.01	26.99	n.d.	11.5	10.3	1.6	8.7	25.10.2005
Brunsbüttel	2-1	53°52.928'	9°10.832'	1.5	693	12.2	101.19	135.82	34.64	n.d.	12.0	10.8	7.3	9.2	26.10.2005
Brunsbüttel	2-3	53°52.928'	9°10.832'	15.0	693	13.5	95.03	130.34	35.31	30.23	11.8	10.1	5.4	8.7	26.10.2005
Elbe, Tonne 53	1-1	53°51.394'	9°01.664'	1.5	704	15.5	81.67	109.12	27.45	n.d.	11.9	10.6	6.7	n.d.	26.10.2005
Elbe, Tonne 53	1-2	53°51.394'	9°01.664'	15.0	704	18.5	68.94	97.00	28.06	20.94	12.1	9.7	3.8	7.3	26.10.2005
Elbe, Tonne 47	1-1	53°50.524'	8°56.071'	1.5	710	17.2	72.85	101.56	28.71	n.d.	12.1	9.5	2.9	n.d.	26.10.2005
Elbe, Tonne 47	1-2	53°50.524'	8°56.071'	13.0	710	21.1	60.10	90.20	30.10	n.d.	11.8	9.6	5.3	n.d.	26.10.2005
Elbe, Tonne 33	1-1	53°51.440'	8°44.381'	1.5	721.6	21.2	50.77	82.96	32.19	n.d.	12.0	10.2	7.5	n.d.	26.10.2005
Elbe, Tonne 33	1-2	53°51.440'	8°44.381'	15.0	721.6	25.8	30.22	64.24	34.02	n.d.	11.4	8.6	6.2	n.d.	26.10.2005
Cuxhaven	1-1	53°53.392'	8°41.560'	1.5	725	23.4	36.61	65.21	28.60	n.d.	11.7	8.8	5.2	n.d.	26.10.2005
Cuxhaven	1-2	53°53.392'	8°41.560'	15.0	725	27.5	22.13	50.67	28.54	n.d.	10.9	9.8	9.0	n.d.	26.10.2005

Table App 1: Salinity gradient in the Elbe estuary, October 2005 (cf. Chapter 3), sampled with RV *Ludwig Prandtl*

station	no.	Lat (°N)	Lon (°E)	depth	stream km	salinity [psu]	c(NO_3^-) [µM]	c(TDN) [µM]	c(DON) [µM]	c(PN) [µM]	δ^{15}N-NO_3^- [‰]	δ^{15}N-TDN [‰]	δ^{15}N-DON [‰]	δ^{15}N-PN [‰]	date
Tesperhude	1	53°40.06	10°42.5	1	579	0.41	113.4	154.5	41.1	69.7	16.8	13.5	4.5	n.d.	22.06.2006
Tesperhude	2	53°40.06	10°42.5	3	579	0.41	114.1	143.8	29.6	96.6	17.1	14.1	2.7	n.d.	22.06.2006
Zollenspieker	1	53°39.58	10°17.99	0.5	598	0.39	114.2	142.8	28.6	77.8	16.7	13.9	2.5	4.3	22.06.2006
Zollenspieker	2	53°39.58	10°17.99	1.5	598	0.39	113.7	146.9	33.2	100.8	16.7	13.7	3.1	9.7	22.06.2006
Köhlbrandhöft, Norderelbe	1	53°54.02	9°93.57	1	625.3	0.4	151.8	202.8	52.3	23.8	12.2	11.1	7.4	3.1	21.06.2006
Köhlbrandhöft, Norderelbe	2	53°54.02	9°93.57	11	625.3	0.4	145.1	199.2	54.1	21.9	12.3	11.1	8.1	1.6	21.06.2006
Köhlbrandhöft, Süderelbe	1	53°53.69	9°93.72	1	625.5	0.4	144.7	195.8	51.0	33.2	13.5	11.7	6.6	3.8	21.06.2006
Köhlbrandhöft, Süderelbe	2	53°53.69	9°93.72	5	625.5	0.4	148.9	192.1	43.2	37.2	13.2	11.7	6.3	3.6	21.06.2006
Seemanshöft	1	53°54.04	9°88.26	1	628	0.35	155.2	212.8	57.6	47.7	12.1	11.0	8.1	3.6	21.06.2006
Seemanshöft	2	53°54.04	9°88.26	8.5	628	0.35	159.1	209.1	50.0	36.6	11.8	11.1	9.0	4.4	21.06.2006
Grauerort	1	53°67.21	9°50.31	1	660.5	0.5	210.4	241.5	32.3	29.3	10	8.9	1.2	6.8	21.06.2006
Grauerort	2	53°67.21	9°50.31	9.5	660.5	0.5	210.0	244.3	35.5	28.2	9.8	8.9	3.5	8.9	21.06.2006
Glückstadt	1	53°78.51	9°38.06	1	675.5	0.8	224.1	254.6	31.7	n.d.	9.4	8.9	4.8	n.d.	21.06.2006
Glückstadt	2	53°78.51	9°38.06	11	675.5	0.8	227.3	252.0	26.0	22.0	9.8	8.8	0.1	9.1	21.06.2006
Brunsbüttel	1	53°87.7	9°17.43	1	693	2.6	190.2	219.5	30.6	71.7	10.8	10.0	4.3	n.d.	21.06.2006
Brunsbüttel	2	53°87.7	9°17.43	13	693	4.3	178.8	205.0	27.5	49.1	11.1	10.2	3.5	n.d.	21.06.2006
Elbe, Tonne 53	1	53°85.51	9°02.02	1	704	8.1	156.5	199.4	43.8	27.0	10.9	10.3	8.2	7.9	21.06.2006
Elbe, Tonne 53	2	53°85.51	9°02.02	17	704	8.7	153.3	193.1	41.7	90.7	10.9	10.4	7.9	n.d.	21.06.2006
Elbe, Tonne 47	1	53°84.3	8°94.21	1	710	10	141.0	191.3	50.7	12.9	11.4	10.2	6.7	n.d.	21.06.2006
Elbe. Tonne 47	2	53°84.3	8°94.21	16	710	12.8	125.4	167.5	41.6	23.7	11.1	10.3	8.1	8.5	21.06.2006
Elbe, Tonne 33	1	53°85.11	8°75.47	1	721.6	19.1	73.2	116.9	44.2	15.1	11.3	10.2	8.2	n.d.	21.06.2006
Elbe, Tonne 33	2	53°85.11	8°75.47	18	721.6	20.2	76.5	110.9	34.8	15.4	11.4	10.2	7.2	9.9	21.06.2006
Cuxhaven	1	53°86.39	8°70.97	1	725	17.2	64.0	95.2	31.5	11.9	10.9	10.1	8.2	n.d.	20.06.2006
Cuxhaven	2	53°86.39	8°70.97	18	725	22.5	56.0	98.8	43.2	11.2	11	10.0	8.5	n.d.	20.06.2006
Elbe, Tonne 22	1	53°97.96	8°60.91	1	735	23.6	49.0	79.4	30.8	14.4	11.4	9.5	6.4	14.2	21.06.2006
Elbe, Tonne 22	2	53°97.96	8°60.91	10	735	25.7	40.2	68.6	28.8	3.6	11.3	9.3	6.3	n.d.	21.06.2006
Elbe, Tonne 12	1	53°99.22	8°43.84	1	746.2	26.1	34.6	60.5	26.3	31.6	11.5	9.4	6.5	8.1	20.06.2006
Elbe, Tonne 12	2	53°99.22	8°43.84	9	746.2	29.9	18.7	47.4	29.1	31.6	11.5	8.5	6.5	8.1	20.06.2006
Elbe, Tonne 02	1	54°02.81	8°22.44	1	759.3	27.8	26.4	51.2	25.3	n.d.	11.5	9.0	6.2	n.d.	20.06.2006
Elbe, Tonne 02	2	54°02.81	8°22.44	14	759.3	31.3	10.3	37.2	27.3	15.4	11.5	8.0	6.5	10.7	20.06.2006
Elbe 1	1	54°03.18	8°11.63	1	765.6	30.7	10.6	31.3	21.1	n.d.	12.0	8.5	6.5	n.d.	20.06.2006
Elbe 1	2	54°03.18	8°11.63	18	765.6	31.6	9.4	36.7	27.7	n.d.	12.1	7.9	6.4	n.d.	20.06.2006
north of Elbe 1	1	54°07.43	8°00.78	1	771.9	30	7.5	28.5	21.4	n.d.	13.9	7.4	5.0	n.d.	20.06.2006
north of Elbe 1	2	54°07.43	8°00.78	15.5	771.9	31.2	8.4	27.8	19.8	n.d.	13.0	8.0	5.7	n.d.	20.06.2006

Table App 2: Salinity gradient in the Elbe estuary, June 2006 (cf. Chapter 3), sampled with RV *Ludwig Prandtl.*

time (UTC)	Lat (°N)	Lon (°E)	stream km	salinity [psu]	c(NO_3^-) [µM]	c(TDN) [µM]	c(DON) [µM]	$\delta^{15}N$-NO_3^- [‰]	$\delta^{15}N$-TDN [‰]	$\delta^{15}N$-DON [‰]	$\delta^{15}N$-PN [‰]	date
13:33:06	53°56.58	9°65.78	644.00	0.52	231.7	256.1	24.5	10.50	9.7	2.6	9.33	20.12.2006
11:59:05	53°77.91	9°38.25	674.00	0.77	228.7	258.4	29.8	10.40	9.4	1.6	8.43	20.12.2006
11:49:06	53°81.12	9°36.69	678.00	1.10	228.9	261.3	32.4	10.28	9.7	5.5	8.32	20.12.2006
11:35:06	53°84.73	9°31.42	683.00	2.05	218.9	253.5	34.6	10.36	9.7	5.4	8.50	20.12.2006
11:13:05	53°87.71	9°20.25	692.00	4.01	204.5	238.2	33.7	10.29	10.2	9.7	8.13	20.12.2006
11:05:06	53°87.8	9°15.24	695.00	5.06	194.7	226.9	32.2	10.48	10.1	7.8	8.22	20.12.2006
10:58:16	53°87.6	9°10.94	698.00	6.55	184.3	215.1	30.8	10.79	10.4	8.0	8.14	20.12.2006
10:49:06	53°86.4	9°05.76	701.50	7.49	161.1	221.2	60.1	10.73	10.4	9.4	8.36	20.12.2006
10:41:15	53°85.37	9°01.45	704.50	8.55	162.4	202.5	40.1	10.58	10.2	8.9	8.01	20.12.2006
10:33:36	53°84.7	8°96.94	707.00	9.47	154.5	206.2	51.7	10.57	10.2	8.9	7.93	20.12.2006
10:28:36	53°84.3	8°93.96	709.50	10.58	147.8	193.0	45.2	10.86	10.3	8.7	7.98	20.12.2006
10:22:15	53°84.02	8°90.15	712.00	11.95	133.4	182.5	49.1	10.54	10.3	9.5	7.96	20.12.2006
10:09:16	53°83.65	8°82.3	717.00	13.74	124.1	168.9	44.8	10.69	10.3	9.3	7.91	20.12.2006
09:59:36	53°84.38	8°76.85	720.30	16.49	103.4	143.1	39.7	10.02	10.1	10.4	7.96	20.12.2006
07:44:16	no data	no data	731.00	18.05	89.6	134.1	44.4	10.34	10.1	9.7	7.73	20.12.2006
07:52:35	no data	no data	733.00	21.10	73.5	113.4	39.9	9.70	9.7	9.7	8.46	20.12.2006
08:00:06	no data	no data	735.00	22.34	65.5	104.8	39.3	9.74	10.0	10.5	8.20	20.12.2006
08:07:15	53°96.2	8°56.61	737.00	23.58	57.1	93.2	36.1	9.13	9.9	11.2	7.96	20.12.2006
09:03:36	53°96.3	8°55.75	738.00	27.22	33.9	63.4	29.4	7.98	9.4	11.0	8.01	20.12.2006
08:11:15	53°96.36	8°55.13	739.00	25.01	50.3	85.4	35.1	9.08	10.0	11.3	8.04	20.12.2006

Table App 3: Salinity gradient in the Elbe estuary, December 2006 (cf. Chapter 3), sampled with RV *Ludwig Prandtl.* Water samples taken from the surface (1 m depth) with the ship's membrane pump.

date	$c(NO_3^-)$ [µM]	c(TDN) [µM]	c(DON) [µM]	c(PN) [µM]	c(DON)/c(TDN) *100 [%]
23.06.2005	78.9	104.0	25.1	n.d.	24.1
11.07.2005	78.4	129.3	50.9	n.d.	39.4
01.08.2005	104.4	141.6	37.2	n.d.	26.3
20.08.2005	50.7	85.2	34.6	n.d.	40.6
20.08.2005	60.7	92.3	31.6	n.d.	34.3
24.10.2005	184.8	218.3	33.5	n.d.	15.3
16.01.2006	372.7	423.4	50.7	n.d.	12.0
20.02.2006	306.2	369.0	62.9	n.d.	17.0
07.03.2006	283.3	327.8	44.5	n.d.	13.6
20.03.2006	421.7	457.9	36.1	n.d.	7.9
31.03.2006	316.4	391.1	74.8	n.d.	19.1
10.04.2006	365.2	427.2	62.0	n.d.	14.5
03.05.2006	238.9	261.2	22.3	n.d.	8.5
23.06.2006	114.1	143.8	29.7	n.d.	20.6
04.07.2006	81.3	122.9	41.6	64.7	33.9
24.07.2006	97.8	145.4	47.6	36.0	32.8
03.08.2006	32.7	75.2	42.5	126.7	56.6
24.08.2006	142.7	175.1	32.3	n.d.	18.5
12.09.2006	97.3	131.6	34.3	n.d.	26.1
10.10.2006	104.7	134.4	29.7	97.8	22.1
03.11.2006	134.1	164.8	30.7	48.9	18.7
28.11.2006	210.8	239.8	29.0	17.4	12.1
21.12.2006	246.2	279.6	33.4	10.9	11.9
12.01.2007	252.3	306.8	54.5	22.8	17.8
19.02.2007	321.2	388.2	67.0	25.2	17.3
14.03.2007	274.8	333.7	59.0	29.6	17.7
27.04.2007	120.7	155.9	35.2	89.7	22.6
30.05.2007	66.3	110.9	44.7	77.4	40.3
11.06.2007	66.9	106.5	39.5	41.6	37.2
04.07.2007	74.2	110.5	36.3	41.9	32.8
31.07.2007	80.8	125.1	44.3	37.4	35.4
27.08.2007	110.2	145.2	35.0	36.6	24.1
20.09.2007	178.7	207.1	28.5	25.7	13.7
16.10.2007	206.5	236.6	30.1	31.2	12.7
14.11.2007	228.4	266.6	38.2	27.5	14.3
06.12.2007	269.5	306.8	37.3	22.0	12.2

Table App 4: concentration data and c(DON) ratio in the Elbe River at the weir of Geesthacht (June 2005 – December 2007, cf. Chapter 4)

date	δ^{15}N-NO_3^- [‰]	δ^{18}O-NO_3^- [‰]	δ^{15}TDN [‰]	δ^{15}DON [‰]	δ^{15}PN [‰]	discharge [m^3/s]
23.06.2005	19.8	n.d.	15.8	3.0	n.d.	462
11.07.2005	18.6	n.d.	14.6	8.5	n.d.	406
01.08.2005	16.4	n.d.	13.7	6.0	n.d.	495
20.08.2005	20.6	n.d.	14.3	5.1	n.d.	418
20.08.2005	20.4	n.d.	14.6	3.5	n.d.	418
24.10.2005	11.3	n.d.	9.8	2.1	n.d.	371
16.01.2006	9.2	0.2	9.2	8.8	n.d.	485
20.02.2006	9.1	2.0	8.7	6.6	n.d.	609
07.03.2006	8.8	1.2	9.2	11.5	n.d.	617
20.03.2006	8.9	n.d.	8.5	3.7	n.d.	853
31.03.2006	8.0	0.7	8.1	8.2	n.d.	1270
10.04.2006	6.4	0.6	6.5	7.3	n.d.	3602
03.05.2006	8.6	1.9	8.0	1.5	n.d.	1526
23.06.2006	17.1	7.6	14.1	2.7	n.d.	512
04.07.2006	19.1	8.4	14.6	5.7	n.d.	385
24.07.2006	17.0	7.2	13.0	4.7	5.9	430
03.08.2006	22.5	12.1	13.4	6.4	8.2	245
24.08.2006	12.0	3.0	10.4	3.3	n.d.	503
12.09.2006	16.9	7.9	13.5	3.8	n.d.	449
10.10.2006	16.9	7.5	14.1	4.2	7.4	320
03.11.2006	13.2	3.5	10.9	0.8	5.9	301
28.11.2006	10.0	0.8	9.7	7.8	9.6	467
21.12.2006	10.3	0.4	10.0	7.7	10.0	406
12.01.2007	10.0	0.8	9.4	6.8	9.1	513
19.02.2007	8.3	0.6	8.2	7.8	8.8	1047
14.03.2007	8.3	1.0	7.8	5.5	8.5	1129
27.04.2007	15.8	7.1	12.9	2.8	5.9	435
30.05.2007	17.8	8.6	13.2	6.4	8.2	389
11.06.2007	19.8	10.3	14.5	5.6	7.8	387
04.07.2007	18.1	8.7	13.6	4.3	7.2	444
31.07.2007	16.2	6.6	12.3	5.2	7.4	466
27.08.2007	14.5	4.9	12.3	5.3	6.6	589
20.09.2007	11.7	2.1	10.8	4.7	7.4	600
16.10.2007	10.4	1.7	9.4	2.8	8.6	766
14.11.2007	9.9	0.9	9.6	8.4	8.3	757
06.12.2007	8.8	0.7	8.6	6.8	8.3	1069

Table App 5: isotope ratios and discharge data in the Elbe River at the weir of Geesthacht (June 2005 – December 2007, cf. Chapter 4)

Curriculum Vitae

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Since February 2005:	Ph.D. student, University of Hamburg, in cooperation with Helmholtz Zentrum Geesthacht (formerly GKSS), Institute for Coastal Research Ph.D. thesis: “Assessing the role of dissolved organic nitrogen in different ecosystems”
October 1995 – October 2004:	Study of Chemistry (Diploma), University of Hamburg
January – October 2004:	Diploma thesis at the Institute for Organic Chemistry: „Identification and synthesis of hydrocarbon patterns of insects“
February – June 2000:	Sokrates student at the Institute for Inorganic Chemistry, University of Southampton, England
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August 1990 – January 1994:	Education as chemical technical assistant at the Gymnasium Altona, Hamburg

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List of papers

Schlarbaum, T., Dähnke, K., and Emeis, K., 2010: Turnover of combined dissolved organic nitrogen and ammonium in the Elbe estuary / NW Europe: Results of nitrogen isotopes investigations. *Mar. Chem.* **119**, 91-107

Schlarbaum, T., Dähnke, K., and Emeis, K., 2010: Dissolved and particulate reactive nitrogen in the Elbe River / NW Europe: a 2-year N-isotope study. *Biogeoscience Discuss.* **7**, 7543-7574, doi:10.5194/bgd-7-7543-2010

Emeis, K.-C., Mara, P., Schlarbaum, T., Möbius, J., Dähnke, K., Struck, U., Mihalopoulos, N., and Krom, M., 2010: N-isotope ratios of nitrate, dissolved organic nitrogen and particulate nitrogen in the Eastern Mediterranean Sea. In press in *Journal of Geophysical Research – Biogeochemistry*

Mara, P., Mihalopoulos, N., Gogou, A., Dähnke, K., Schlarbaum, T., Emeis, K.-C., and Krom, M., 2009. Isotopic composition of nitrate in wet and dry atmospheric deposition on Crete in the eastern Mediterranean Sea. *Global Biogeochem. Cycles* **23**(GB4002), 1-11

Paetsch, J., Serna, A., Dähnke, K., Schlarbaum, T., Johannsen, A., and Emeis, K.C., 2010. Nitrogen cycling in the German Bight (SE North Sea) – Clues from modelling stable nitrogen isotopes. *Continental Shelf Research* **30**, 203-213