

**Identification and Quantification of Diffuse Nitrogen Inputs
by Means of Stable Nitrogen and Oxygen Isotopes in Nitrate:
Investigations in the Warnow River System**

Identifizierung und Quantifizierung von diffusen Stickstoffeinträgen
anhand der stabilen Isotope von Stickstoff und Sauerstoff im Nitrat:

Untersuchungen im Einzugsgebiet der Warnow

Dissertation zur Erlangung des akademischen Grades doctor rerum naturalium

(Dr. rer. nat.) der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität Rostock

vorgelegt von Barbara Deutsch

geb. am 26.09.1973 in Marl

aus Rostock

Rostock, im Mai 2005

Gutachter:

PD Dr. Maren Voß

Institut für Ostseeforschung Warnemünde, Sektion Biologische Meereskunde

PD Dr. Stefan Forster

Universität Rostock, FB Biowissenschaften, Meeresbiologie

Prof. Dr. Gerhard Gebauer

Universität Bayreuth, Bayceer, FG Bodenprozesse

Tag der Verteidigung: 03. März 2006

INDEX

I.	SUMMARY	4
II.	INTRODUCTION.....	6
	Anthropogenic nitrogen in the environment	6
	Coastal eutrophication.....	9
	The Baltic Sea ecosystem.....	11
	Nitrogen emissions from agriculture.....	15
	Stable isotopes in Hydrology and Ecology	19
III.	AIM OF THIS PHD-THESIS.....	28
IV.	MATERIAL AND METHODS	31
	Study area.....	31
	Stable isotope measurements.....	32
V.	VARIATIONS IN THE $\delta^{15}\text{N}$ AND $\delta^{18}\text{O}$ VALUES OF NITRATE IN DRAINAGE WATER OF TWO FERTILIZED FIELDS IN MECKLENBURG-VORPOMMERN (GERMANY).....	35
VI.	ASSESSING THE IMPACT OF NITRATE FROM TILE DRAINAGE FOR ADJACENT SURFACE WATERS: A STABLE ISOTOPE APPROACH.....	50
VII.	QUANTIFICATION OF DIFFUSE NITROGEN INPUTS INTO A SMALL RIVER SYSTEM USING STABLE ISOTOPES OF OXYGEN AND NITROGEN IN NITRATE.....	59
VIII.	ANTHROPOGENIC NITROGEN INPUT TRACED BY MEANS OF $\delta^{15}\text{N}$ VALUES IN MACROALGAE: RESULTS FROM IN-SITU INCUBATION EXPERIMENTS	72
IX.	FINAL CONCLUSIONS AND FUTURE OUTLOOK.....	89
X.	REFERENCES	91
XI.	DANKE	106
XII.	ERKLÄRUNG.....	107
XIII.	LEBENS LAUF	108

I. SUMMARY

The presented PhD-thesis is part of the scholarship programme “The Southern Baltic Sea and its Coasts in Change” financed by the German Federal Environmental Foundation (DBU). In this interdisciplinary programme 15 young scientists worked on the anthropogenic or natural impacts on the coastal areas of the southern Baltic Sea, and the consequences for this sensible Ecosystem. The scholarships covered a wide range of scientific fields; geology, law, socio-economy, paleo-geography, and marine biology.

Aim of this thesis was the identification and quantification of diffuse nitrogen sources for the Warnow river-system which is a tributary to the Baltic Sea and influenced by high nitrogen inputs ($>4000 \text{ t a}^{-1}$) mainly from agriculture. Due to the interdisciplinary approach which combined agricultural and marine research it should be assessed whether combined measurements of stable isotope ratios of nitrogen and oxygen in nitrate (so-called “dual isotope approach”) can be used to quantify and trace diffuse nitrate inputs.

Four studies have been carried out and the first concentrated on the reasons for the variability of the stable isotope ratios of nitrogen and oxygen in drainage water nitrate. It was assumed that the kind of fertilizer and microbial processes modify the stable isotope ratios. Over a period of 17 months the variations in drainage water nitrate from two conventional farmed soils were investigated with weekly to bimonthly resolution. Nitrification was the dominating process in the soils, and other processes like mixture of soil water nitrate with nitrate from mineral fertilizers could be identified but were of minor importance. Ammonia volatilization, a process responsible for large N losses from agricultural land, was also identified.

The second study concentrated on the main discharge period with a much higher temporal resolution than in the first study. This led to a better resolution of short-term variations in the isotope values. A close relationship between atmospheric deposition, discharge and nitrate concentration in the drainage water could be demonstrated. Additionally, the importance of

the drainage water as N source was investigated for an adjacent ditch and a brook, which followed the tile drain outlet. As observed in the second study, nitrification was the major process in the soil.

The third study tested, if the dual isotope approach in combination with a linear mixing model reliably quantifies diffuse nitrate inputs in comparison to other modelling approaches. The stable isotopes of nitrogen and oxygen were measured in three main nitrate sources as well as the river for a period of 5 months. The concentration-weighted mean values were applied in a mixing model, and the results showed that 86 % of the river nitrate derived from drainage water. A comparison with estimates from a nutrient emission model showed good agreement.

In the fourth study a simple method to trace anthropogenic nitrogen in river-systems and estuaries was applied, and its reliability was tested. Former studies had shown that macroalgae are suitable detectors for anthropogenic N because they do not fractionate during the uptake of nitrogen and thus directly reflect the isotope ratios of assimilated nitrogen. Two incubation experiments with one brown alga and two red algae species in the Warnow river estuary revealed a significant correlation of $\delta^{15}\text{N}$ in NO_3^- and in the algae. Furthermore, it was shown that the red algae are more suitable to trace anthropogenic N. Especially in undisturbed parts of the estuary, where relatively constant nitrate isotope values were measured, the red algae well reflected the isotope values of the river nitrate.

Altogether, the results of this PhD-thesis show, that the application of stable isotope ratios of nitrogen and oxygen in nitrate provides a good tool to characterize, quantify and trace diffuse nitrogen inputs in river-systems. The method can be used to support nutrient emission models, and can be applied for the monitoring of water restoration measurements.

II. INTRODUCTION

Anthropogenic nitrogen in the environment

Anthropogenic nitrogen (N) fixation exceeds natural terrestrial N fixation since the late 20th century, only 100 years after the discovery of natural N fixation (Galloway & Cowling, 2002). The key to this rapid increase was the invention of the Haber-Bosch process in 1913, which converts atmospheric nitrogen (N_2) into ammonia (NH_3). This allows an unlimited production of reactive nitrogen (N_r), which includes all biologically, photochemically and radiatively active N compounds in the atmosphere and biosphere of the Earth (Galloway & Cowling, 2002). In 1990 the total amount of N fixed by the Haber-Bosch process was $\sim 85 \text{ Mt N a}^{-1}$ (Mt = megatons = 10^6 tons), most of which was used for food production in the form of mineral fertilizers ($\sim 82 \text{ Mt N a}^{-1}$; Fixen & West, 2002). Presently, over 50% of the food consumed worldwide is produced under addition of fertilizer nitrogen from the Haber-Bosch process (Smil, 2001), but only a small amount of this nitrogen actually reaches the final consumer. Figure II.1 depicts that 86 and 96 N atoms of 100 N atoms artificially produced are lost to the environment in a vegetarian and carnivorous diet, respectively (Galloway & Cowling, 2002).

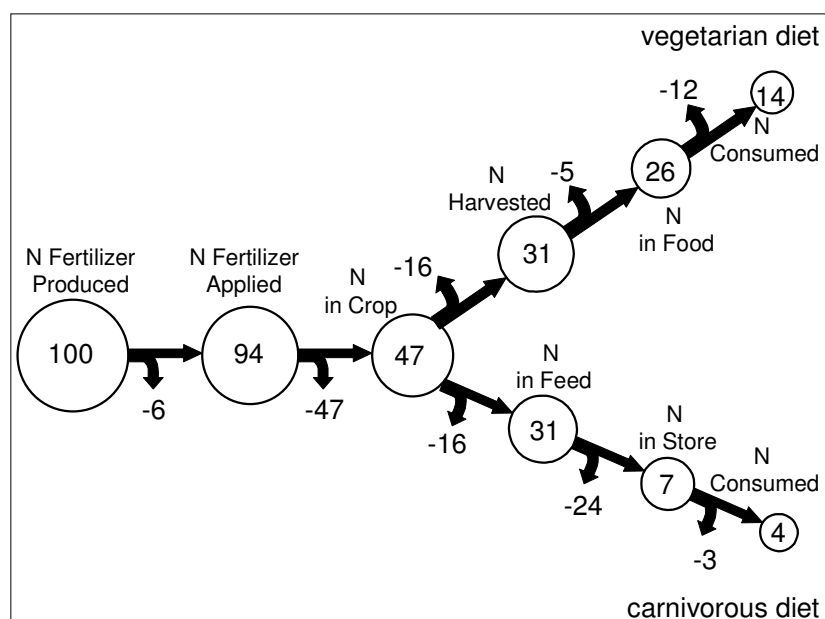


Fig. II.1: Losses of N produced by the Haber-Bosch process for vegetarian and carnivorous diet (redrawn after Galloway & Cowling, 2002)

For both diets, losses also occur during transport, storage and application (6 atoms), after the application of fertilizer (47 atoms), and during harvest and crop residue (16 atoms). The final production of a carnivorous diet leads to a higher loss of N (24 atoms) than the production of a vegetarian diet (5 atoms), mainly because of the animal metabolism and a higher amount of unusable residuals. Consumption and excretion leads to further loss of N.

Another pathway how anthropogenic nitrogen is lost to the environment is the combustion of fossil fuels, which accounts for $\sim 21 \text{ Mt N a}^{-1}$ in 1990 (Galloway & Cowling, 2002). The total amount of N_r created by anthropogenic activities was $\sim 140 \text{ Mt N a}^{-1}$ in 1990, including the cultivation-induced N_r creation of $\sim 33 \text{ Mt N a}^{-1}$ from rice and legume cultivation (Galloway & Cowling, 2002). Natural N fixation is estimated to $90\text{-}130 \text{ Mt N a}^{-1}$ for terrestrial ecosystems (Stedman & Shetter, 1983) and to $80\text{-}400 \text{ Mt N a}^{-1}$ for the marine environment (Carpenter & Romans, 1991).

The cycle of anthropogenic nitrogen in the environment is summarized in Figure II.2.

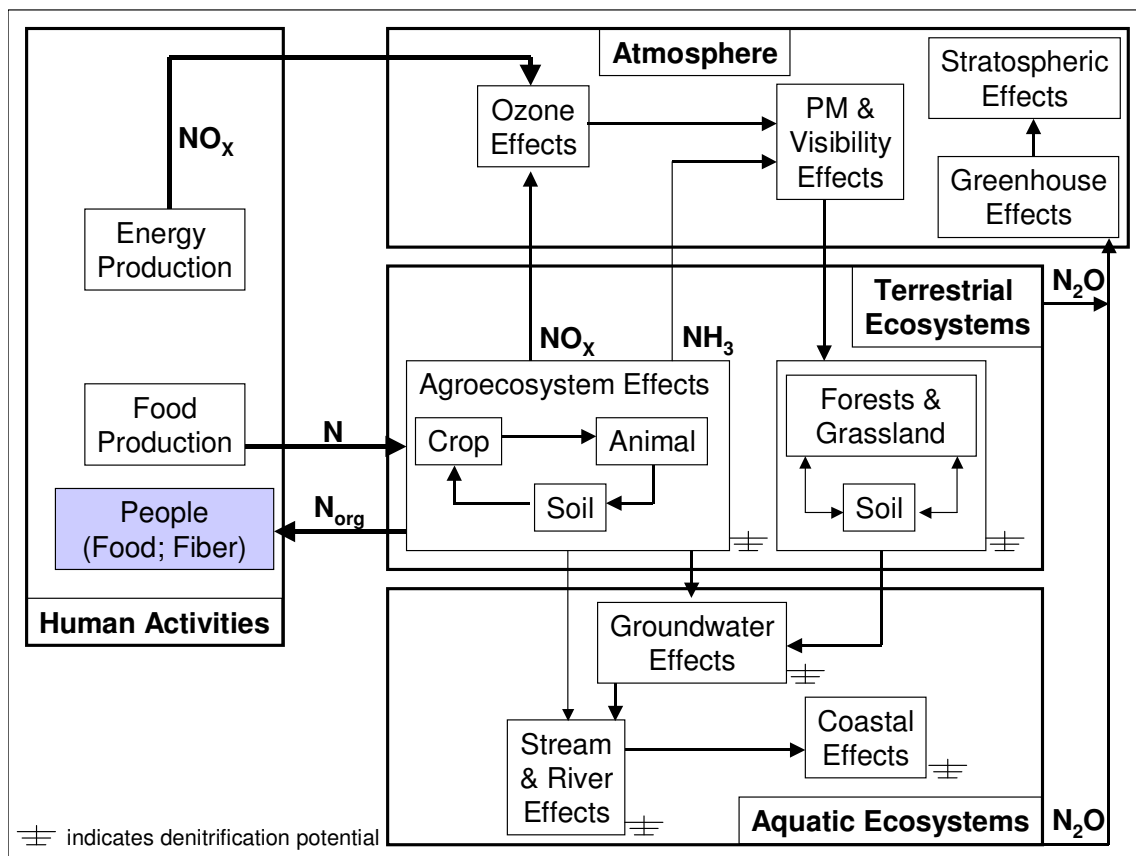


Fig. II.2: Pathways of human induced reactive nitrogen (N_r) in the atmosphere, terrestrial ecosystems, and aquatic ecosystems of the earth (redrawn after Galloway & Cowling, 2002)

Nitrogen oxide (NO_x) emissions contribute to ground level ozone (smog) and react with ammonia (NH₃), moisture and other compounds to form nitric acid, responsible for forests damage. Furthermore, NO_x emissions increase the concentrations of fine-dust particles (PM_{2.5}), which are harmful to human health and finally react with common organic chemicals to form toxic substances (Egmond *et al.*, 2002). Nitrogen produced during the Haber-Bosch process is applied to the agricultural areas and a portion is gathered in the form of organic N. The remainder is lost to the atmosphere and to aquatic ecosystems. Losses to the atmosphere occur in the forms of NO_x, NH₃, and nitrous oxide (N₂O), which is a greenhouse gas contributes to global warming. Nitrate (NO₃⁻) and ammonium (NH₄⁺) are the main N forms, which are lost to groundwater, streams, or rivers, and finally reach the oceans. The only way to remove these reactive N-forms from the environment, are microbial denitrification ($2\text{NO}_3^- + 12\text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O}$) and anaerobic ammonium oxidation (anammox; $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}$). Both processes yield non-reactive N₂ as a final product, but also other reactive N forms are produced as by-products. These occur naturally as well as man-made in sewage treatment plants. There is no data about the global N₂ output via denitrification and anammox. For the European agricultural areas the N₂ production via denitrification is estimated to ~8 Mt N a⁻¹ (Egmond *et al.*, 2002), and ~6 Mt N a⁻¹ for the United States (Howarth *et al.*, 2002). Globally, an amount of ~14 Mt N a⁻¹ is denitrified to N₂ within agricultural areas, which is ~ 8 % of the total N applied (Smil, 1999). Studies from watershed landscape units have shown, that denitrification rates might be much higher. 48 % of the total N input was denitrified according to van Breemen *et al.* (2002), and even higher portions were reported by Seitzinger *et al.* (2002). The remaining N is stored or transported to other ecosystems. In the aquatic environment an enhanced input of N leads to a variety of changes, summarized in the generic term of eutrophication.

Coastal eutrophication

The process of eutrophication describes the enhanced accumulation of organic matter by primary production as a result of increased nitrogen and phosphorous inputs (Nixon, 1995). In general, two pathways of nitrogen and phosphorous inputs are distinguished: the direct discharge of N and P from industry or sewage treatment plants, named “point sources”, and “diffuse” inputs, which are emitted over large areas what makes them difficult to locate and - consequently - to attribute to a certain source. These are runoff from fields and meadows, groundwater, and atmospheric deposition (de Jonge & Elliott, 2001). The first organisms responding to such an enhanced input of nutrients are primary producers, like phytoplankton, benthic macrophytes, filamentous algae, and macroalgae (Rabalais, 2002).

In the early stages of eutrophication research with a focus on freshwater ecosystems a strong positive correlation between nutrient load and primary production was reported. This was also found for several coastal waters such as the Adriatic Sea (Solic *et al.*, 1997), the Belt Sea (Rydberg *et al.*, 1990), and the Wadden Sea (de Jonge, 1990). Other studies pointed out that this is not necessarily the case for all coastal areas receiving high nutrient loads. A compilation of data for the relationship between annual N loading and phytoplankton production in 51 estuaries, carried out by Borum (1996) showed only a poor correlation. This indicates, that some estuaries seem to be more sensible to nutrient enrichment and resulting eutrophication than others.

The responses of coastal areas to increased nutrient loads is presented in a model by Cloern (2001; Fig. II.3). He distinguishes between reversible, direct and indirect responses, and a filter is to represent the characteristics of an area, in terms of its' sensibility to increased nutrient loads. Direct responses are increases in chlorophyll concentrations, primary production, macroalgal biomass and a higher sedimentation rate of organic carbon. Changes in the Si:N and Si:P ratios, in the structure of the phytoplankton community and the more frequent occurrence of toxic and harmful algal blooms, which are reported from the Gulf of

Mexico (Dortch *et al.*, 1997) and in Tolo Harbour (Hong Kong; Lam & Ho, 1989) are also attributed to enhanced nutrient loads.

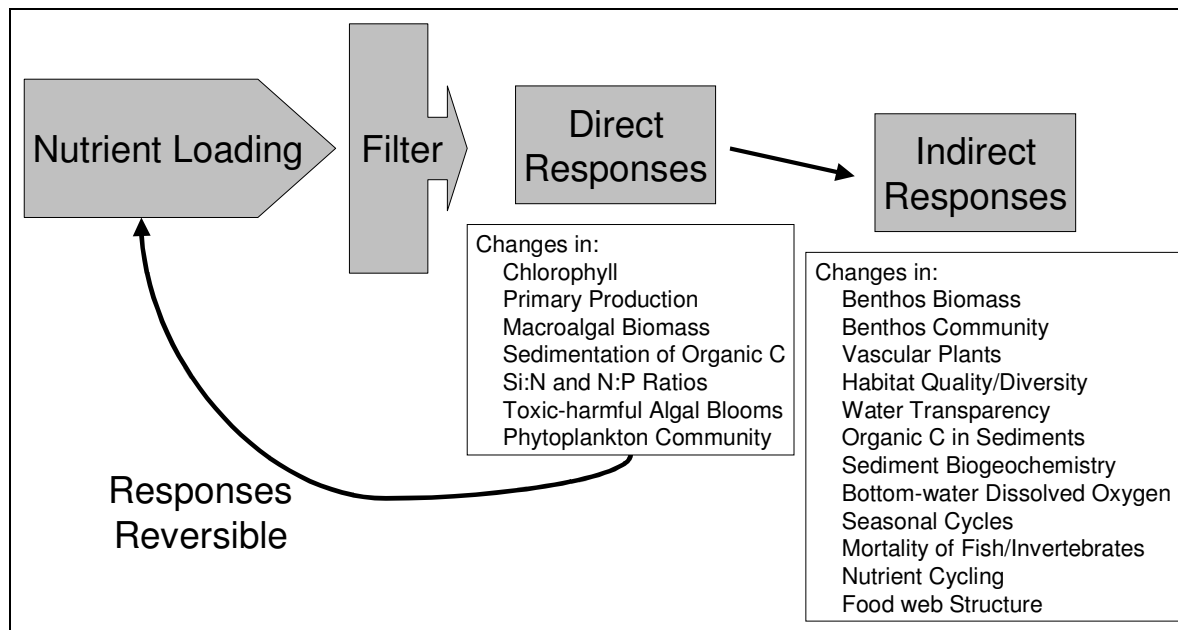


Fig.II.3: A model, which describes the possible responses of coastal areas to enhanced nutrient inputs, redrawn after Cloern (2001).

Indirect responses are the consequences of the direct changes. Hypoxic and anoxic conditions of the bottom waters and associated changes in benthic community structure and mortality of fishes and invertebrates are well documented for areas with stratified water columns such as the northern Gulf of Mexico (Rabalais *et al.*, 2001) and the Black Sea (Mee, 2001). They are a result of enhanced oxygen consumption during the decomposition of the sedimented dead planktonic matter. This also alters the sediment biogeochemistry, with an upwards movement of the redoxcline and a subsequent release of hydrogen sulphide and phosphate from the sediments (Rabalais, 2002). Likewise, increased primary production results in a higher turbidity of the water column. This leads to unfavourable growth conditions for the submerged benthic vegetation and often results in a loss of feeding and nursery areas for fish and invertebrates (Rabalais, 2002).

A massive reduction of the nutrient inputs can yield a recovery of eutrophied aquatic ecosystems. The rapid decrease of fertilizer use (-50% N; -60% P-fertilizer) after the economic collapse of the former Soviet Union in 1990 resulted in substantially decreased

nutrient inputs into the eutrophied Black Sea. As a consequence a 30% reduction in phytoplankton biomass and a reduction in summer anoxia of the Black Sea's shelf were observed (Mee, 2001). After a 40% reduction of nitrogen and phosphorous inputs to Chesapeake Bay there are signs for a slight recovery, indicated by the return of seagrass to some regions (Boesch, 2001).

It is not fully known why some estuaries are more sensible to enhanced nutrient inputs than others. Estuaries with short water residence times seem to be less sensitive to eutrophication (Nixon *et al.*, 1996). Also if primary production is light-limited, e.g. because of high concentrations of suspended sediment, high nutrient concentrations seem to have minor consequences (Cloern, 2001). Furthermore, a high rate of particle filtration by benthic suspension feeders can balance an increased phytoplankton primary production (Cloern, 1982). Tidal water column mixing seems to be a major factor in regulating effects of eutrophication. A comparison of 40 estuaries showed that chlorophyll concentrations per unit of dissolved inorganic nitrogen (DIN) were on average 10 times higher in microtidal than in macrotidal estuaries (Monbet, 1992). This would explain the extreme responses to nutrient enrichment in areas with a small tidal energy, such as the northern Gulf of Mexico, Adriatic, Black Sea, and the Baltic Sea (Cloern, 2001).

The Baltic Sea ecosystem

The Baltic Sea drainage basin covers an area of more than 1.7 Mio. km², which is more than four times larger than the surface area of the Baltic Sea. Fourteen states fully or partially lie in the drainage basin, which is inhabited by more than 85 Mio. people. Due to its structure and topography the Baltic Sea is divided into seven major subregions (Fig. II.4). Most people (64%) live in the drainage basin of the Baltic Proper, which results in an average population

density of 95 inhabitants/km² (Sweitzer *et al.*, 1996). The northern part of the drainage basin is sparsely populated, with 5 inhabitants/km² in the drainage basin of the Bothnian Bay and 11 inhabitants/km² in the Bothnian Sea drainage basin, respectively. The drainage basin of the Baltic Proper is dominated by agricultural land-use (~39% arable land and ~12% pasture), whereas in the basins of the Bothnian Bay, Bothnian Sea, and Gulf of Finland forested areas dominate (72%, 66%, and 53% respectively) and only few areas are used for agriculture (3%, 4%, and 24 %, respectively; Sweitzer *et al.*, 1996).

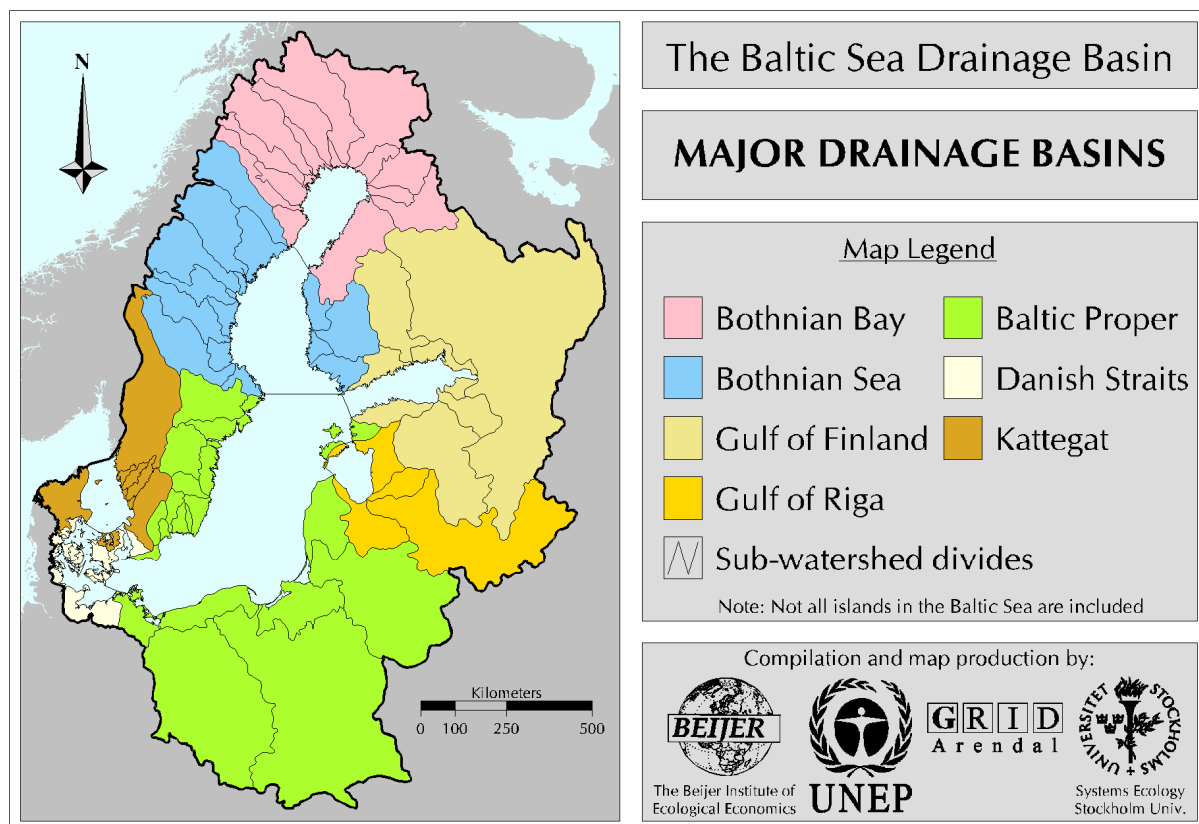


Fig. II.4: Map of the Baltic Sea drainage basin with subregions (taken from www.grida.no/baltic)

Because of its topography with several deep basins separated by sills, the narrow connection to the North Sea via the shallow Kattegatt and the Danish Straits, and the high population density in the southern parts the Baltic Sea is very sensible to anthropogenic influences. High freshwater input via rivers and atmospheric deposition results in a positive water balance. This leads to a large freshwater outflow (1250 km³ a⁻¹; Krauss, 2001), comparable to the freshwater discharge of the Mississippi River (Wulff *et al.*, 2001). Likewise, there is a

permanent inflow of saltwater ($740 \text{ km}^3 \text{ a}^{-1}$; Krauss, 2001). Consequently, a horizontal salinity gradient exists in the surface water with 8 PSU in the central and western parts of the Baltic Sea and 4 PSU in the northern parts (Krauss, 2001). Furthermore, there is a permanent stratification between 60 – 80 m in the water column of the deep basins that separates the less saline water of the upper layer from the more saline and dense water near the bottom (Matthäus, 1995). This stratification restricts the oxygen supply to the deep waters of the Baltic Sea. Whereas the upper layer is mixed in winter and is in equilibrium with the atmosphere, the deep water is only renewed and supplied with oxygen by saltwater inflows from the North Sea, termed “Major Baltic Inflows” (MBI; Schinke & Matthäus, 1998). MBI occurred relatively frequently until the mid-1970s, but have become rare since. The last inflows took place in 1983, 1993 and 2001 (Nausch *et al.*, 2003). Between such events oxygen concentrations decrease and anoxic areas spread out. In 1940 the oxygen free areas of the Baltic Proper covered $\sim 20000 \text{ km}^2$, but have since then expanded to a current area of $\sim 70000 \text{ km}^2$. This implies that one third of the Baltic Proper bottom area suffers from intermittent oxygen depletion (Jansson & Dahlberg, 1999). This increase in the area where anoxia prevails not only results from fewer inflow events since the mid-1970s but is enhanced by increased biological production from anthropogenic nutrient input. In 2000 the total loads of nitrogen (N_{total}) and phosphorous (P_{total}) to the Baltic Sea were estimated to be 814 000 t and 42 010 t, respectively, with 40% of the total nitrogen and 55% of the total phosphorous originating from the drainage basin of the Baltic Proper (HELCOM, 2003). The lot of these nutrients originates from diffuse sources (60% of N_{total} ; 52% of P_{total}) and reaches the Baltic Sea via the rivers. In 2000 the three major rivers in the Baltic Proper area (Vistula, Nemunas, and Odra) contributed by approx. 70% to the N load and approx. 53% to the P load of this area (HELCOM, 2003). Stalnacke *et al.* (1999) estimated that the riverine N input exceeds the combined contributions from atmospheric deposition, point emissions along the coast, and nitrogen fixation by marine organisms. Recent studies, however, suggest that the amount of N

fixed by cyanobacteria might be in the same order of magnitude as the riverine N input (Schneider *et al.*, 2003; Voss *et al.*, in press).

It is not fully known how much nitrogen that enters the coastal areas of the Baltic Sea reaches the central basins but there is evidence that most of the N remains in the coastal areas and there is only little export (Voss *et al.*; in press). The consequences of the excess nutrient input to the coastal areas of the Baltic Sea have been reported in numerous studies. Increased primary production and higher particle load of the water column enhanced turbidity as shown by a significant decrease in Secchi depth in the Baltic Proper from 1969 – 1991 of $\sim 0.05 \text{ m a}^{-1}$ (Sanden & Hakansson, 1996). A similar result related to the increase in turbidity is the shoaling of the maximal depth at which *Fucus vesiculosus* grows in the Åland Sea from 11.5 m in 1943/44 to 8.5 m in 1984 (Kautsky *et al.*, 1986). Furthermore, the loss of seagrass-beds (*Zostera marina*) in the shallow areas of the Baltic is reported (Schramm, 1996; Baden *et al.*, 2003). *F. vesiculosus* and *Z. marina* serve as protection and spawning areas for invertebrates and fish, and with the loss of these habitats the respective species may become endangered. The extension of the anoxic zones in the deep basins of the Baltic Sea has consequences for the reproduction of the Baltic cod, because the cod's eggs float in a defined density level in the water column. If the water in this depth horizon is anoxic the eggs die off (Vallin & Nissling, 2000). Additionally, anoxic sediments release large amounts of phosphorous to the overlying water, which is absorbed to iron (III) hydroxides under oxic conditions (Jansson, 2001). This additional P source is considered to be partly responsible for the development of massive Cyanobacteria blooms in the Bornholm and Gotland Sea (Nausch *et al.*, 2003).

A simulation with an ecosystem model of the Baltic Sea showed that a 50% reduction of riverine nutrient loads would lower the DIN and phosphate concentrations in the water column of the Baltic Sea within 10 years (Neumann *et al.*, 2002). However, a reduction of the chlorophyll concentrations, which is a sign for decreased primary production, was only found

for the coastal areas and the western Gotland Basin (~ -30%). In the other areas chlorophyll remained constant or increased, mainly because of an increase of N-fixing cyanobacteria compensating the decrease of other phytoplankton.

Although considerable measures have been taken since the 1970s, to reduce anthropogenic N and P loads, no decrease in the annual riverine nitrogen and phosphorous loads was observed in the period from 1970 - 1993 (Grimvall & Stalnacke, 2001). With the EU Water Framework Directive, which came into effect in 2000, a “good” ecological and chemical quality of all surface and groundwaters should be achieved by 2015. Regarding nutrients a “good” quality means, that “nutrient concentrations do not exceed the levels established so as to ensure the functioning of the ecosystem and the achievement of the values specified ... for the biological quality elements” (EC, 2000).

Since it is known that there is a strong positive correlation between riverine nitrogen loads and agricultural land use in the drainage area (Grimvall & Stalnacke, 2001; Voss et al., unpublished data) it is clear, that we have to set a major focus on the reduction of diffuse N losses from agriculture.

Nitrogen emissions from agriculture

Nitrogen is applied to the fields either in inorganic (nitrate, ammonium, urea) or organic form (animal or green manures, grain legumes, leguminous leys and certain organic wastes). Urea and ammonia are the world’s most widely traded and used fertilizer, because of their low production costs. China is the largest producer of ammonia and urea, and with a total production of ~ 42 Mt N in 2000 it supplied half of the world commercial fertilizer consumption of ~ 82 Mt N (Table II.1; Fixen & West, 2002). The total amount of manure applied to agricultural soils in 1995 was 33 Mt (Bouwman & Boumans, 2002).

Once applied, the fertilizer N contributes to the complex cycle of N in agricultural soils, which is summarized in figure II.5.

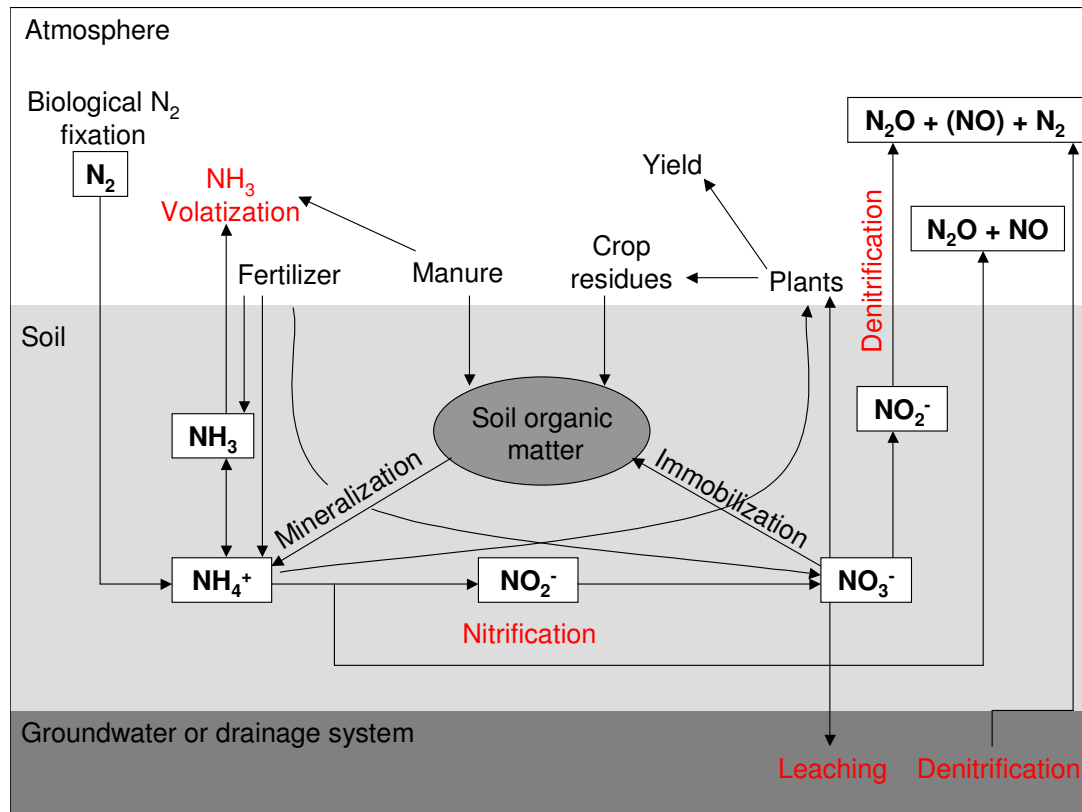


Fig.II.5: A simplified illustration of the N cycle in agricultural soils; the pathways for N losses to the environment are coloured red. Redrawn after Hofman & Cleemput (2004).

Plants usually assimilate nitrogen in the forms of ammonium or nitrate, which is supplied by the fertilizers or by the mineralization of soil organic matter. Although agricultural soils receive large amounts of inorganic N, inside the soil most of the N is present in organic form as proteins, amino sugars and purine and pyrimidine derivatives (Hofman & Cleemput, 2004). Organic N is continuously mineralized to ammonium by micro organisms ($N_{\text{org}} \rightarrow \text{NH}_4^+$), which is either assimilated by plants or is microbially nitrified to nitrate ($\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$). If not assimilated or immobilized, the highly water-soluble nitrate can be transported below the root zone by the downward soil water flow and then is lost to drainage-systems or the groundwater (Lammel, 1990; Göbel, 2000). This major process for agricultural N emissions every year accounts for 14-20 Mt N lost to the aquatic environment (Table II.1; Smil, 1999). Most of the nitrate derives from mineralization and subsequent nitrification of soil organic N in autumn and winter. The crops are harvested at that time and the soils are still

warm enough for microbial degradation to take place, and moisture needed comes with autumn rains (Kirchmann *et al.*, 2002). Several studies have shown that about 90% of nitrate leaching from soils after harvest in autumn derives from mineralization of soil organic N, and only 10% originates directly from the inorganic nitrate fertilizers not completely assimilated after application in spring (Bergström, 1987; MacDonald *et al.*, 1989). An important additional N source are crop residues which remain in the soils after harvest and become part of the soil organic N pool (Kirchmann *et al.*, 2002). Likewise, the mineralization of animal manure can contribute to large amounts of mineralized N, as 30% of applied manure can leach as nitrate within 3 years after application (Bergström & Kirchmann, 1999).

Table II.1: Application and losses of nitrogen in agriculture

Fertilizer consumption	Amount [Mt N a⁻¹]	References
inorganic (in 2000)	82	(Fixen & West, 2002)
organic (in 1995)	33	(Bouwman & Boumans, 2002)
Total	115	
N losses		
NO ₃ ⁻ leaching	14-20	(Smil, 1999)
NH ₃ volatilization		
from inorganic fertilizers	11	(Bouwman & Boumans, 2002)
from organic fertilizers	8	(Bouwman & Boumans, 2002)
NO and N ₂ O (in 1995)	5.5	(IFA/FAO, 2001)
Total	38.5 - 44.5	

A special case are nitrate losses via “preferential flow” caused by heavy rainfall. These increase the soil water flow via small channels, fissures, and cracks created by animals or during dry periods, and they result in a rapid transport of nitrate to the drainage systems or the groundwater. If such events occur during autumn and spring or directly after application of nitrate fertilizers large amounts of nitrate are lost (Lammel, 1990).

As stated above urea and ammonium are the most applied fertilizers. Urea needs to be hydrolysed to ammonium to make it available for plant uptake. Besides lower production costs, a further advantage of urea and ammonium fertilizers is the lower risk of leaching because ammonium is strongly adsorbed to the soil particles.

In the soil ammonium is in equilibrium with atmospheric ammonia. An increase in the soil pH - for example during the hydrolysis of urea - leads to a displacement of the $\text{NH}_4^+/\text{NH}_3$ equilibrium in favour of the NH_3 form (Hofman & Cleemput, 2004). Ammonia then volatilizes to the atmosphere and reaches other terrestrial and aquatic ecosystems via atmospheric deposition (Paerl, 1995).

As shown in figure II.5 further gaseous N losses occur during the processes of nitrification and denitrification. Whereas the loss of unreactive N_2 is not relevant to the environment, NO and N_2O are partly responsible for the greenhouse effect and the destruction of stratospheric ozone (Hofman & Cleemput, 2004).

During the last two decades intensive research on the reduction of N loss in agriculture has been carried out. One method is the determination of the soils' N-demand by means of knowledge of the N_{org} content of the soils (N_{min} -method). Changes in agricultural practices have profound effects on the N-amount lost, and reductions of up to 80% have been reported, if "catch crops" were sown after harvest, which allows them to assimilate the nitrate generated during autumn and winter (Lewan, 1994). Nitrification inhibitors and urease inhibitors suppress the formation of nitrate, and consequently reduce the risk of leaching. Other management practices are summarized in the term "Precision Farming" (Kirchmann *et al.*, 2002), which includes site-specific nitrogen management and will contribute to a further reduction of the nitrogen losses from agriculture.

Stable isotopes in Hydrology and Ecology

Fundamentals

Stable isotopes provide well established tools for investigating hydrological processes. δD - H_2O ($^2\text{H}/^1\text{H}$) and $\delta^{18}\text{O}$ - H_2O values are used to characterize water flowpaths and develop water mass budgets. Another important field of application is the understanding of nutrient cycles and the identification of contamination sources, such as sewage or manure (Gonfiantini *et al.*, 1998; Kendall, 1998; Kendall & Caldwell, 1998).

The term isotope defines atoms of the same chemical element with the same number of protons but different numbers of neutrons. Isotopes can be classified into two groups: stable isotopes (i.e. ^{12}C with 6 protons and 6 neutrons and ^{13}C with 6 protons and 7 neutrons) that do not decay in geological timescales, and non-stable (radioactive) isotopes (i.e. ^{14}C with 6 protons and 8 neutrons). Stable isotopes are a component of all elements and occur in different portions, with the “light” isotope (less neutrons) being more abundant. Nitrogen has two stable isotopes (^{14}N ; ^{15}N), and in atmospheric N_2 the ratio of “heavy” ^{15}N to “light” ^{14}N is 1:272 (Kendall, 1998).

Isotope-ratios of low-mass elements, for example oxygen, nitrogen or carbon are reported as δ values in permil (‰), and are given relative to a standard of a known composition.

The calculation is as follows:

$$\delta [\text{‰}] = 1000 (R_{\text{Sample}} / R_{\text{Standard}} - 1),$$

where R represents the ratio of the heavy to the light isotope.

To ensure an international comparability between the different isotope labs, all isotope values are reported relative to international reference standards (Table II.2) with a defined isotope value. Samples are analyzed at the same as the reference, or are measured along with internal laboratory standards, which are calibrated relative to the international standards.

Table II.2: International reference standards for stable isotope measurements

Isotope	Ratio measured	Reference Standard	Abundance ratio of standard	Reference
¹³ C	¹³ C/ ¹² C	PDB (Pee Dee Belemnite)	1.1237 * 10 ⁻²	(Craig, 1957)
¹⁵ N	¹⁵ N/ ¹⁴ N	atmospheric N ₂	3.677 * 10 ⁻³	(Mariotti, 1983)
¹⁸ O	¹⁸ O/ ¹⁶ O	VSMOW (Vienna Standard Mean Ocean Water)	2.0052 * 10 ⁻³	(Craig, 1961)

Because of their different masses, the stable isotopes of an element have slightly different chemical and physical properties. The dissociation energy in molecules containing heavy isotopes is higher, which results in a higher energy demand to split a chemical bond (Hoefs, 2004). Fractionation processes can alter the isotopic composition of chemical compounds, and two types can be distinguished.

Equilibrium fractionation occurs when processes or chemical reactions are reversible. In these reactions the heavy isotope accumulates in the compound with the higher oxidation state. In the sulfate/sulfide equilibrium for example sulfate becomes enriched in $\delta^{34}\text{S}$ (Kendall & Caldwell, 1998).

If chemical reactions are unidirectional, kinetic fractionation occurs. An example is denitrification where the substrate (NO_3^-) gets enriched in the heavier isotope relative to the product (N_2) because the lighter isotope reacts faster than the heavy one (Mariotti *et al.*, 1981).

The extent of fractionation is reported as fractionation factor:

$$\alpha = R_p / R_s$$

where R_p is the ratio of the heavy isotope in the product, and R_s in the substrate.

Often the extent of fractionation is reported as isotope enrichment factor:

$$\epsilon_{p-s} = (\alpha - 1) * 1000$$

To assess how the isotope values of the substrate and product develop, it is important to distinguish whether processes take place in open or closed systems (Kendall & Caldwell,

1998). The changes of the δ values in open systems in equilibrium are described by the so called “Rayleigh equations”, named after Lord Rayleigh, who first observed the fractional distribution of substances with different vapour pressures. Although these equations should only be used for open equilibrium systems, they can also be used to approximate the evolution of isotope values during kinetic unidirectional reactions in closed systems, where the amount of substrate is finite (Mariotti *et al.*, 1988).

The calculations for the δ values of the substrate (δ_S), the instantaneous generated product (δ_{Pi}), and the accumulated product (δ_{Pa}) are given below:

$$\delta_S \approx \epsilon_{P-S} * \ln f$$

$$\delta_{Pi} \approx \delta_S + \epsilon_{P-S}$$

$$\delta_{Pa} \approx -\epsilon_{P-S} ((f * \ln f)/(1-f)),$$

where f is the fraction of unreacted substrate, and ϵ_{P-S} is the enrichment factor. To calculate δ values for kinetic fractionation in open systems with a continuous supply of substrate and remove of the product models are needed (Brandes *et al.*, 1998; Voss *et al.*, 2001)

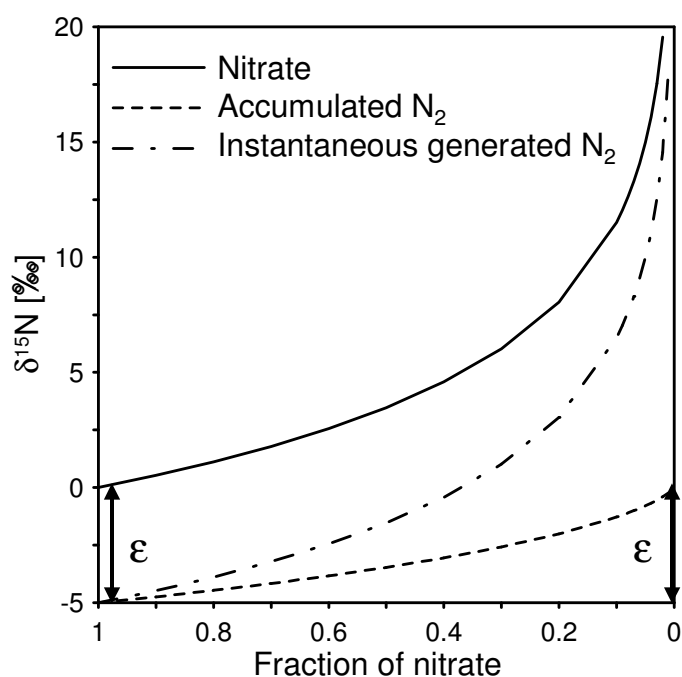


Fig. II.6: δ values for substrate, instantaneous and accumulated product during denitrification.

Figure II.6 illustrates the development of the $\delta^{15}\text{N}$ values of nitrate, accumulated N_2 and the instantaneous generated N_2 during denitrification in closed systems.

The magnitude of ϵ varies between the different processes. Especially for enzymatic processes, there is a high dependency on temperature and substrate concentration. Table

II.3 summarizes ranges of ϵ_{S-P}

values for $\delta^{15}\text{N}$ for several processes in the nitrogen cycle.

Tab. II.3: ϵ -values for $\delta^{15}\text{N}$ of several processes in the nitrogen cycle summarized in the compilation of Robinson (2001). The ϵ -values are reported in the S-P-form.

Process	$\epsilon_{\text{S-P}}$ (‰)
N_2 fixation via nitrogenase	0 - 6
NH_3 volatilization	40 - 60
N_2O and NO production during nitrification	35 - 60
N_2O and N_2 production during denitrification	28 - 33
NO_3^- assimilation into organic N by plants	0 - 19
NH_4^+ assimilation into organic N by plants	9 - 18
NO_3^- or organic N assimilation by microbes	13
NH_4^+ assimilation by microbes	14 - 20
NH_4^+ production from organic matter decomposition (ammonification)	0 - 5
NO_3^- production during nitrification	15 - 35
Organic N assimilation by animals (deamination and transamination)	1 - 6

Stable isotopes of nitrogen and oxygen in nitrate

Nitrate that derives from different sources shows characteristic nitrogen and oxygen isotope values, because of fractionation during formation or the reaction of nitrate in the N cycle (Tab. II.4). Thus, the combined determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in nitrate is a powerful tool to identify nitrate sources in aquatic environments and aquifers (Amberger & Schmidt, 1987; Wassenaar, 1995; Spoelstra *et al.*, 2001; Burns & Kendall, 2002; Campbell *et al.*, 2002; Mayer *et al.*, 2002).

Table II.4: $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate from various sources

Source	$\delta^{15}\text{N-NO}_3$ [‰]	$\delta^{18}\text{O-NO}_3$ [‰]	References
Nitrification in soils	-18 to 5	-2 to 15	Amberger & Schmidt, 1987; Kendall, 1998; Mayer <i>et al.</i> , 2001
Sewage and manure	10 to 20	-2 to 15	Heaton, 1986; Wassenaar, 1995; Fogg <i>et al.</i> , 1998; Karr <i>et al.</i> , 2002
Atmospheric deposition	-10 to 5	30 to 70	Freyer, 1991; Kendall, 1998
Mineral fertilizers	0.5 to 5	~ 23	Freyer & Aly, 1974; Bedard-Haughn <i>et al.</i> , 2003

Nitrate originating from nitrification in soils usually has $\delta^{15}\text{N}$ values between -18 to 5 ‰ (Kendall, 1998). During the unidirectional nitrification process, kinetic fractionation leads to ^{15}N -depleted nitrate and ^{15}N -enriched residual ammonium. As shown in Table II.3, the enrichment factor covers a wide range between 15 and 35 ‰, depending on soil temperature and ammonium concentration. However, in N-limited systems, the fractionation during nitrification is minimal ($\epsilon \approx 0$ ‰), and the $\delta^{15}\text{N}$ of soil nitrate is close to the $\delta^{15}\text{N}$ value of soil organic N (Kendall & Aravena, 1999). The reason is a low mineralization rate which leads to low concentrations of ammonium, that is directly nitrified to nitrate without fractionation. If the ammonium concentration is increased e.g. due to fertilizer application, the nitrification rate enhances (Atxotegi *et al.*, 2003) and the nitrification becomes the rate-limiting step during the conversion of organic N to nitrate (Kendall & Aravena, 1999). This results in large fractionation in $\delta^{15}\text{N}$, as observed by Feigin *et al.* (1974) directly after the application of anhydrous ammonia on an agricultural field. The $\delta^{15}\text{N}\text{-NO}_3$ values decreased from 2.5 ‰ to -10 ‰, and $\delta^{15}\text{N}\text{-NH}_4$ values simultaneously increased.

The $\delta^{18}\text{O}$ values of the nitrate generated during nitrification in soils usually range between -2 to 15 ‰ (Amberger & Schmidt, 1987; Kendall, 1998; Mayer *et al.*, 2001), because at least one oxygen atom derives from atmospheric O_2 ($\delta^{18}\text{O} = 23.5$ ‰; Kroopnick & Craig, 1972), and up to two oxygen atoms from the soil water (Hollocher *et al.*, 1981; Andersson & Hooper, 1983). Mayer *et al.* (2001) found that a reduced nitrification rate, as often observed in N-limited systems, like the forests they studied, can result in a larger portion of O derived from atmospheric O_2 . They concluded that theoretically all three O atoms incorporated in NO_3^- can origin from atmospheric O_2 , which results in a $\delta^{18}\text{O}$ value of 23.5 ‰. These findings, together with observed variations in the $\delta^{18}\text{O}$ values of soil water can explain the large range reported for $\delta^{18}\text{O}$ values of nitrate generated during nitrification.

The $\delta^{18}\text{O}$ values of soil water are determined by the $\delta^{18}\text{O}$ values of precipitation and the equilibrium fractionation during evapotranspiration (Hsieh *et al.*, 1998). Precipitation usually has $\delta^{18}\text{O}$ values between -22 and 0 ‰ (IAEA, 1981). Seasonal differences in temperature leads to higher values during summer and lower ones in winter because of equilibrium fractionation (Ingraham, 1998). This also leads to a global distribution of precipitation- $\delta^{18}\text{O}$ with lower values at higher latitudes.

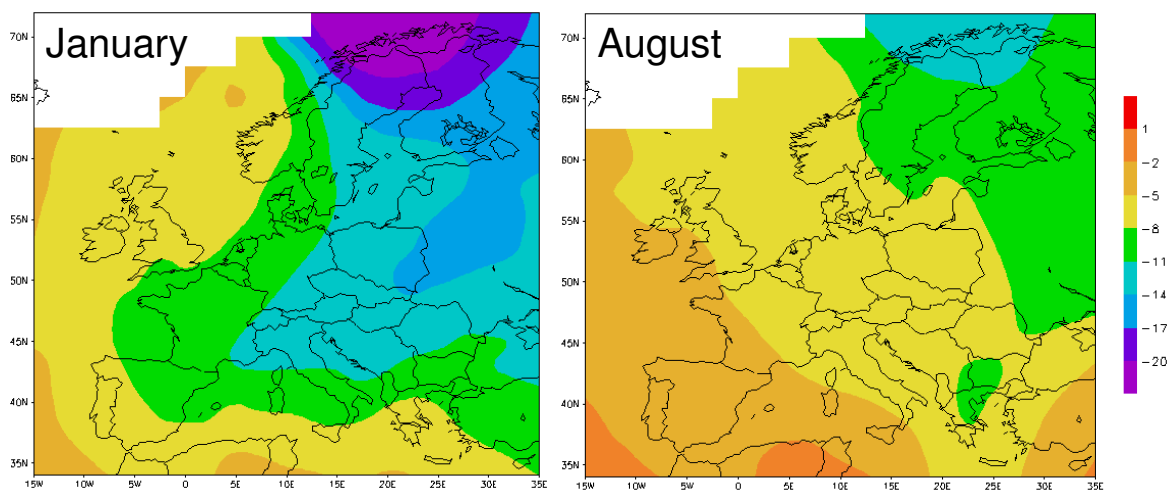


Fig. II.7: Amount weighted $\delta^{18}\text{O}$ values in precipitation across Europe in January and August (IAEA, 2001)

Evapotranspiration leads to increased $\delta^{18}\text{O}$ values of soil water because ^{18}O -depleted vapour is lost. Hsieh *et al.* (1998) found in their study on Hawaii lower $\delta^{18}\text{O}$ values at the soil surface and increased values with depth during periods of rain, and increased surface values of up to 8 ‰ higher during dry periods. They further concluded, that there may exist large variation in the $\delta^{18}\text{O}$ values of soil water between arid and humid environments. Soil water $\delta^{18}\text{O}$ values range between -25 and 4 ‰ (Kendall, 1998).

Mineral fertilizers usually have a narrow range for $\delta^{15}\text{N}$ that mirrors their production from atmospheric N_2 via the Haber-Bosch process (0.5 to 5 ‰). Ammonium and urea have slightly higher values (Freyer & Aly, 1974; Bedard-Haughn *et al.*, 2003). The $\delta^{18}\text{O}$ values of mineral nitrate fertilizers are around 23 ‰, close to the $\delta^{18}\text{O}$ of atmospheric O_2 (23.5 ‰).

Nitrate from sewage and manure typically shows $\delta^{15}\text{N}$ values $> 10 \text{ ‰}$ (Heaton, 1986; Wassenaar, 1995; Fogg *et al.*, 1998; Karr *et al.*, 2002). These high $\delta^{15}\text{N}$ values are a result of ammonia volatilization, which leads to large fractionations between ^{15}N -depleted ammonia and ^{15}N -enriched residual ammonium ($\epsilon = 40 - 60 \text{ ‰}$; Wassenaar, 1995; Robinson, 2001). Three steps are responsible for this large fractionation (Kendall & Aravena, 1999): 1st the equilibrium fractionation between ammonium and ammonia in solution, 2nd the equilibrium fractionation between aqueous and gaseous ammonia, and 3rd the kinetic fractionation caused by the diffusive loss of ^{15}N -depleted ammonia. In soils the ^{15}N -enriched residual ammonium may then be converted to ^{15}N -enriched nitrate via nitrification (Heaton, 1986; Kendall & Aravena, 1999).

Differences in groundwater-nitrate below agricultural soils treated either with inorganic or mixed fertilization were observed by Amberger & Schmidt (1987). If only mineral fertilizers were applied $\delta^{15}\text{N-NO}_3$ values were lower (3.0 to 5.9 ‰) compared to the area with organic and inorganic fertilization ($\delta^{15}\text{N} = 10.6 \text{ ‰}$), and $\delta^{15}\text{N-NO}_3$ values of 10 ‰ are reported in soil water nitrate close to an animal waste site (Fogg *et al.*, 1998). But ammonia volatilization not only occurs after application of animal waste. $\delta^{15}\text{N-NO}_3$ values 12.4 ‰ higher than the $\delta^{15}\text{N}$ value of the applied fertilizer were found in groundwater below a golf course treated with mineral ammonium and nitrate fertilizers (Flipse & Bonner, 1985).

$\delta^{15}\text{N}$ values in nitrate from atmospheric deposition ranges between -10 to 5 ‰ and shows a seasonal variations with low values in spring and summer and higher values during winter (Heaton, 1986; Freyer, 1991). These variations are explained with increased emissions of isotopically depleted nitrogen oxides from agriculture during summer, and a increased proportion of enriched nitrous oxides from fossil fuel combustion during winter ($\delta^{15}\text{N} = 6-9 \text{ ‰}$; Heaton, 1990), but it is still unclear if this is the determining factor. Little is known about the processes, that generate the $\delta^{18}\text{O-NO}_3$ values of atmospheric deposition. Usually they

show a large variability between 30 and 70 ‰. Explanations for this wide range are fractionations during nitrate formation in thunderstorms, incomplete combustion of fossil fuels, and photochemical reactions in atmosphere (Kendall, 1998).

There are several other processes, that change the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate. One important process is denitrification, leading to ^{15}N -depleted N_2 . Denitrification occurs in suboxic waterbodies, soils or sediments. Böttcher *et al.* (1990) reported an increase in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in groundwater nitrate, with a ratio of 2:1, and calculated enrichment factors ($\epsilon_{\text{P-S}}$) of -15.9 ‰ for $\delta^{15}\text{N}$ and -8.0 ‰ for $\delta^{18}\text{O}$. Higher factors of up to -33 ‰ for $\delta^{15}\text{N}$ are also reported in the compilation of Robinson (2001) as well as lower factors (-5 to -8 ‰; Mariotti *et al.*, 1988). The variation is explained by differences in the denitrification rate. Slow rates during low ambient temperatures and low concentrations of electron donors are suggested to result in larger fractionations; vice versa rapid denitrification leads to small fractionation. In relatively impermeable aquifers the enrichment factor may be reduced because of low diffusion rates of nitrate (Mariotti *et al.*, 1988). A recent study showed that the 2:1 enrichment ratio found by Böttcher *et al.* (1990) in groundwater may not be applicable to all environments. In the marine environment an 1:1 increase was observed (Sigman *et al.*, 2003). Several studies have shown that watersheds which are highly impacted by anthropogenic N inputs have high $\delta^{15}\text{N-NO}_3$ values > 8 ‰ (Heaton, 1986), and positive correlations between $\delta^{15}\text{N-NO}_3$ and percentage of agricultural and urban land are reported (McClelland & Valiela, 1997; Mayer *et al.*, 2002)

Although recent studies have shown that the dual-isotope approach with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in nitrate is a powerful tool to describe N related processes, there are only few studies that deal with $\delta^{18}\text{O-NO}_3$ values in soil water. One of the difficulties in measuring $\delta^{18}\text{O-NO}_3$ values in agricultural nitrate is that the dominant “nitrification $\delta^{18}\text{O-NO}_3$ -signal” may overlay fertilizer induced effects (Mengis *et al.*, 2001). Most studies concentrate on $\delta^{18}\text{O-NO}_3$ values

in groundwater near agricultural areas, and a problem for these investigations is the time delay between leaching from the soil and appearance in the groundwater. This can take days to several months, and during this time fractionation by e.g. denitrification can modify the isotopic signature and falsify the isotope values of the soil water nitrate. To avoid these problems measurements can be carried out in water which is collected in drainage systems. Tile drainage is a common agricultural practice in areas with water-saturated soils like the ones in Mecklenburg-Vorpommern. Usually, the systems are installed in soil depths between 50 – 100 cm and significantly reduce the residence time of the soil water.

III. AIM OF THIS PHD-THESIS

Aim of the presented PhD thesis was the identification and quantification of diffuse nitrate inputs for the Warnow river by means of stable isotope measurements in nitrate. Since the studies of Grimvall & Stalnacke (2001) and Voss et al. (unpublished data) reported a positive correlation between the riverine N load to the Baltic Sea and agricultural land-use in river drainage basins a major focus of this PhD-thesis was set on diffuse nitrate losses from agriculture. Likewise, the processes in the soils that stand behind these losses were identified. Due to a new interdisciplinary approach which combined agricultural and marine research the importance of agricultural nitrate inputs for the coastal waters of the Baltic Sea was verified. To trace the agricultural nitrate from the field runoff to the coastal waters four studies were carried out on agricultural fields as well as in the river and its estuary.

Besides the identification of nitrate sources, which has been carried out in several studies before (Amberger & Schmidt, 1987; Wassenaar, 1995; Spoelstra *et al.*, 2001; Burns & Kendall, 2002; Campbell *et al.*, 2002; Mayer *et al.*, 2002), the dual-isotope approach ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) was applied for the first time to quantify nitrate inputs into a river-system. Finally, an assessment was given whether the determination of stable isotope ratios in nitrate can be used as a tool for water resources management.

All studies were carried out in the drainage basin of the Warnow river, which is dominated by agricultural land use (68% of the total area). The river yearly receives more than 4000 t nitrogen, mainly from diffuse sources (Pagenkopf, 2001), and because of the high proportion of water-saturated soils in the drainage area, it is assumed that almost half of the agricultural area is artificially drained. This results in high N losses via tile drainage, which account for 46% of the total diffuse losses (Pagenkopf, 2001).

The studies are presented in the chapters V to VIII, each structured as a manuscript with introduction, material and methods, results, and discussion section.

In first study, the major processes that affect the isotope values of the drainage water nitrate were identified. Two tile drain outlets beneath conventionally farmed fields were sampled weekly to bimonthly for a period of 17 months. Additionally, the isotopic composition of the applied mineral fertilizers was measured to identify fertilizer induced variations in the isotope values of the drainage water nitrate. The results of this study are published in Aquatic Sciences.

The second study concentrated on the impact of drainage water nitrate from a conventionally farmed soil on the adjacent surface waters. In contrast to the first study sampling took place with a high temporal resolution during the first main discharge period of the hydrological year 2003/2004 (six weeks). This time resolution was found to be essential to resolve processes which modify the isotope signatures. Sampling was carried out daily to weekly. Additionally, nitrate isotope values were measured in an adjacent ditch and a brook, to evaluate the importance of the drainage water nitrate for the following surface waters.

Aim of the third study was the identification and quantification of diffuse nitrate inputs into a sub-basin of the Warnow river. Three major nitrate sources (drainage water, groundwater and atmospheric deposition), as well as the river itself were sampled regularly during 5 months in winter to minimize the impact of fractionation processes in the river nitrate. The mean isotope values of the sources were used in a mixing-model to determine the percentage of each sampled source to the river nitrate.

In the fourth study a different approach was used to trace anthropogenic N along an estuarine gradient. The results of the third study showed, that the Warnow river has elevated $\delta^{15}\text{N-NO}_3$ values, which is also reported from other other river systems with high anthropogenic N loads (McClelland & Valiela, 1997; Mayer *et al.*, 2002). Several studies had shown, that the $\delta^{15}\text{N}$ values in macroalgae' tissues, grown in polluted rivers or estuaries can be used to trace anthropogenic N (Costanzo *et al.*, 2000; Gartner *et al.*, 2002; Savage & Elmgren, 2004). This is due to the fact that macroalgae only show small or no fractionation during uptake of

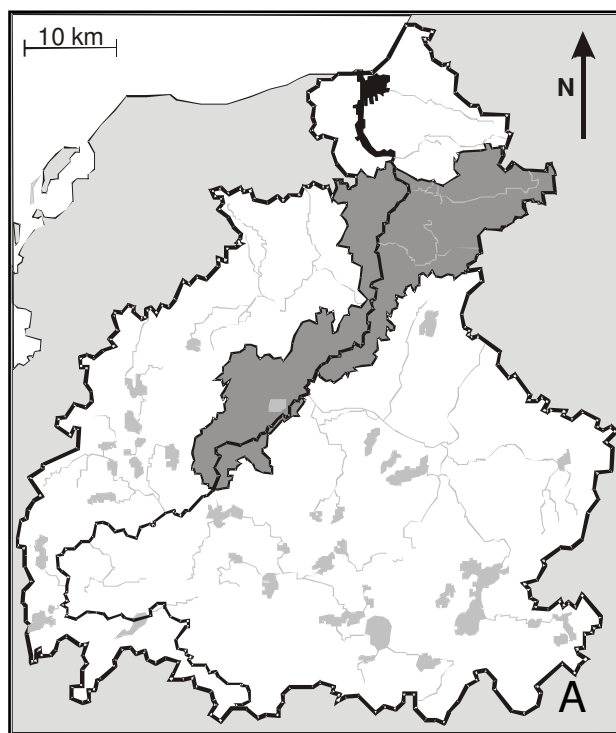
nitrogen (Högberg, 1997). Thus, they may directly reflect the $\delta^{15}\text{N}$ value of the riverine DIN, but this confirmation is lacking in all other studies.

Three macroalga species, grown at a unpolluted brackish site, were incubated during two experiments along the salinity and nitrate gradient in the Warnow estuary. The incubated species were the brown-alga *Fucus vesiculosus* (Phaeophyta) and the two red-algae *Polysiphonia sp.* and *Ceramium rubrum* (Rhodophyta). Incubations were carried out in gauze-bags fixed to shipping-signs, during 14 and 10 days, respectively. With the parallel determination of the $\delta^{15}\text{N}$ values of the river nitrate it should be tested, whether the algae adapt to the $\delta^{15}\text{N}$ values of the river nitrate and which species is the most suitable for such experiments.

IV. MATERIAL AND METHODS

Study area

The Warnow river is located in Mecklenburg-Vorpommern (northeastern Germany) and flows into the Southern Baltic Sea at the City of Rostock (Figure IV.1.A, B). With a length of 149 km and a drainage area of 3270 km², mainly dominated by agricultural and forested areas (63 % and 24 % respectively) it is the second largest river system of Mecklenburg-Vorpommern



(Thiele & Mehl, 1995). The Warnow is characterized as polytrophic (Börner *et al.*, 1994) due to a high amount of nutrient inputs (Nitrogen: 4140 t/yr, Phosphorous: 200 t/yr), and a production rate of 650 gC/m²*yr. The river-system is divided into

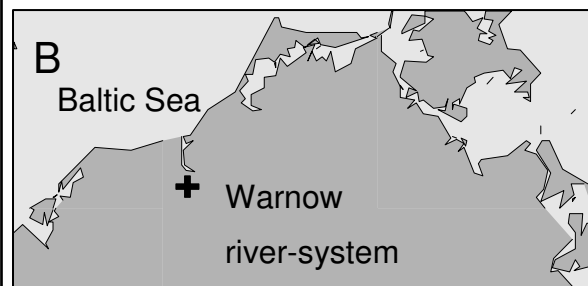


Figure IV.1: Site map of the Warnow river system (A) located in northeastern Germany (B). The dark gray area shows the sub basin 'Middle Warnow'

seven sub basins, and the investigated sub basin 'Middle Warnow' covers an area of 444 km². Land use in the sub basin is dominated by agriculture, with 49 % arable land, and 23 % pasture (Pagenkopf, 2001).

According to the Mesoscale Agricultural Mapping Programme (MMK) 75 % of the agricultural land in the sub basin 'Middle Warnow' are waterlogged soils, and statements given by Bockholt & Kappes (1994) allow a estimation, that up to 50% of the agricultural area in the sub basin 'Middle Warnow' is artificially drained.

Stable isotope measurements

The determination of stable isotope ratios is carried out in a Isotope Ratio Mass Spectrometer (IRMS). For the measurement, the element of interest needs to be converted into a gas. For nitrogen and carbon isotopes the samples are combusted with O₂ at 1020°C in an elemental analyzer. The resulting gases (N₂, CO₂, SO₂, nitrous oxides, and water vapour) are transported with a He-flow through a reduction tube where the nitrous oxides and SO₂ are reduced to N₂ and sulfur. Afterwards the water is removed with a water trap, and a gas-chromatography column (GC) separates the N₂ from CO₂. A split interface reduces the amounts of N₂ and CO₂ and ~1% finally reach the IRMS. For oxygen isotope determination the O is bound to carbon to form CO in a pyrolysis furnace with glassy carbon as C source at 1350°C. The gas is transported with the He-flow through a GC column and reduced in a split interface. Inside the IRMS, the gases are ionized in the source with electrons, accelerated and then enter the magnetic sector. Dependent on their masses the ions are separated and hit a collector (Mulvaney, 1993). A schematic illustration of δ¹⁵N determination is shown in figure IV.2.

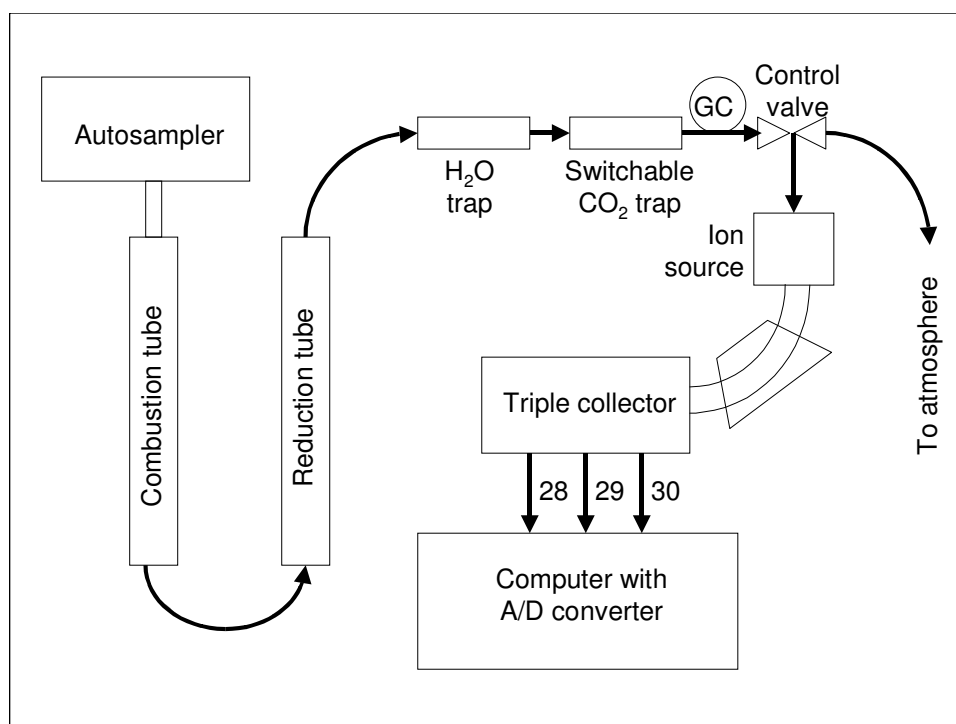


Fig.IV.2: Schematic diagramm which illustrates ¹⁵N-analysis in a IRMS (redrawn after Mulvaney (1993))

The isotope values are reported relative to reference gases (N_2 , CO_2 , CO), which are calibrated against international standards and are measured along with every sample. Furthermore, daily measurements of internal lab standards allows a correction of small equipment specific variations. The standards used in IOW are shown in Table IV.1.

Table IV.1: International and internal standards used for determination of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{18}O$ values

Standard	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	$\delta^{18}O$ [‰]
International Standards			
IAEA-N1 (ammonium-sulfate)	0.4 ±0.07		
IAEA-N2 (ammonium-sulfate)	20.3 ±0.09		
IAEA C6 (sucrose)		-10.43 ± 0.13	
NBS 22 (oil)		-29.74 ± 0.12	
USGS 24 (graphite)		-15.99 ± 0.11	
IAEA KNO_3			25.1 ±0.6
IAEA C3 (Cellulose)			32.2 ±0.2
USGS 34 (KNO_3)			-27.9 ± 0.75
Internal Lab Standards			
Peptone	5.8 ±0.20	-22.11 ± 0.17	
Acetanilide	-1.7 ± 0.2	-29.81 ± 0.19	
Merck- KNO_3	-0.4 ±0.16		24.6 ±0.7

For the determination of the $\delta^{15}N$ and $\delta^{18}O$ values, nitrate of the freshwater samples was converted into silver nitrate ($AgNO_3$) using the method of Silva *et al.* (2000).

Samples were passed through a cation exchange resin (5 ml AG 50W-X4, H^+ -form; Biorad), followed by an anion exchange resin (2 ml AG1-X8, Cl^- -form; Biorad). At least 60 μmol nitrate was finally collected on the anion exchange resin. The resins containing nitrate were stored in a refrigerator for several weeks until further preparation.

Samples were eluted from the anion exchange resin with 15 ml 3M HCl, and neutralized with approx. 6 g Ag_2O to obtain a pH of 5.5 – 6. The precipitated $AgCl$ and remaining Ag_2O was removed by filtration (0.45 μm membrane filter). 2 ml of 1M $BaCl_2$ solution was added to remove SO_4^{2-} and PO_4^{3-} . Precipitated $BaSO_4$ and $Ba_3(PO_4)_2$ were removed by filtration (0.45 μm membrane filter). The sample was passed through a cation exchange resin (5 ml AG

50W-X4, H⁺-form; Biorad) to eliminate the excess Ba²⁺. Then a second neutralization with Ag₂O (approx. 1-2 g) was carried out and the resulting AgCl and excess Ag⁺ was removed by filtration (0.45 μm membrane filter). The solution, now containing Ag⁺ and NO₃⁻, was freeze-dried and the remaining solid AgNO₃ was weighed into silvercaps for determination of δ¹⁵N-, and δ¹⁸O-NO₃-isotopic composition.

Reproducibility was tested with a AgNO₃, which was produced along with the samples to determine the accuracy and precision of the sample preparation. The standard contained Merck-KNO₃ (1000 μM NO₃⁻) and SO₄⁻ (620 μM).

The isotope values of the produced AgNO₃ (δ¹⁵N = -0.4 ± 0.36 ‰; n = 18 and δ¹⁸O = 23.5 ± 0.77; n = 23) were close to the isotope values of the Merck-KNO₃ (Table IV.1), which verified a preparation without isotopic fractionation.

Preparation procedure for δ¹⁵N-NO₃ values from brackish samples was carried out with the diffusion method of Sigman *et al.* (1997) In brief, nitrate is reduced to ammonium using Devarda's alloy and converted to ammonia at a pH~11. Ammonia is trapped on an acidified filter which is packed between two teflon-membranes. The filter then is dried and packed into a silver cap for combustion.

The δ¹⁵N analysis was carried out with a Thermo Finnigan Delta Plus IRMS after combustion in a Flash EA at a temperature of 1020°C. Samples for δ¹⁸O were also analysed in a Thermo Finnigan Delta Plus IRMS after pyrolysis in a Thermo Finnigan TC/EA. Temperature of combustion was 1350°C.

V. VARIATIONS IN THE $\delta^{15}\text{N}$ AND $\delta^{18}\text{O}$ VALUES OF NITRATE IN DRAINAGE WATER OF TWO FERTILIZED FIELDS IN MECKLENBURG-VORPOMMERN (GERMANY)

Introduction

The excessive input of nitrate into rivers and the resulting eutrophication of coastal waters is still an unsolved problem. Diffuse nitrate sources predominate, such as nitrate contaminated groundwater, runoff from drained farmland, or atmospheric deposition. In some German rivers, these sources account for more than 90% of the total nitrogen input (Behrendt *et al.*, 1999). The major problem for an efficient reduction of N inputs is the difficulty to identify and localise sources of nitrate. Moreover, most input data are estimations by means of models. An alternative method is the identification of diffuse inputs by using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}\text{-NO}_3$ values, and was successful in many river-systems and aquifers (Wassenaar, 1995; Kendall, 1998; Spoelstra *et al.*, 2001). Detailed knowledge of the isotopic composition of potential sources is essential. For instance, the isotopic composition of nitrate that leaches from agricultural soils undergoes a number of alterations. These alterations are related to differences in stable isotope ratios of applied fertilizers as well as to microbial processes in soils, like nitrification and denitrification. The $\delta^{15}\text{N}$ values of mineral fertilizers have a narrow range of 0.5 – 5‰ for NO_3^- and slightly lower values for NH_4^+ and urea (Bedard-Haughn *et al.*, 2003). $\delta^{18}\text{O}$ values of mineral fertilizers are near 23 ‰ (Amberger & Schmidt, 1987), reflecting the use of atmospheric oxygen for their production ($\delta^{18}\text{O} = 23.5$ ‰; Kroopnick & Craig, 1972). Nitrate derived from animal waste and manure typically shows $\delta^{15}\text{N}$ values over 10 ‰ because of the volatile loss of ^{15}N depleted NH_3 (Iqbal *et al.*, 1997; Kendall, 1998). This fractionation can also occur during the application of mineral fertilizer that mainly consists of reduced N forms (Bedard-Haughn *et al.*, 2003).

The mineralisation of soil organic N, including nitrification, leads to ^{15}N depleted nitrate; values range from -10 to 6 ‰, depending on the soil $\delta^{15}\text{N}$ value (Bremner & Tabatabai, 1973). The $\delta^{18}\text{O}$ value of generated nitrate typically varies from 2 to 14 ‰ (Mayer *et al.*, 2001). During denitrification ^{15}N -depleted N_2 is produced, resulting in increasing $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in the residual nitrate. The increase is 2 fold higher in $\delta^{15}\text{N}$ than in $\delta^{18}\text{O}$ (Böttcher *et al.*, 1990).

Tile drainage, a common agricultural practice to improve moisture and aeration conditions, shortens the residence time of water in the biologically active unsaturated zone and, therefore, enhances diffuse pollution of adjacent surface water bodies with nutrients or pesticides (Tomer *et al.*, 2003). Often, tile drainage is accompanied by flow anomalies, causing further unexpected acceleration of water flux and solute transport towards the tile drains (Lennartz *et al.*, 1999).

The goal of this study was to investigate the variability in stable isotope ratios of drainage water nitrate from two conventionally treated agricultural fields, and to identify the major sources and processes responsible for these variations. The input of nitrate to surface water bodies via tile drains plays an important role in regions with gleysols and stagnic cambisols from moraine substrates, such as those in Mecklenburg-Vorpommern, Germany. For a period of 17 months the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of drainage water nitrate leaching from two fertilized fields in the catchment area of the Warnow river (north-east Germany) were determined. Additional measurements of the isotopic composition of mineral fertilizers applied on these fields provided further information about processes in the investigated soils and the period until a response in drainage water nitrate could be observed.

Material and methods

The two sampled collector-drain outlets are located on the Farmers Cooperative Papendorf (Latitude $54^{\circ} 26' \text{N}$, Longitude $11^{\circ} 49' \text{E}$), 15 km south of Rostock in the sub area 'middle

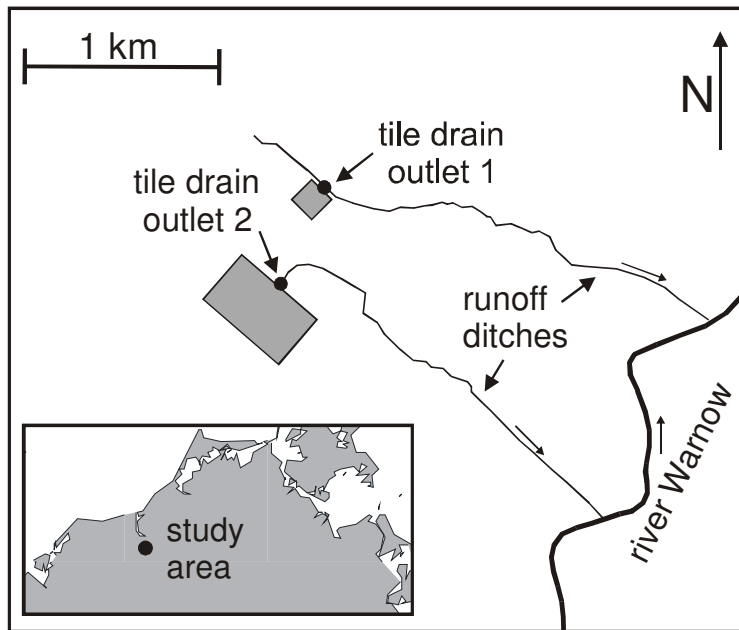


Figure. V.1: Location of the drainage tiles (dots) and agricultural fields (grey areas) investigated. The insert shows the coastal area of Mecklenburg-Vorpommern with the study area.

Warnow' (Fig. V.1). Soil textures are mainly sandy loams (24%) and loamy sands (46 %) from moraine substrates. Dominant soil types are cambisols, luvisols and gleysols. The drained area is 2.5 ha for tile drain 1 and 15 ha for tile drain 2. The drainage systems were installed ~ 100 y ago and are located at a soil depth between 60 - 80 cm. Tile drain outlet 2 also drained two small ponds in the

fields. Cultivated crops were winter wheat and winter barley in 2002 and sugar beet and maize in 2003 for tile drain 1. In tile 2, winter wheat and winter barley were grown in 2002 and 2003. Crop rotation was identical in both fields, with 4 y of cereals followed by 1 y of sugar beet and maize. Application of nitrogen was carried out with mineral as well as organic fertilizers (Table V.1). Organic fertilizer was applied every fifth year before to the cultivation of sugar beets and maize. The last application above tile 2 was in fall 1998. During hydrological years 2001/2002 and 2002/2003, the meteorological station near the sampled tile outlets (distance ~ 5 km) recorded a precipitation of 612 and 423 L m^{-2} , respectively. The precipitation during the hydrological year 2002/2003 was 242 L m^{-2} lower than the long-term

mean of 665 L^{-2} . Only half of the normal amount was recorded from November 2002 to April 2003. The long-term mean evaporation in the sampling area is $499 \text{ L m}^{-2} \text{ a}^{-1}$.

Table V.1: Date, amount and composition of the applied fertilizer

Tile drain 1			
Date	Amount applied [kgN ha ⁻¹]	Fertilizer	Nitrogen compounds
03-25-2002	85	AHL	28% N (7% NH ₄ ⁺ ; 7% NO ₃ ⁻ , 14% amide)
04-23-2002	70	Urea	46% N (46% amide)
05-29-2002	79	KAS	27% N (13.5 % NH ₄ ⁺ ; 13.5 % NO ₃ ⁻)
August 2002	232	manure	
Tile drain 2			
Date	Amount applied [kgN ha ⁻¹]	Fertilizer	Nitrogen compounds
03-27-2002	66	AHL	28% N (7% NH ₄ ⁺ ; 7% NO ₃ ⁻ , 14% amide)
04-22-2002	61	NTS	27% N (6% NH ₄ ⁺ ; 8% NO ₃ ⁻ , 13% amide)
05-28-2002	148	NTS	27% N (6% NH ₄ ⁺ ; 8% NO ₃ ⁻ , 13% amide)
03-18-2003	79	AHL	28% N (7% NH ₄ ⁺ ; 7% NO ₃ ⁻ , 14% amide)
04-28-2003	90	Urea	46% N (46% amide)

Water sampling was carried out from the end of January 2002 to the beginning of May 2003 at weekly to bimonthly intervals. Discharges at the tile drain outlets were not measured.

Water samples (1L) were stored in pre-acidified polyethylene bottles; each filtered through a $0.45 \mu\text{m}$ membrane filter immediately after sampling and frozen at -20°C until further preparation. Preparation of the nitrate samples was carried out after Silva *et al.* (2000).

The solid fertilizers (KAS, Urea) were homogenized and weighed into silver cups for determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values. For determination of $\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ values of fluid fertilizers (AHL, NTS), the same preparation method was used as for drainage water samples. For determination of $\delta^{15}\text{N}$ values, the fluid fertilizers were cooled to avoid ammonia volatilisation, pipetted into silver caps and immediately measured.

Results

Table V.2: Concentrations of nitrate and ammonium in the drainage water from each tile drain.

Date	Tile drain 1		Tile drain 2	
	NO_3^- [μM]	NH_4^+ [μM]	NO_3^- [μM]	NH_4^+ [μM]
01-25- 2002	953	0.0	not sampled	
02-01- 2002	694	0.0	not sampled	
02-08- 2002	745	0.0	not sampled	
03-09- 2002	645	0.0	not sampled	
03-15- 2002	not sampled		538	0.0
04-06- 2002	714	1.1	636	1.2
04-19- 2002	730	1.1	655	1.3
04-23- 2002	517	0.0	1138	3.8
04-28- 2002	697	0.4	905	1.1
05-06- 2002	696	0.0	not sampled	
05-13- 2002	not sampled		1051	0.4
05-25- 2002	723	0.3	828	0.6
06-05- 2002	692	0.3	not sampled	
06-12- 2002	712	0.2	not sampled	
07-31- 2002	739	0.0	1567	1.8
09-01- 2002	664	0.0	1471	50.7
10-29- 2002	663	0.0	1105	0.0
12-04- 2002	844	0.0	533	1.4
12-20- 2002	813	n. d.	1462	150.7
01-17- 2003	837	6.4	151	6.4
02-13- 2003	848	0.0	518	31.6
03-14- 2003	966	1.6	574	100.5
05-02- 2003	575	15.0	1144	0.0

In tile drain 1, nitrate concentrations varied from 517 to 966 μM (Table V.2, Fig. V.2).

The lowest concentration was found in April 2002, the highest in March 2003.

Lower nitrate concentrations were observed during summer and higher ones in autumn and winter. The second fertilizer application (Table V.1) led to a noticeable 180 μM increase in the nitrate concentration, whereas the other three applications seemed to have no effect on outflow nitrate concentrations. Most samples showed ammonium concentrations below 1.6 μM . Ammonium reached high concentrations of 6.4 and 15.0 μM only in January and May 2003, respectively.

Tile drain 2 showed a higher variability in NO_3^- concentrations than tile 1 (Table V.2, Fig. V.3). After application of fertilizer,

increases in nitrate between +98 and +739 μM were observed. The highest nitrate concentration (1567 μM) was found at the end of July 2002, after the third fertilizer application. The concentration then decreased to 533 μM until the beginning of December 2002, rapidly rising to 1462 μM . A minimum value of 151 μM was reached in January 2003 and, subsequently, concentrations increased until the end of the sampling period. Ammonium

concentrations showed a higher variability in tile 2, ranging between 0 to 150.7 μM . Values remained nearly constant below 3.8 μMol until September 2002 and then peaked at 50.7 μM . A maximum value was reached in December 2002. After a decline, they increased to a third peak in March 2003.

Mineral fertilizers applied had $\delta^{15}\text{N}$ values for total nitrogen between -3.8 and 0.7 ‰ (Table V.3). The $\delta^{15}\text{N}\text{-NO}_3$ values for both of the fluid fertilizers (AHL, NTS) are slightly higher compared to values of total nitrogen (0.8 and 4.4 ‰, respectively). The $\delta^{18}\text{O}\text{-NO}_3$ values for nitrate containing AHL, NTS and KAS fertilizers was 25.7, 19.4 and 20.5 ‰, respectively.

The $\delta^{15}\text{N}\text{-NO}_3$ values of tile 1 ranged between 8.3 and 14.5 ‰, with a mean value of 10.6 ± 1.5 ‰ (Fig. V.2). They decreased from 11.6 to 8.3 ‰ until the second

fertilizer application, and then increased to a maximum value at the end of May 2002. After

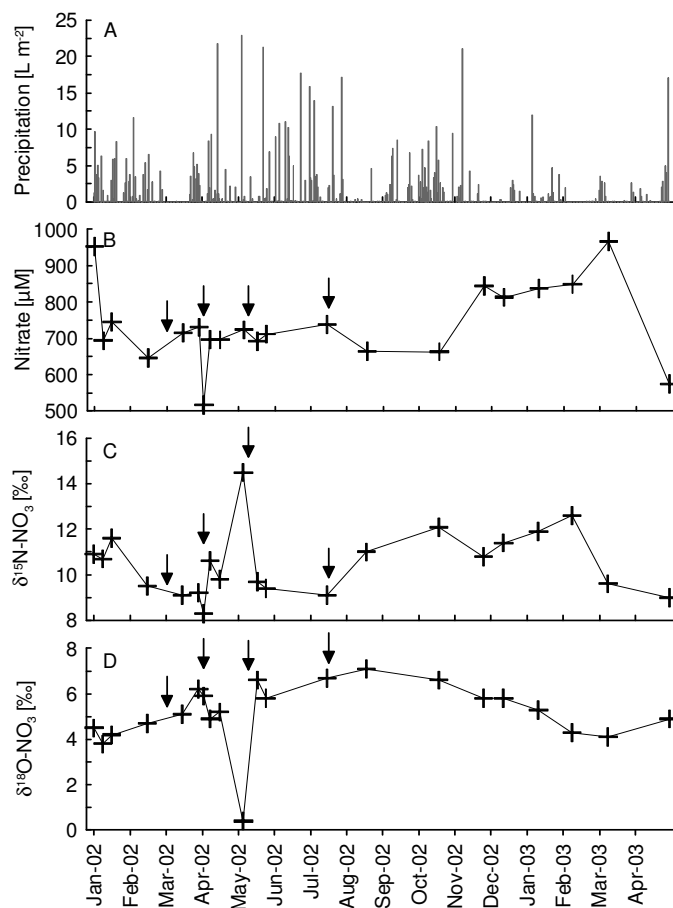


Figure V.2: Changes in variables for tile drain outlet 1 during the sampling period from January 25 2002 to May 2 2003. A) precipitation as recorded ~ 5 km west of the sampling area; B) nitrate concentration; C) $\delta^{15}\text{N}\text{-NO}_3$; D) $\delta^{18}\text{O}\text{-NO}_3$. The small arrows indicate the fertilizer application.

Table V.3: Isotopic composition of the applied mineral fertilizers (¹ data from Amberger & Schmidt (1987))

Fertilizer	$\delta^{15}\text{N}_{\text{total}}$ [‰]	$\delta^{15}\text{N}\text{-NO}_3$ [‰]	$\delta^{18}\text{O}\text{-NO}_3$ [‰]
AHL	-3.8	0.8	25.7
NTS	0.1	4.4	19.4
KAS (+CaO)	-1.7		20,5 ¹
Urea	0.7		

fertilizer application, and then increased to a maximum value at the end of May 2002. After

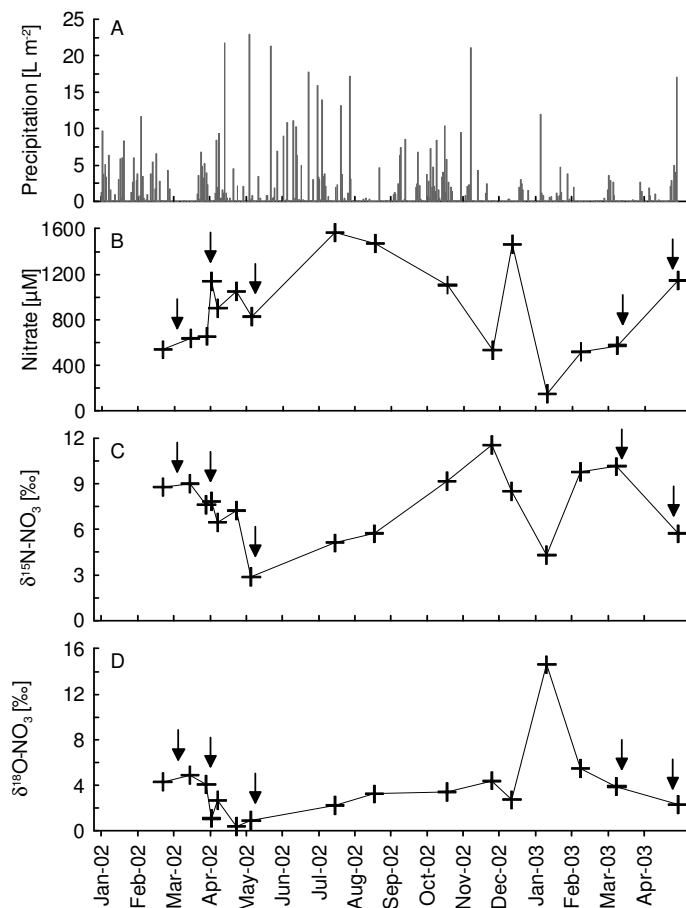


Figure V.3: Changes in variables for tile drain outlet 2 during the sampling period from January 25 2002 to May 2 2003. A) precipitation as recorded ~5 km west of the sampling area; B) nitrate concentration for tile drain outlet 2; C) $\delta^{15}\text{N-NO}_3$ for tile drain outlet 2; D) $\delta^{18}\text{O-NO}_3$ for tile drain outlet 2. The small arrows indicate the fertilizer application.

Fig. V.3). They decreased from 9.4 to 2.9 ‰ until May 25. After the third fertilizer application, values increased (+8.6‰), and a maximum value was reached on December 4. Values then decreased again until January 2003 and then increased. The fourth fertilizer application led again to a decrease in $\delta^{15}\text{N-NO}_3$ values (-4.4 ‰). A student's t-test indicated that tile 2 had significantly lower $\delta^{15}\text{N}$ values ($p < 0.001$) compared to tile 1. The trend in $\delta^{18}\text{O-NO}_3$ values was similar to that of $\delta^{15}\text{N-NO}_3$ values, with the exception of January 2003. Values ranged between 0.4 and 14.6 ‰ with a mean of 3.8 ± 3.2 ‰. The third fertilizer application also resulted in slightly increasing values. After December 2002, a strong increase

the third application of fertilizer, a decrease (-4.8‰) occurred, and the last application resulted in slightly increasing $\delta^{15}\text{N-NO}_3$ values until February 2003. The $\delta^{18}\text{O-NO}_3$ values varied between 0.4 and 7.1 ‰, showing a mean value of 5.1 ± 1.4 ‰. They ranged between 3.8 and 6.2 ‰ until the beginning of May 2002, then they declined to a minimum value. After the third fertilizer application, values increased to a maximum in September 2002 and then decreased slightly until March 2003.

In drainage water of tile 2, $\delta^{15}\text{N-NO}_3$ values varied between 2.9 and 11.5 ‰ (mean value 7.6 ± 2.3 ‰,

was found, but in this case was associated with a decline in $\delta^{15}\text{N}\text{-NO}_3$ values. The highest $\delta^{18}\text{O}\text{-NO}_3$ value occurred in January 2003.

Discussion

All samples from tile drain outlet 1 were in the nitrate range derived from manure and sewage (Fig. V.4), whereas nitrate in some samples of tile drain 2 seemed to originate from nitrification in soils. Rennie *et al.* (1976) found $\delta^{15}\text{N}\text{-NO}_3$ values in soil water of non-fertilized soils to vary between 2.7 and 12.2 ‰, and values up to 14.2 ‰ were recorded if fertilizer was applied (Flipse & Bonner, 1985). Amberger & Schmidt (1987) reported differences in $\delta^{15}\text{N}$ values in groundwater nitrate beneath

agricultural soils depended on the fertilizers used. If only mineral fertilizers were applied, they found lower $\delta^{15}\text{N}$ values (3.0 – 5.9 ‰) than in areas - such as ours - with applications of mineral fertilizer as well as manure ($\delta^{15}\text{N} = 10.6$ ‰). The $\delta^{18}\text{O}$ values ranged from 5.7 to 13.5 ‰ (Amberger & Schmidt, 1987). Nitrate concentrations in our study were generally similar to those reported for other agricultural regions in Germany. Feige & Röthlingshöfer (1990) measured nitrate concentrations of 161 to 2177 μM in drainage water beneath cropland treated with conventional farming practices. Next to our study area, 71-1800 μM NO_3^-

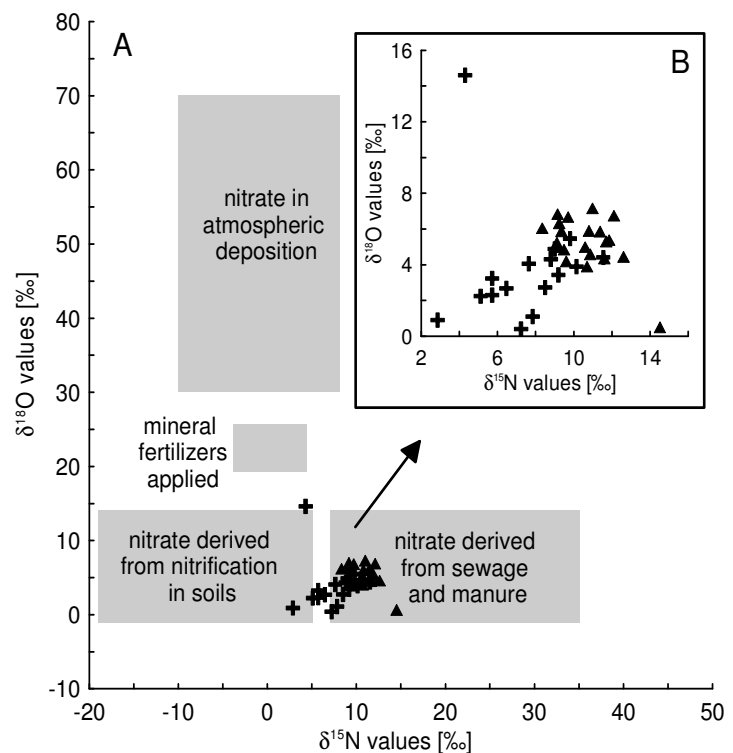


Figure V.4: A) ranges of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate from different sources (redrawn after (Mayer *et al.*, 2002) from the fertilizers applied and isotope values of drainage water nitrate (triangles: tile drain 1; crosses: tile drain 2. Insert (B) shows the tile drain isotope values in more detailed).

beneath barley fields solely treated with mineral fertilizers were reported (Bockholt *et al.*, 1992). Values of up to 2140 μM were found beneath maize, when additional application of manure took place. A correlation between nitrate concentration and precipitation, as reported from other studies in Germany (Lammel, 1990; Göbel, 2000) was not found in our data.

There are several processes that can modify the concentration and isotope values of drainage water nitrate. These are nitrification and denitrification, ammonia volatilisation, and mixing with nitrate from other sources, e.g. mineral fertilizers or atmospheric nitrate deposition. Nitrification, the last step in the mineralisation of soil organic N, leads to a release of nitrate which is usually ^{15}N -depleted compared to the residual ammonium (Kendall, 1998). The extent of this fractionation depends greatly on the ammonium concentration. High concentrations, e.g. after application of fertilizer containing ammonium, leads to an increase in the nitrification rate (Zaman *et al.*, 1999), resulting in a greater fractionation and low $\delta^{15}\text{N}$ - NO_3 values of nitrate (Kendall, 1998). If the availability of ammonium in soils decreases, $\delta^{15}\text{N}$ - NO_3 values of the formed nitrate increase towards pre-fertilization values (Feigin *et al.*, 1974). Nitrate generated during nitrification derives one oxygen atom from O_2 and two from soil water (Hollocher *et al.*, 1981; Andersson & Hooper, 1983). $\delta^{18}\text{O}$ values of nitrate generated during nitrification in acid forest floors range between 2 and 14 ‰ (Mayer *et al.*, 2001), and Amberger & Schmidt (1987) found values lower than -2 ‰ in groundwater under forests and agricultural areas. Both tile drains in our study had $\delta^{18}\text{O}$ - NO_3 values ranging from 0.4 to 14.6 ‰, suggesting that nitrification was the dominant process. The variation in $\delta^{18}\text{O}$ - NO_3 values may be due to changes in $\delta^{18}\text{O}$ values of soil water.

During denitrification, ^{15}N -depleted N_2 is produced and lost to the atmosphere. This causes an enrichment in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the residual nitrate (Böttcher *et al.*, 1990), covering a wide range, depending on the denitrification rate, temperature, and substrate concentrations (Böttcher *et al.*, 1990). The amount of nitrogen lost is heavily dependent on soil conditions (water content, nitrate concentration), crops cultivated and fertilizer application. In the central

European climate, increased N losses during denitrification are temporary events lasting a couple of days or weeks in spring and autumn (Benckiser, 1996). In our study, denitrification can only be presumed in drainage water from tile 2 from the end of May until the beginning of December 2002. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate increased by 8.6 and 3.5 ‰, respectively; with a ratio of 2.5:1. A decrease in nitrate concentration, usually associated with denitrification, was only observed from late July to the beginning of December 2002; decreasing by 1034 μM . From late July to beginning of December, the enrichment factors ($\epsilon_{\text{N}_2\text{-NO}_3}$) for ^{15}N and ^{18}O can be calculated using a simplified Rayleigh equation (Mariotti *et al.*, 1988):

$$\epsilon = (\delta_s - \delta_{s_0}) / \ln (s/s_0) \quad (1)$$

where

δ_s = delta value in the substrate (‰)

δ_{s_0} = initial delta value in the substrate (‰)

s = substrate concentration (μMol)

s_0 = initial substrate concentration (μMol)

The resulting enrichment factors are -5.9 ‰ for $\delta^{15}\text{N}$ and -2.0 ‰ for $\delta^{18}\text{O}$. Böttcher *et al.* (1990) found greater enrichment values of -15.9 ‰ for $\delta^{15}\text{N}$ and - 8.0 ‰ for $\delta^{18}\text{O}$, while Mariotti *et al.* (1988) reported ϵ values for $\delta^{15}\text{N}$ from -4.7 to -5.0 ‰, similar to our calculated value. This result suggests that denitrification took place in this period, but it remained unclear whether this occurred in soils or in the existing ponds.

To verify whether the application of fertilizer led to changes in isotope values and concentrations of drainage water nitrate, some additional factors must be considered. These factors are the elapsed time and the amount of precipitation between application and sampling. Both agricultural fields received similar N amounts from fertilizers during the sampling period (466 kg N ha⁻¹ above tile 1, 444 kg N ha⁻¹ above tile 2). The highly soluble nitrate compound of the fertilizer is transported to deeper soil layers with downward soil

water movement. Flow velocity depends on soil conditions as well as precipitation intensity. If precipitation intensity is too high, nitrate is transported directly to the tile drains and is no longer available for plants.

After the first application of fertilizer, little precipitation (0.1 L m^{-2}) occurred between application and sampling (Table V.4). This resulted in a reduced or halted soil water flow and, consequently, no traces of fertilizer were observed in drainage water nitrate from both tile drains. Nitrate increased slightly and isotope values showed only small changes.

The second application above tile 1 led to a remarkable increase in nitrate concentration ($+180 \mu\text{M}$) and $\delta^{15}\text{N-NO}_3$ value ($+2.3 \text{ ‰}$). Urea was applied, and needed to be hydrolysed to ammonium, nitrified and transported to the tile drain within 5 days (period between application and sampling). This timing seems plausible since Atxotegi *et al.* (2003) found an increase in soil nitrate concentration within 5 days after application of urea fertilizer in experimental plots in Iowa. Our data further suggests that the increase in drainage water nitrate was a result of fertilization. Precipitation intensity during the 5 days was 11.6 L m^{-2} , and might have been enough to transport the generated nitrate to the tile drain. Other evidence

Table V.4: Amount of fertilizer applied, elapsed time and precipitation between fertilizer applications and sampling, and changes in the nitrate concentration and isotope values of the drainage water nitrate from each tile drain.

Tile drain	Fertilizer application	Amount applied [kgN ha^{-1}]	Days between application and sampling	Precipitation between application and sampling [L m^{-2}]	Change in nitrate concentration [μM]	Change in $\delta^{15}\text{N-NO}_3$ [‰]	Change in $\delta^{18}\text{O-NO}_3$ [‰]
1	1	85	12	0.1	+ 69	- 0.4	+ 0.4
	2	70	5	11.6	+ 180	+ 2.3	- 1.0
	3	79	7	4.1	- 31	- 4.8	+ 6.2
	4	232	< 30	< 44.2	-75	+ 1.9	+ 0.4
2	1	66	8	0.1	+ 98	+ 0.2	+ 0.6
	2	61	1	0	+ 483	+ 0.2	- 3.0
	3	148	64	158.5	+ 739	+ 2.2	+ 1.3
	4	79					
	5	90	4	26.1	+570	-4.4	- 1.6

for the influence of urea fertilizer is the increase in $\delta^{15}\text{N-NO}_3$ values by 2.3 ‰, which might have been a result of ammonia volatilisation. Hydrolysis of urea to ammonium leads to a temporary rise in the soil pH. This rise favours the formation of ^{15}N -depleted ammonia that volatilises into the atmosphere. The residual ammonium is enriched in ^{15}N and subsequently converted into ^{15}N -enriched nitrate. This process was observed by Kreitler *et al.* (1978) and Iqbal *et al.* (1997) after the application of manure and mineral fertilizer that contained urea compounds, resulting in $\delta^{15}\text{N-NO}_3$ values >10 ‰. Flipse & Bonner (1985) found $\delta^{15}\text{N}$ values in groundwater nitrate of two fertilized sites that were about 12.4 ‰ higher than the applied fertilizer and explained the enrichment as a loss in ^{15}N -depleted ammonia. If the increase in the nitrate concentration of tile drain1 was a result of nitrification of soil organic N or of the applied fertilizers without the effect of ammonia volatilisation, a decrease in $\delta^{15}\text{N-NO}_3$ should have occurred; this was not the case.

After the third application above tile 1, no change in nitrate concentration but in isotope values was observed. $\delta^{15}\text{N-NO}_3$ decreased from 14.5 to 9.7 ‰ and $\delta^{18}\text{O-NO}_3$ increased from 0.4 to 6.6 ‰. This result indicates a mixture of soil water nitrate with nitrate derived from the KAS fertilizer. The KAS fertilizer had a $\delta^{15}\text{N}$ value for total N (nitrate and ammonium) of -1.7 ‰ and a $\delta^{18}\text{O}$ value for nitrate of 20.5 ‰. These values arise from their production from atmospheric nitrogen ($\delta^{15}\text{N} = 0$ ‰) and oxygen ($\delta^{18}\text{O} = 23.5$ ‰; Kroopnick & Craig, 1972; Amberger & Schmidt, 1987). A mixture of soil water nitrate with nitrate from mineral fertilizers could lead to the observed changes in $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values of the drainage water nitrate. It seems that the moderate amount of precipitation (4.1 L m⁻²) was sufficient to displace the nitrate to the tile drain within 7 days.

The last fertilizer applied above tile drain 1 was cow manure. Unfortunately, the exact date of the application is unknown and thus there is uncertainty about the time gap between application and sampling, and about the amount of precipitation during the period. The transformation of organic nitrogen to nitrate is a slow process and takes much longer than the

generation of nitrate from urea in mineral fertilizers (Atxotegi *et al.*, 2003). This fact may be the reason that no increase in nitrate concentration was observed, although a high amount of manure was applied (232 kg N ha^{-1}). Additionally, ammonia volatilisation from manure may be indicated by the increase in $\delta^{15}\text{N-NO}_3$ values.

The sampling dates for tile drain outlet 2 did not allow statements of how the second, third and fourth fertilizer application influenced drainage water nitrate. Elapsed time between the second application and sampling was only one day and no precipitation occurred suggesting that the increase in nitrate concentration was not fertilizer-induced. Direct input of fertilizer, which contained ammonium, nitrate and urea, into the drained ponds and a rapid transport to the tile drain can be excluded because no increase in ammonium concentration was observed. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O-NO}_3$ values indicated no mixture with fertilizer-derived nitrate. After the third application above tile drain 2, 64 days elapsed before sampling. In combination with the high amount of precipitation (158.5 L m^{-2}), a direct influence of fertilizer was not traceable. The last application was urea, and the time gap between application and sampling were 4 days. During this period precipitation was 26.1 L m^{-2} , with 17 L m^{-2} the day before sampling and nitrate concentration increased by $570 \mu\text{M}$. However, no increase in $\delta^{15}\text{N-NO}_3$ values was observed. Air temperatures on both fertilization dates were comparable, such that ammonia volatilisation was possible in the field above tile drain 2. The strong decrease in the $\delta^{15}\text{N-NO}_3$ value (-4.4 ‰) indicates that the nitrate was derived from nitrification. The larger drainage area or a silting up of the tile may lead to a longer residence time of soil water, whereby the nitrate generated after urea fertilization did not reach the tile drain outlet.

Nitrate input from atmospheric deposition is another source that can affect drainage water nitrate. Nitrate in atmospheric deposition is characterized by $\delta^{15}\text{N}$ values between -15 to 15 ‰ and $\delta^{18}\text{O}$ values $> 30 \text{ ‰}$ (Durka *et al.*, 1994). In rain samples 15 km north of the study site, $\delta^{15}\text{N}$ values between 0.4 and 3.7 ‰ and $\delta^{18}\text{O}$ values between 38.0 and 60.7 ‰ were found (Deutsch *et al.*, in prep.). Nitrate concentrations were low ($17 - 139 \mu\text{M}$) compared to

drainage water nitrate. In January 2003, a mixture of drainage water nitrate with water containing low nitrate concentrations and high $\delta^{18}\text{O}$ values was observed in tile drain 2. Nitrate decreased by 1311 μMol and the $\delta^{18}\text{O}\text{-NO}_3$ value increased by 11.9 ‰. We assume snowmelt occurring a few days before sampling caused the changes. Nitrate in snow has similar $\delta^{18}\text{O}$ values as in rainwater (Campbell *et al.*, 2002). Moreover, the mixture of two waters with different isotope values and concentrations was only observed in the drainage water of tile drain 2. This finding emphasizes the role of the small ponds located in the drainage area. A further influence of nitrate from atmospheric deposition on the isotope signatures throughout the sampling period can be excluded because of the low $\delta^{18}\text{O}\text{-NO}_3$ values in tile 2 during the remaining sampling period. Altogether, the relative small amount of nitrate and ammonium in atmospheric deposition compared to the high amount of fertilizers applied by agriculture argues against a high influence of atmospheric deposition.

Conclusions

The dual-isotope approach with $\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ is a powerful tool to identify microbial processes or fertilizer-induced effects that influence the nitrate concentration in drainage water beneath agricultural fields. Changes in isotope values and in nitrate concentration could be attributed to the application of fertilizers, particularly in the small drainage area of tile 1. Although nitrate comprised only a small share of the fertilizers applied, the discharge water in the tile drain outlets had high nitrate concentrations. This result is clearly due to nitrification; except if there were events such as snowmelt or rain soon after nitrate fertilization that washed out the newly added NO_3 fertilizer. This particular importance of nitrification in the investigated soils was also confirmed by low $\delta^{18}\text{O}\text{-NO}_3$ values. The higher variability in nitrate concentrations and isotope values in tile 2 might be due to the influence of the small ponds or because of different microbial activity in the soils.

Furthermore, the larger the drainage area, the longer the residence time of drainage water. Nitrate that reached the drainage system near the outlet needs much less time than nitrate from further away. Presumably, this effect would be enhanced further by silting of the tile drains. We cannot judge whether differences in the microbial activity or in the residence times of the drainage water from both tiles existed. Although $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the drainage water nitrate from both tiles differed, their isotope values can be distinguished from other diffuse nitrate sources, such as atmospheric deposition.

VI. ASSESSING THE IMPACT OF NITRATE FROM TILE DRAINAGE FOR ADJACENT SURFACE WATERS: A STABLE ISOTOPE APPROACH

Introduction

Diffuse nitrogen inputs from agriculture are the major N source for rivers systems and aquifers in many areas of the world (Mengis *et al.*, 1999; Galloway & Cowling, 2002). Although only a small amount of nitrate is directly applied to the field, it is – amongst DON – the dominant N-form lost to aquatic environments (Addiscott *et al.*, 1992). Mainly responsible for high nitrate losses are microbial processes in the soils. Mineralization of soil organic N followed by nitrification result in high nitrate concentrations especially during autumn and winter when crops are harvested and the soil is still warm enough for microbial activity (Kirchmann *et al.*, 2002). The highly water-soluble nitrate is then shifted downwards with the soil water flow and reaches drainage-systems and/or groundwater. Especially in artificially drained agricultural areas this is an efficient pathway because of short residence times of the soil water in the biologically active unsaturated zone (Tomer *et al.*, 2003). Several studies have shown that the concentration of nitrate is positively correlated to water discharge (Lammel, 1990; Göbel, 2000). From the drainage system NO_3^- is lost to rivers and reaches coastal areas, where it contributes to eutrophication (Addiscott *et al.*, 1992; Galloway & Cowling, 2002).

A common method to trace diffuse nitrate inputs is the determination of stable isotope ratios of nitrogen and oxygen in nitrate (Amberger & Schmidt, 1987; Wassenaar, 1995; Spoelstra *et al.*, 2001; Burns & Kendall, 2002; Campbell *et al.*, 2002; Chang *et al.*, 2002; Mayer *et al.*, 2002). Studies have shown that enhanced agricultural activity results in increased $\delta^{15}\text{N}$ values in nitrate of the adjacent surface waters, while nitrate in undisturbed rivers shows $\delta^{15}\text{N}$ values

of -1.5-4.5 ‰ (McClelland & Valiela, 1998). A positive correlation between percentage of land used for agriculture and the $\delta^{15}\text{N-NO}_3$ values for 16 watersheds in the United States is reported from Mayer *et al.* (2002). The volatile loss of ammonia from organic and inorganic fertilizers, and denitrification in soils lead to higher $\delta^{15}\text{N}$ values of rivers compared to natural watersheds (Mengis *et al.*, 1999; Mayer *et al.*, 2002). The stable isotope ratios of oxygen in nitrate are successfully applied to identify microbial processes like nitrification and denitrification (Böttcher *et al.*, 1990; Mayer *et al.*, 2001) or to identify mixtures with rain water nitrate, or nitrate from mineral fertilizers (Amberger & Schmidt, 1987; Durka *et al.*, 1994; Campbell *et al.*, 2002).

In this study the variations in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of drainage water nitrate were investigated during the main discharge period of the hydrological year 2003/2004 (one runoff event). Sampling took place from end of January to middle of March at a high temporal resolution in a small, predominantly rural catchment near Rostock in north eastern Germany. To evaluate the share of drainage water nitrate to the adjacent surface waters additional samples were taken in the adjacent ditch and brook.

Material and methods

Water samples were taken in the catchment area of the river Zarnow 15 km south-east of the city of Rostock (Mecklenburg-Vorpommern, Germany) from Jan 30, 2004 to Mar 13, 2004. Sampling stations for discharge measurements and water sampling were located at the drain outlet of a tile drained field site (4.2 ha), at an adjacent ditch draining around 180 ha used for crop production by conventional farming and the brook Zarnow with a catchment of about 16 km² (Fig. VI.1). At the tile drain outlet, the water level was recorded in a Venturi flume in 15 minute intervals, while an automatic sampler (ISCO) took samples every three hours, which were then merged to 22 daily composite samples. At the other stations, manual sampling was

carried out daily to twice a week depending on meteorological conditions. Here, 10 samples were selected for isotopic analysis. All samples were collected in 1L preacidified PE-bottles

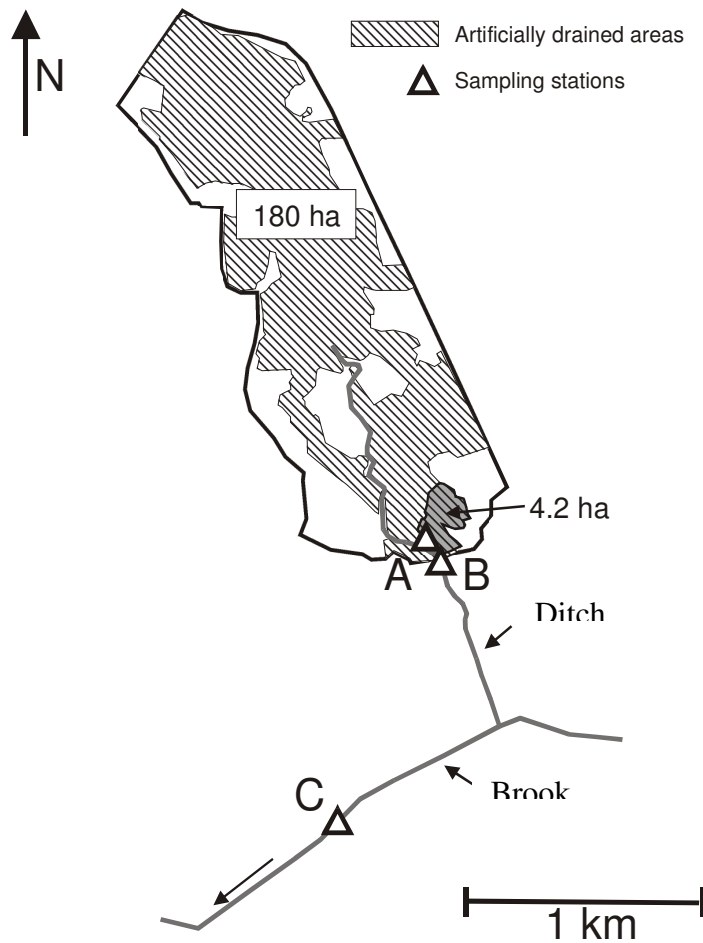


Figure. V.1: Map of the investigation area; the sampled stations are marked with triangles and letters (A tile drain outlet; B adjacent ditch, and C brook).

and frozen at -20°C until further preparation. Analysis for nitrate was carried out by ion chromatography. Further investigations included a soil survey and the operation of a weather station (Kahle *et al.*, 2005). Cultivated crops on the tile drained field site were corn in 2003 and winter wheat in 2004. Application of fertilizers was carried out in organic and inorganic forms, altogether 240 kg N ha^{-1} in 2003.

For isotope analysis the nitrate of the water samples was converted into AgNO_3 using the method of Silva *et al.* (2000).

Results

At January 30, nitrate concentrations in the tile drain outlet was $686 \mu\text{M}$ (Fig. VI.2B) and then increased to the maximum value of $2040 \mu\text{M}$ with the increased drain discharge (Fig. VI.2A). After February 7 concentrations decreased to $874 \mu\text{M}$ until the end of the sampling period. During the whole sampling period high discharge rates were corresponding to high nitrate concentrations. Between the tile drain discharge and the nitrate concentration, a positive linear

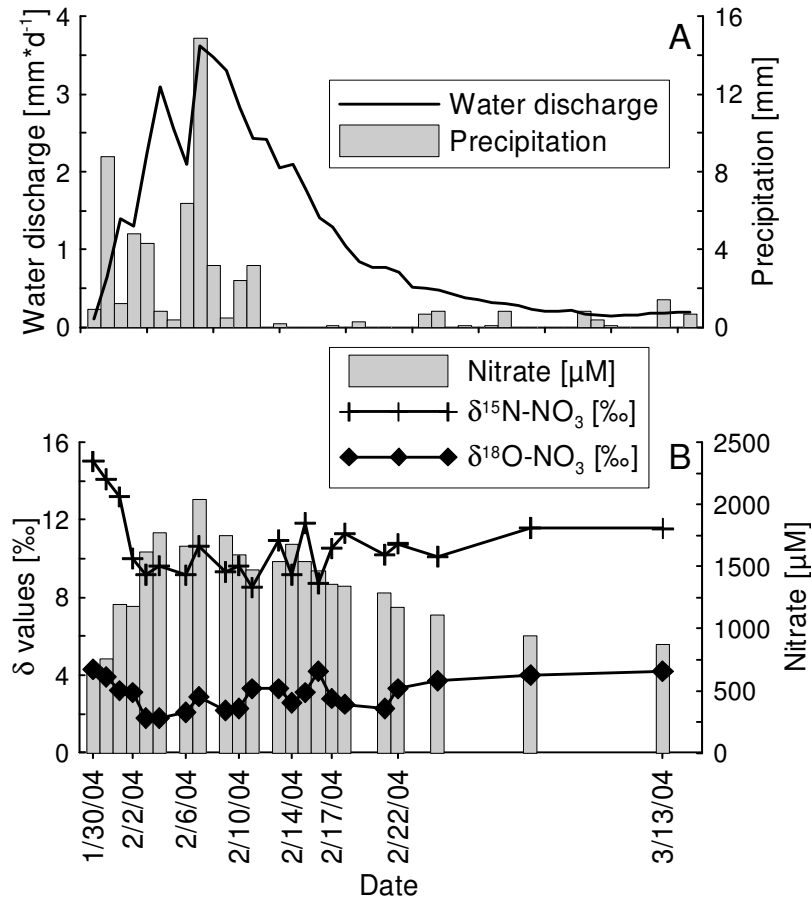


Figure VI.2: Amount of precipitation and drain discharge for the tile drain outlet (A), as well as concentration and isotope values of the drainage water nitrate (B).

relationship ($r^2 = 0.84$; $p < 0.001$; Fig. VI.3) was observed. The $\delta^{15}\text{N-NO}_3$ values started out at 15 ‰, dropped to 9.2 ‰ within the first five days, and then fluctuated between 8.5 and 11.8 ‰ (Fig. VI.2B). A similar pattern was observed for the $\delta^{18}\text{O-NO}_3$ values. At the start of the sampling period the value was 4.3 ‰ and decreased within five days to 1.8 ‰, then the values fluctuated between 1.8 and 4.2 ‰ (Fig. VI.2B).

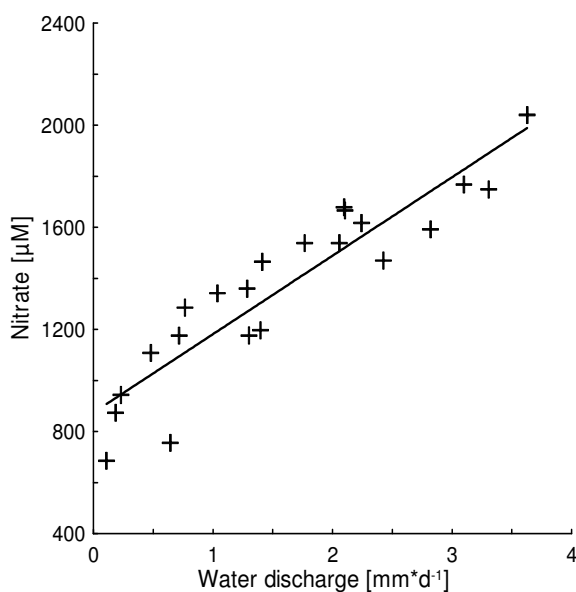


Figure VI.3: Correlation between water discharge and nitrate concentration for the tile drain outlet.

The adjacent ditch and the brook showed lower nitrate concentrations than the tile drain outlet (Fig VI.4A and B). In the ditch, nitrate varied between 574 and 1652 μM, the brook had concentrations between 312 and 947 μM. The $\delta^{15}\text{N-NO}_3$ values ranged between 7.2 and 11.0 ‰ in the ditch, and between 7.5 and 12.1 ‰ in the brook. The

values started low and increased during the sampling period. A close similarity was indicated by a linear correlation between the $\delta^{15}\text{N-NO}_3$ values from the ditch and the brook ($r^2= 0.74$; $p< 0.01$; $y = 1.1534x - 0.7343$). For the ditch, $\delta^{18}\text{O-NO}_3$ values were in a range of 2.3 to 4.1 ‰, and showed an increase until end of the sampling period. The $\delta^{18}\text{O}$ values for the brook varied between 2.6 and 9.1 ‰, and showed a strong increase over time.

Discussion

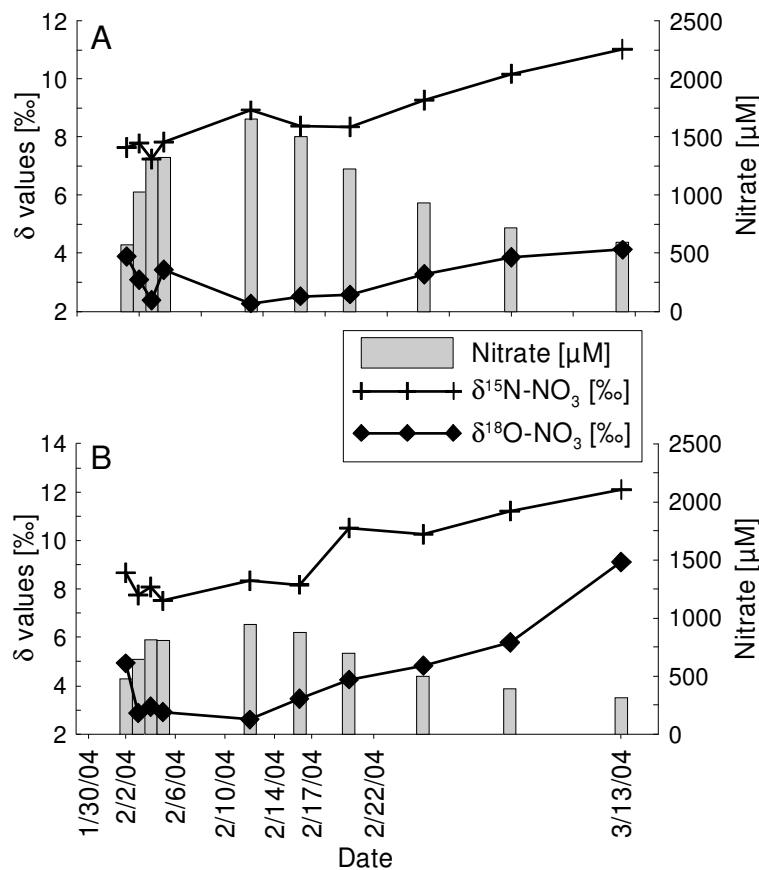


Figure. VI.4: Nitrate concentrations and nitrate stable isotope values for the ditch (A) and the brook (B).

Considering that the sampling was carried out during the first main discharge period within the hydrological winter 2003/2004, it was expected that the nitrate in the drainage water derived from the nitrification of soil organic N during winter and autumn (Göbel, 2000; Kirchmann *et al.*, 2002). This can be proved by means of the $\delta^{18}\text{O-NO}_3$ values, which are in the range for nitrification reported from other studies (-2 to 15 ‰;

Amberger & Schmidt, 1987; Kendall, 1998; Mayer *et al.*, 2001). The $\delta^{15}\text{N-NO}_3$ values of the drainage water reflected the mixture of inorganic and organic N application, realized in 2003.

This agrees with the $\delta^{15}\text{N-NO}_3$ values of 10.6 ‰ in groundwater beneath pasture treated with organic and inorganic N reported from Amberger & Schmidt (1987).

The strong decrease of the $\delta^{15}\text{N-NO}_3$ values (-5.8 ‰) and the moderate decrease in the $\delta^{18}\text{O-NO}_3$ values (-2.5 ‰) of the drainage water during the first five days (Fig. 2) may be explained by two reasons: The first is a mixture of soil water nitrate with nitrate from rain, but the decreased $\delta^{18}\text{O-NO}_3$ values argue against this theory. Nitrate in rain usually shows $\delta^{15}\text{N}$ values between -15 and 5 ‰, and $\delta^{18}\text{O}$ values > 30 ‰ (Freyer, 1991; Durka *et al.*, 1994; Spoelstra *et al.*, 2001; Deutsch *et al.* in prep.), and a mixture should lead to higher $\delta^{18}\text{O-NO}_3$ values in drainage water than we measured.

The second possible reason is a change in the nitrate isotope values because of an increased nitrification rate. Nitrate, which is generated during nitrification shows lower $\delta^{15}\text{N}$ values, than the residual ammonium due to fractionation (Kendall, 1998). The extent of this fractionation strongly depends on the nitrification rate with larger fractionation at higher rates. The $\delta^{18}\text{O}$ values of the generated nitrate usually vary between -2 and 15 ‰ (Amberger & Schmidt, 1987; Mayer *et al.*, 2001). Up to two oxygen atoms incorporated into nitrate derive from the soil water ($\delta^{18}\text{O} = -25$ to 4 ‰; Kendall, 1998) and at least one oxygen comes from atmospheric O_2 (Hollocher *et al.*, 1981; Andersson & Hooper, 1983), which shows $\delta^{18}\text{O}$ values around 23.5 ‰ (Kroopnick & Craig, 1972). The proportion of oxygen from soil water or atmospheric O_2 incorporated into nitrate depends on the nitrification rate, and at low rates up to two oxygen atoms can derive from atmospheric oxygen (Mayer *et al.*, 2001). Therefore, an increase in the nitrification rate can lead to a higher amount of incorporated oxygen from the soil water and consequently to a decrease in the $\delta^{18}\text{O}$ values of the generated nitrate.

Furthermore, the $\delta^{18}\text{O}$ value of the soil water can be modified by a larger portion of rain water which in northern Germany during winter shows amount-weighted mean $\delta^{18}\text{O}$ values between -11 to -8 ‰ (IAEA, 2001). Additionally, changes in the $\delta^{18}\text{O-O}_2$ values in soils due to

bacterial respiration can not be excluded. Unfortunately, no $\delta^{18}\text{O}$ values of the soil water were measured, which would have helped to explain this pattern. The parallel curves of the nitrate concentrations in the tile drain outlet, the ditch, and the brook suggest that drainage water is the dominant N-source for the adjacent surface waters. The time lag between highest nitrate concentration in the tile and the maximum concentrations in the ditch and the brook was 5 days.

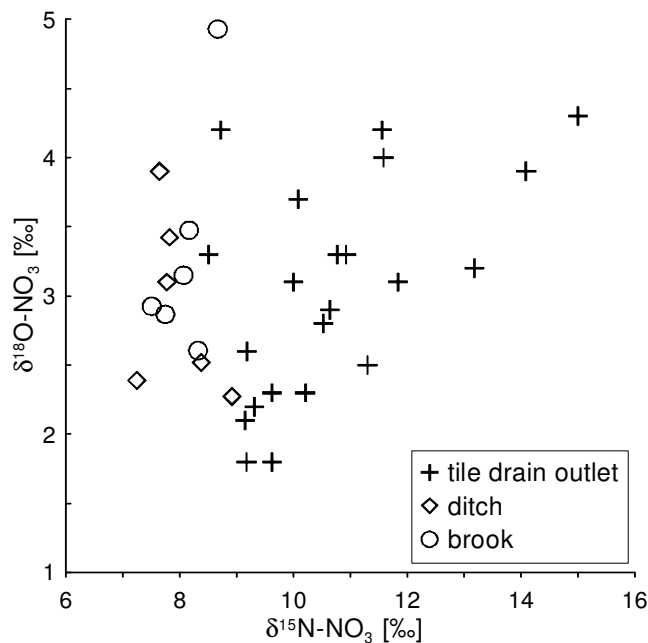


Figure.VI.5: $\delta^{15}\text{N-NO}_3$ plotted against $\delta^{18}\text{O-NO}_3$ for the tile drain outlet (crosses), the ditch (diamonds) and the brook (circles). Samples from the last four sampling dates from the ditch and the brook were excluded.

A comparison of the $\delta^{15}\text{N-NO}_3$ values from the tile drain outlet, ditch, and brook shows that the values were significantly higher in the tile drain outlet (ANOVA, $p < 0.001$; Fig. VI.5). For this comparison the last four values from the ditch and the brook were excluded, which will be further discussed below. The lower $\delta^{15}\text{N-NO}_3$ values and nitrate concentrations in combination with similar $\delta^{18}\text{O-NO}_3$ values in

the ditch and the brook indicate a second N-source besides drainage water nitrate. Assimilation of nitrate or denitrification can - in our opinion - be excluded because these processes usually are associated to increasing $\delta^{15}\text{N}$ and $\delta^{18}\text{O-NO}_3$ values (Mariotti *et al.*, 1988; Böttcher *et al.*, 1990; Kendall, 1998). Rainwater nitrate can be excluded as second source, because the $\delta^{18}\text{O}$ values of all samples were nearly equal. A mixture with nitrate from natural soils or forests might be possible, since the lower nitrate concentration does not point to a nitrate input from agricultural soils, treated only with mineral fertilizers. Amberger &

Schmidt (1987) reported a $\delta^{15}\text{N-NO}_3$ value in groundwater beneath a forest of -1.2‰ , and Mayer *et al.* (2002) measured values between 3.7 and 5.4‰ in 4 watersheds in the northeastern U.S., which are dominated by forested areas. Deutsch *et al.* (in prep.) found in groundwater close to the sampling area a concentration weighted mean $\delta^{15}\text{N-NO}_3$ value of 0.6‰ , and $\delta^{18}\text{O-NO}_3$ of 1.4‰ , which indicates that groundwater nitrate might be a possible mixing source. Assuming a conservative mixing, a linear mixing model can be applied with the concentration weighted mean $\delta^{15}\text{N-NO}_3$ values of the drainage water (10.4‰), ditch (8.1‰), and the brook (8.1‰). The estimated contributions of drainage water nitrate to the nitrate in the ditch and brook were 76% .

As mentioned above, the last four samples from the ditch and the brook were excluded from the comparison, because the simultaneous increase of the $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values in indicate a fractionation process. Denitrification as well as N-uptake by phytoplankton lead to fractionation in the $\delta^{15}\text{N}$ as well as in the $\delta^{18}\text{O}$ values. An increase in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values with a ratio of 2:1 during denitrification in groundwater was found by Böttcher *et al.* (1990). The ratio in the adjacent ditch is 1.8:1 ($\epsilon(^{15}\text{N}) = 3.7\text{‰}$; $\epsilon(^{18}\text{O}) = 2.1\text{‰}$), which shows that denitrification may be the reason, but N-uptake during primary production cannot be excluded (Kendall, 1998). The increase in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the nitrate in the brook is 0.3:1 ($\epsilon(^{15}\text{N}) = 2.0\text{‰}$; $\epsilon(^{18}\text{O}) = 6.0\text{‰}$), which does not point to denitrification, but maybe to N-uptake by primary producers.

The results of this study well confirmed the results from other studies, which showed that the main portion of nitrate which leaches from agricultural soils within hydrological winter derives from nitrification. The strong positive correlation between nitrate concentration and drain discharge in the tile drain outlet documented the dependency between weather conditions and amount of N lost via leaching. The high influence of drainage water as N source for the adjacent surface waters was shown by means of the $\delta^{15}\text{N}$ values and the

synchronous course of the nitrate concentrations. The high temporal resolution of drainage water sampling allowed to record short-term variations in the concentration and isotope values of drainage water nitrate and infer the processes behind these changes.

VII. QUANTIFICATION OF DIFFUSE NITROGEN INPUTS INTO A SMALL RIVER SYSTEM USING STABLE ISOTOPES OF OXYGEN AND NITROGEN IN NITRATE

Introduction

Although inputs of nutrients have been reduced in the recent years, the excessive load of nitrogen and phosphorous is still one of the major ecological problems of the Baltic Sea. In 2000 an amount of $814 \cdot 10^3$ t nitrogen entered the Baltic Sea, and more than 84% of the total N load derived from rivers (HELCOM, 2003). Worldwide, almost 70% of the riverine N load consists of dissolved organic nitrogen (DON; Meybeck, 1982) but experiments showed that its bioavailability may be as low as 2-16% (Stepanauskas & Leonardson, 1999), while nitrate is rapidly consumed. Diffuse nitrate inputs such as fertilizer runoff from farmland, atmospheric deposition and groundwater input are especially hard to reduce, because they are emitted over large areas which makes their identification exceedingly difficult. Nowadays, a powerful tool to distinguish different nitrate sources is the determination of stable isotope ratios of nitrogen and oxygen (Wassenaar, 1995; Spoelstra *et al.*, 2001; Chang *et al.*, 2002; Mayer *et al.*, 2002). The $\delta^{15}\text{N}$ values of nitrate from different sources often show overlapping ranges, but the additional measurement of the $\delta^{18}\text{O}$ values allows a more precise classification. Nitrate derived from sewage and manure is isotopically distinct from atmospheric nitrate in $\delta^{15}\text{N}$ (+7 to +20 ‰; -10 to +8 ‰ respectively), as well as in $\delta^{18}\text{O}$ (<15 ‰ compared to +25 to +75 ‰; Wassenaar, 1995; Kendall, 1998). Nitrate originating from mineral fertilizers shows $\delta^{15}\text{N}$ values of 0 ± 4 ‰ (Kendall, 1998), and $\delta^{18}\text{O}$ values of 22 ± 3 ‰ (Amberger & Schmidt, 1987) because of their production from atmospheric nitrogen ($\delta^{15}\text{N} = 0$ ‰) and oxygen ($\delta^{18}\text{O} = +23.5$ ‰). However, the isotopic composition of nitrate collected in drainage tiles and runoff ditches does not reflect exactly the isotope values of the fertilizer

applied, but is altered due to isotope fractionation processes of the light and heavy isotopes (Kendall & Aravena, 1999). Flipse & Bonner (1985) demonstrated that groundwater nitrate under fertilized fields showed $\delta^{15}\text{N}$ values up to 12.4 ‰ higher than the fertilizers applied and explained this difference as the volatile loss of ^{15}N -depleted ammonia from the fertilizer, containing reduced nitrogen forms. Another fractionation process is denitrification, which cause increases in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the residual nitrate with an enrichment of $\delta^{18}\text{O} : \delta^{15}\text{N}$ close to 1:2 (Böttcher *et al.*, 1990). Nitrate uptake by plants (Högberg, 1997), and soil N-mineralization, including ammonification and nitrification (Iqbal *et al.*, 1997; Mayer *et al.*, 2001) may also modify the isotope signature of nitrate.

The objective of this study was to test whether the isotopic composition of nitrate from three diffuse nitrate sources can be used to quantify the diffuse nitrate inputs into a sub basin of the river Warnow (444 km²). The river Warnow is the second largest river in Mecklenburg-Vorpommern (Germany) which also supplies the city of Rostock (200 000 inhabitants) with drinking water. The three nitrate sources drainage water from fertilized fields, groundwater, and atmospheric deposition, as well as the river itself were sampled regularly from November 2002 to April 2003. The nitrate of the samples was analysed for concentration, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values, and data were tested in a three source mixing-model (Phillips & Koch, 2002), to determine the percentage of every sampled nitrate source to the river nitrate. For the successful appliance of a conservative isotope mixing-model, it is necessary that the isotope values of the mixture are not altered due to fractionation processes. For this purpose, sampling was carried out during late fall and winter. At low water temperature microbial activity is reduced (Pfenning & McMahon, 1996) and therefore alteration of the river nitrate isotope values due to fractionation processes is minimal. An assessment was given whether the stable isotope approach used in this study can also be used as an additional method for nitrate source quantification.

Material and Methods

From November 2002 to April 2003 two tile drain outlets, located on the area of a farmers' co-operative near Rostock, were sampled monthly to bimonthly (Figure VII.1). The drainage

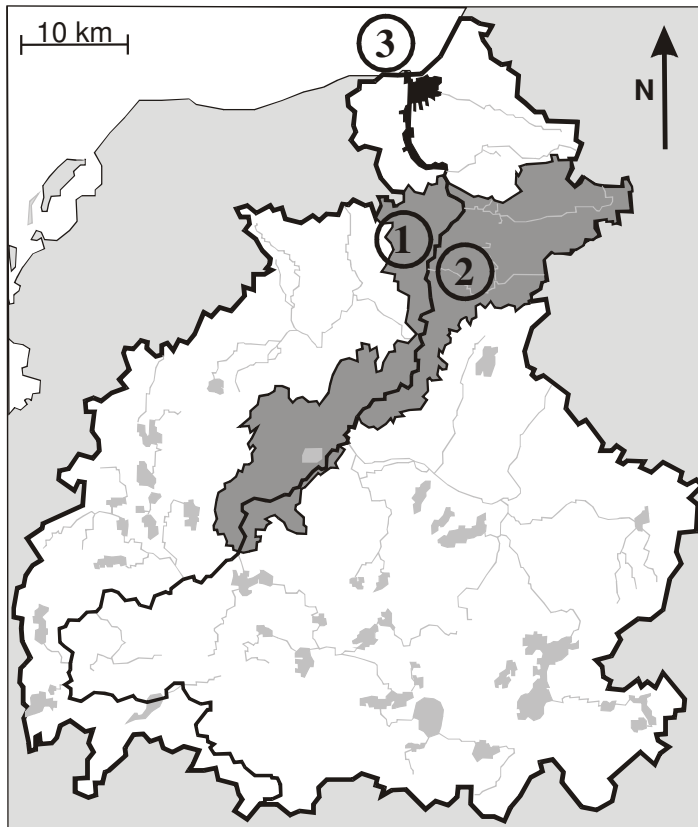


Figure. VII.1: Site map of the Warnow river system. The dark gray area shows the sub basin 'Middle Warnow'. Numbers in boxes point the sampling sites (1: tile drain outlets, ditches, and river sampling; 2: groundwater sampling; 3: rain sampling).

system is located at a soil depth of 60 to 100 cm. The soil textures are mainly sandy loams (46%) and sands (24%) from moraine substrates, the dominant soil types are cambisols, luvisols and gleysols. The drained areas investigated are approximately 2.5 ha (outlet 1) and 15 ha (outlet 2), respectively. Outlet 2 was also supplied with surface water of two small ponds located in the field. For a detailed map of the tile drain outlets see Figure V.1. Cultivated crops were winter wheat/winter

barley in 2002 and sugar beets/corn in 2003 (outlet 1) and for tile drain outlet 2 winter wheat in 2002 and winter barley in 2003. Fertilizer application and the composition of fertilizer used are shown in Table VII.1. Application of manure on both areas takes place every fifth to sixth year, and the last application on the soil above outlet 2 was in autumn 1998 with a similar amount as applied above tile 1 in 2002. The distance between the tile drains and the river Warnow is approximately 2 km.

Table VII.1: Application date, amount, and composition of the fertilizers used on the soils above the sampled drainage tiles.

drainage tile 1			
date	amount applied [kgN/ha]	fertilizer	nitrogen compounds
03-25-2002	85	AHL	28% N (7% NH ₄ ; 7% NO ₃ , 14% amide)
04-23-2002	70	urea	46% N (46% amide)
05-29-2002	79	KAS	27% N (13.5 % NH ₄ ; 13.5 % NO ₃)
August 2002	232	manure	
drainage tile 2			
date	amount applied [kgN/ha]	fertilizer	nitrogen compounds
03-27-2002	66	AHL	28% N (7% NH ₄ ; 7% NO ₃ , 14% amide)
04-22-2002	61	NTS	27% N (6% NH ₄ ; 8% NO ₃ ; 13% amide)
05-28-2002	148	NTS	27% N (6% NH ₄ ; 8% NO ₃ ; 13% amide)
03-18-2003	79	AHL	28% N (7% NH ₄ ; 7% NO ₃ , 14% amide)
04-28-2003	90	urea	46% N (46% amide)

Table VII.2: Water temperatures of the Warnow river measured close to the sampling location.

Date	Temperature [°C]
10-28-02	8.6
11-12-02	5.0
11-27-02	5.7
12-10-02	0.1
01-09-02	1.3
01-22-03	3.7
02-04-03	1.5
02-19-03	1.8
03-05-03	3.5
03-18-03	6.5

To investigate whether the application of mineral fertilizers affects the isotope values of the drainage water nitrate, the isotopic composition of the fertilizers used (Table VII.1) was additionally determined.

The two adjacent ditches (ditch 1 and 2), connecting the field runoff with the river Warnow were sampled additionally to examine the possible alteration of the nitrate isotope values due to isotope fractionation processes during the passage of the drainage water to the river.

River samples were taken from January to April 2003 near Rostock at the same time as those from the drainage tiles. Sampling took place from a landing stage at a maximum depth of 0.3

m. Water temperature measured close to the sampling location decreased from 8.6 °C (October 2002) to 0.1 °C in December 2002 and then increased to 6.5 °C in March 2003 (Table. VII.2; unpublished data, Office for the Environment and Nature, Rostock).

Groundwater samples were taken at a sampling site near the village of Reez close to a wood and arable land from November 2002 to April 2003 (Figure VII.1). The well had a depth of 8.4 m and sampling was carried out with a submerged pump at a maximum discharge flow of 5 L/min. The aquifer had a thickness of approx. 5 m, and there is a direct discharge into the river.

Atmospheric deposition was collected from October 2002 to April 2003 at the German Meteorological Service in Rostock-Warnemünde. Samplers were funnel shaped (diameter 24 cm) with a vial below. After every rainfall they were immediately emptied and subsampled for nutrient analysis. Because of low nitrate concentrations, samples of consecutive rainfall events were combined for isotope analysis.

Samples were prepared for isotope analysis after the method of Silva *et al.* (2000).

To estimate the contribution of the nitrate sources to the Warnow river a mixing model based on mass balance equations was used (Phillips & Koch, 2002). The equations are:

$$\delta^{15}\text{N}_W = f_D\delta^{15}\text{N}_D + f_G\delta^{15}\text{N}_G + f_R\delta^{15}\text{N}_R \quad (1)$$

$$\delta^{18}\text{O}_W = f_D\delta^{18}\text{O}_D + f_G\delta^{18}\text{O}_G + f_R\delta^{18}\text{O}_R \quad (2)$$

$$1 = f_D + f_G + f_R \quad (3)$$

The subscripts D, G and R represent the three sampled sources: drainage tile (D), groundwater (G), rain (R), and W represents the Warnow river. f is defined as the fraction of the respective source. The isotope values used were the concentration weighted mean values.

Results

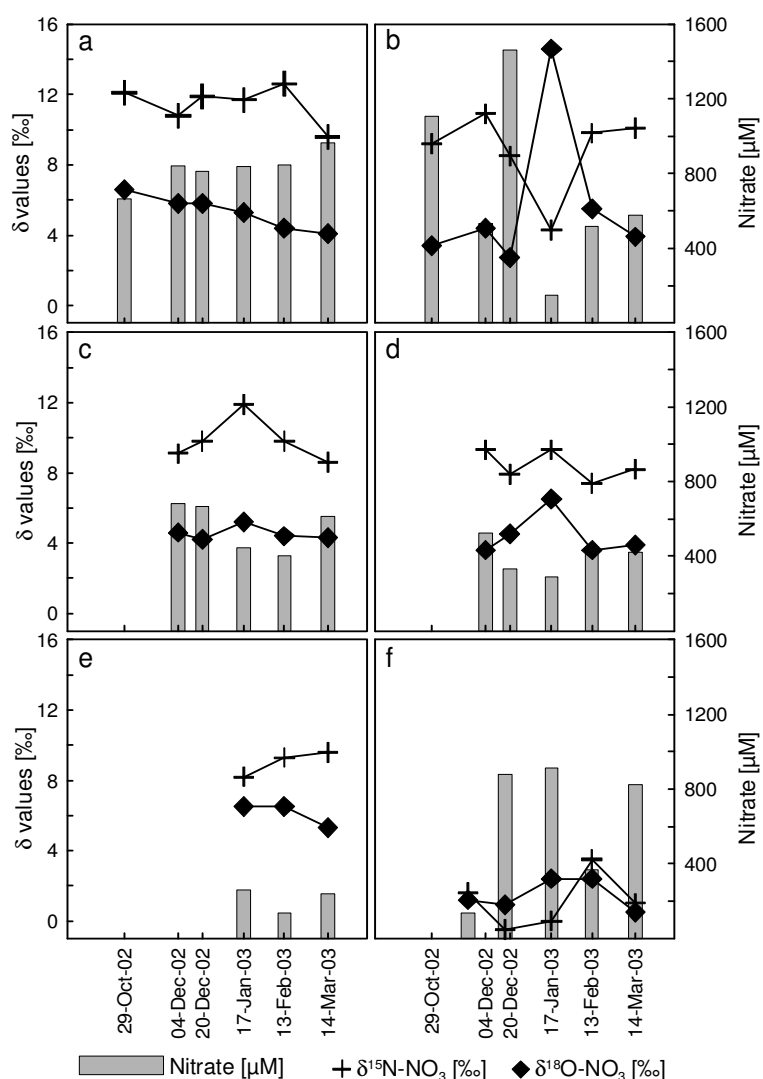


Figure VII.2: Nitrate concentration, $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values of the drainage tiles (a, b), runoff ditches (c, d), river (e) and groundwater samples (f) during the sampling period.

The highest nitrate concentrations were found in the two tile drain outlets and in the groundwater, with a maximum value of 1462 μM in outlet 2 at the end of December 2002 (Figure VII.2a, b, f). Outlet 2 and the groundwater showed high variability in nitrate concentrations with no trend. In tile drain 1 nitrate slightly increased from 663 to 966 μM during the sampling period (Figure VII.2a). The nitrate drinking water limit of 806 μM (50 mg NO_3/l) was exceeded in 59% of the drainage and groundwater samples. Mean concentrations of outlets 1, 2, and the groundwater were 829, 724, and 625 μM , respectively. The ditches had mean concentrations of 562 (ditch 1) and 404 μM (ditch 2; Figure VII.2c, d). Rain samples showed the lowest concentrations with 17-139 μM (mean 63 μM ; Figure VII.3), and there was no correlation between nitrate concentration and amount of precipitation. The river Warnow had concentrations between 135 and 259 μM (Figure VII.2e).

The concentration-weighted mean $\delta^{15}\text{N-NO}_3$ values of the tile drain outlets 1 and 2 were 11.4 and 9.2 ‰, and mean $\delta^{18}\text{O-NO}_3$ values were 5.3 and 4.0 ‰. As shown in Figures VII.2a and b, the two outlets differed in their isotopic ratios. While the $\delta^{15}\text{N-NO}_3$ values in outlet 1 were rather constant (9.6 to 12.1 ‰) and the $\delta^{18}\text{O-NO}_3$ values slightly decreased from 6.6 to 4.1 ‰, outlet 2 showed high variability in the $\delta^{18}\text{O-NO}_3$ values in January 2003, and decreasing $\delta^{15}\text{N}$ values. The increase in the $\delta^{18}\text{O}$ values was also visible in ditch 2 (Figure VII.2d), and co-occurred with increasing $\delta^{15}\text{N-NO}_3$ values. Nitrate in ditch 1 showed an increase in $\delta^{15}\text{N}$ but no change in the $\delta^{18}\text{O}$ values (Figure VII.2c). The concentration-weighted mean $\delta^{15}\text{N-NO}_3$ values of ditch 1 and 2 were 9.7 and 8.4 ‰, the mean $\delta^{18}\text{O-NO}_3$ were 4.5 and 4.2 ‰, respectively.

The mineral fertilizers had $\delta^{15}\text{N}$ values between -3.8 and 4.4 ‰ and $\delta^{18}\text{O-NO}_3$ values of 19.4 - 25.7 ‰ (Table VII.3).

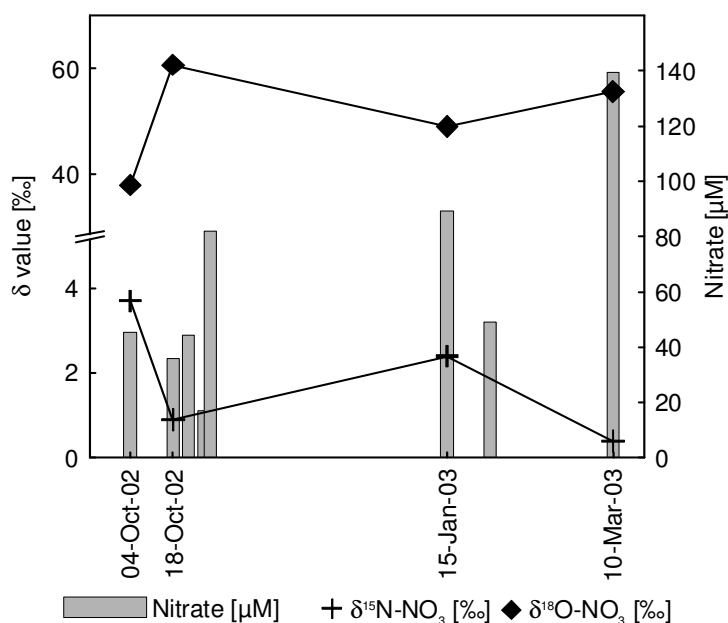


Figure VII.3: Nitrate concentration, $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values of the rain samples. Notice the break of the y-axis.

Table VII.3: Isotopic composition of the total nitrogen as well as of the nitrate compounds of the mineral fertilizers applied on the catchment area of the drainage tiles in 2002 and 2003.

fertilizer	$\delta^{15}\text{N}_{\text{total}}$ [‰]	$\delta^{15}\text{N-NO}_3$ [‰]	$\delta^{18}\text{O-NO}_3$ [‰]
AHL	-3.8	0.8	25.7
NTS	0.1	4.4	19.4
KAS (+CaO)	-1.7		20.5 ¹
Urea	0.7		

The isotopic composition of the river nitrate was close to that of the two tile drain outlets and the ditches, with a concentration weighted mean $\delta^{15}\text{N}$ of 9.0 ‰, and $\delta^{18}\text{O}$ of 6.0 ‰ (Figure VII.2e). A single-factor analysis of variance (ANOVA) of these five groups of samples indicated no significant difference for both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values. Groundwater nitrate had concentration-weighted mean $\delta^{15}\text{N}$ values of 0.6 ‰ and $\delta^{18}\text{O}$ of 1.4 ‰ (Figure VII.2f). Nitrate in rain samples showed $\delta^{15}\text{N}$ values between 0.4 and 3.7 ‰ (cwm. 0.1 ‰) and $\delta^{18}\text{O}$ values of 49.1 to 60.7 ‰ (cwm. 51.7 ‰; Figure VII.3). A single-factor ANOVA confirmed that 3 groups can be significantly distinguished from each other by means of their $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in nitrate ($p < 0.05$; Figure VII.4).

The mixing model indicated that the nitrate in the sub basin 'Middle Warnow' mainly consists of drainage water (86%) and 11% originates from groundwater (Table VII.4), although both sources showed similar mean nitrate concentration. The atmospheric deposition had a share of 3% in the river nitrate.

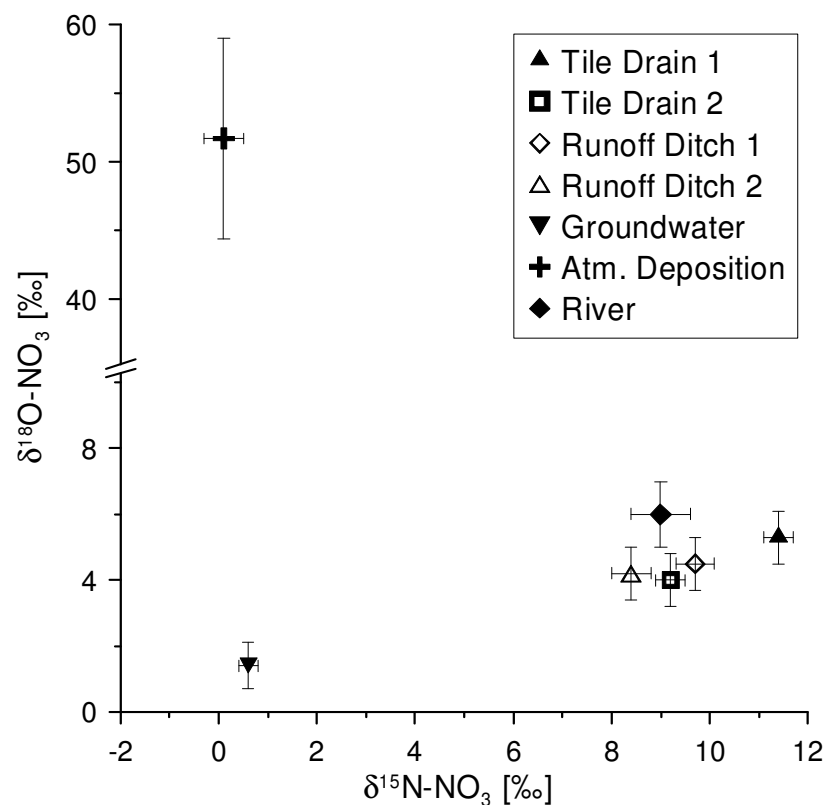


Figure VII.4: cwm $\delta^{18}\text{O-NO}_3$ vs. cwm $\delta^{15}\text{N-NO}_3$ for all samples, notice the break of the y-axis.

Table VII.4: Concentration weighted mean values of $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ used in the mixing model and the resulting fractions of each source with standard error.

source	$\delta^{15}\text{N-NO}_3$ [‰]	$\delta^{18}\text{O-NO}_3$ [‰]	fraction [f]
drainage water	10.4	4.7	0.86 ± 0.07
groundwater	0.6	1.4	0.11 ± 0.07
rain	0.1	51.7	0.03 ± 0.02
mixture			
river	9.0	6.0	

Discussion

The tile drain outlets showed the highest nitrate concentrations of all sampling sites. This was expected because of the long-term application of mineral and organic fertilizer. The $\delta^{15}\text{N-NO}_3$ values in the drainage water are in the range reported for nitrate in agricultural soils with both application of mineral fertilizers as well as manure (Amberger & Schmidt, 1987; Iqbal *et al.*, 1997). The mineral fertilizers used in our area showed typical $\delta^{15}\text{N}$ values between -3.8 and 0.7 ‰ for total N, and the nitrate compound of the AHL and NTS fertilizers had 0.8 and 4.4 ‰, respectively. Application of manure and mineral fertilizers, which contain urea and/or ammonium compounds leads to volatile loss of ^{15}N -depleted ammonia, and consequently to ^{15}N -enriched nitrate generated by nitrification (Heaton, 1986; Kendall, 1998). The $\delta^{18}\text{O-NO}_3$ values for both outlets were in a range given for nitrate generated during the nitrification process, which shows $\delta^{18}\text{O}$ values between 2 to 14 ‰ (Kendall, 1998; Mayer *et al.*, 2001). Values from 5.7 to 10.5 ‰ have been recorded in groundwater beneath agricultural land only treated with mineral fertilizers (Amberger & Schmidt, 1987). Mineral fertilizers are made of atmospheric O_2 , which shows $\delta^{18}\text{O}$ values of 23.5 ‰ (Kroopnick & Craig, 1972), and the mineral fertilizers applied on our fields showed $\delta^{18}\text{O-NO}_3$ values between 19.4 and 25.7 ‰.

The decrease in $\delta^{18}\text{O-NO}_3$ in outlet 1 and the simultaneous increase in the nitrate concentration might be the result of an increase in nitrification rate during the sampling period, because nitrate that leaches from beneath agricultural fields during fall and winter mainly derives from the mineralization of soil organic matter and the following nitrification process (Lammell, 1990). The low $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values indicate that no or only reduced denitrification occurred in the soils. Böttcher *et al.* (1990) found $\delta^{15}\text{N-NO}_3$ values up to 78 ‰, and $\delta^{18}\text{O-NO}_3$ values up to 44 ‰ in groundwater recharged under arable land with increasing sampling depth. He suggested denitrification as the responsible process. In our study only nitrate of outlet 2 showed a simultaneous increase in the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values (ratio of 1: 1.7) between the first and second sampling date, associated to a strong decrease in nitrate concentration. This might be an indicator for denitrification in the soil.

Because of the similar soil textures and fertilizing practice, the greater variability in nitrate isotopic composition and in nitrate concentrations of outlet 2 compared to outlet 1 can only be explained by differences in the drained area. While outlet 1 reflected the drained agricultural area, outlet 2 was further supplied with surface water from two small ponds. Dilution and mixing during heavy rainfall or snowmelt events could have altered the concentrations and the isotopic composition of the nitrate. Rainwater nitrate, as well as nitrate in snow is characterized by high $\delta^{18}\text{O}$ values > 30 ‰ (Durka *et al.*, 1994; Campbell *et al.*, 2002). This is probably the reason for $\delta^{18}\text{O-NO}_3$ peak of outlet 2 on January 17, 2003. The meteorological data reported a snowmelt event a few days before sampling, so the decrease in the nitrate concentration and the increase in the $\delta^{18}\text{O-NO}_3$ might be the result of a mixture of nitrate-poor snowmelt water with low $\delta^{15}\text{N-NO}_3$ and high $\delta^{18}\text{O-NO}_3$ values with the soil water.

The adjacent ditches, which connect the tile drain outlets with the river, showed similar isotope values in nitrate than the outlets, which suggests that the nitrate discharged from the drainage tiles enters the river without isotopic fractionation. The lower nitrate concentrations of the ditches compared to the tile drain outlets can be the result of dilution with nitrate free

water. Processes like denitrification or assimilation, which also cause decreasing nitrate concentrations and can occur without fractionation of the isotope values (Mariotti *et al.*, 1988; Högberg, 1997) cannot be excluded. However, these processes seem unlikely because of the low water temperatures (Table VII.2). Pfenning & McMahon (1996) reported a 77% decrease in the denitrification rate during their laboratory experiments when the incubation temperature was lowered from 22 to 4° C.

Nitrate in the groundwater seems to be generated during the nitrification of mineralized soil organic N (Amberger & Schmidt, 1987; Kendall, 1998), which is indicated by the low $\delta^{18}\text{O-NO}_3$ values (0.5 to 2.4 ‰). However, the very low $\delta^{15}\text{N-NO}_3$ values (-0.5 and 3.5 ‰) and the partially high nitrate concentrations (>50 % of all groundwater samples exceeded the nitrate drinking water limit) could also be a result of a long-term input of mineral fertilizer nitrogen (Freyer & Aly, 1974).

The rain samples had the lowest nitrate concentrations of all sampled sources (17 to 139 μM) and are in the range reported for other regions in Germany with similar moderate industrial pollution (Beilke & Uhse, 2002). Also, their isotope values ($\delta^{18}\text{O} = 39.0$ to 60.7 ; $\delta^{15}\text{N} = 0.4$ to 3.7) are typical for nitrate from atmospheric deposition (Durka *et al.*, 1994; Campbell *et al.*, 2002). $\delta^{15}\text{N-NO}_3$ values between -4.3 to 2.4 ‰ were found in rain samples collected in Jülich (Germany), and a seasonal trend with higher values during fall and winter was observed (Freyer, 1991).

The elevated $\delta^{15}\text{N}$ values of the river nitrate well reflect the agricultural land use of the sub basin. A positive correlation between percentage of land used for agriculture and urban purposes and mean $\delta^{15}\text{N}$ values of nitrate for 16 river-systems in the northeastern U.S is reported from Mayer *et al.* (2002). They determined a mean $\delta^{15}\text{N}$ value of 8.4 ‰ for the Schuylkill river with 38.4 % of land used for agriculture.

Although fractionation processes like denitrification and assimilation in the river cannot absolutely be excluded during the sampling period, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in the river

nitrate do not indicate such a process. Whereas the increase in the $\delta^{15}\text{N-NO}_3$ value might be an indicator for assimilation or denitrification, the simultaneous decrease in the $\delta^{18}\text{O-NO}_3$ values argue against fractionation. We therefore assume that fractionation of the river nitrate did hardly occur during the sampling period.

Table VII.5: Comparison of the estimated proportion of each source on the Warnow river nitrate in the subbasin 'Middle Warnow', given from the modified MONERIS model and this study.

pathways	percentage of input	
	MONERIS	this study
drainage tiles	80	86 ± 7
groundwater	15	11 ± 7
atmospheric deposition	5	3 ± 2

The results from the mixing model and estimated inputs of nitrogen for the subbasin 'Middle Warnow' given from a modified version of the MONERIS model (Behrendt *et al.*, 1999) for the period 1995 – 1999 show good agreement (Table VII.5; Pagenkopf,

2001). The model estimates N inputs from point sources and seven diffuse pathways, which are drainage water, groundwater, atmospheric deposition, erosion, urban areas, and surface runoff. To allow a comparison of our results with the model data, the estimated inputs from drainage water, groundwater, and atmospheric deposition given by the modified MONERIS model were added together and set as 100%.

Our estimate only represents the fall and winter period, whereas the model calculates annual mean values. Several studies have shown that the main portion of nitrate leaches from agricultural soils during fall and winter, when most nitrate derives from mineralization of soil organic N (Kirchmann *et al.*, 2002). Combined with the high amount of water discharge due to low evapotranspiration and high rainfalls during this period, more nitrate is leached to the drainage system.

According to the Mesoscale Agricultural Mapping Programme (MMK) our investigated agricultural soils (water-saturated sandy loams and sands) account for 11% of the total agricultural area in the sub basin, which is dominated by water saturated loams (50%) and

peat soils (20%). It cannot be excluded that drainage water from loam and peat soils show elevated $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values because of a higher influence of denitrification, but results from a second study carried out on agricultural fields in the sub basin showed similar isotope values in drainage water nitrate (Deutsch et al., unpublished data).

Uncertainty mainly exists for the groundwater values, since only one aquifer was sampled. Changes in the land use above the aquifers as well as denitrification can alter the $\delta^{15}\text{N}$ as well as $\delta^{18}\text{O}$ values of the groundwater nitrate. Only a 2 ‰ enrichment in the $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values as reported by Böttcher *et al.* (1990), would increase the contribution of the groundwater nitrate from 11 to 15%.

Although based on few samples, the study shows that the stable isotope ratios of nitrogen and oxygen in nitrate can be used to determine the sources of nitrate inputs to a river. Given that the various sources differ substantially in their isotopic composition, the mixing model approach provides a trustworthy hint on the different source contributions. The choice of the winter season for sampling may have supported the quality of our results since low temperatures reduce microbial fractionation processes.

VIII. ANTHROPOGENIC NITROGEN INPUT TRACED BY MEANS OF $\delta^{15}\text{N}$ VALUES IN MACROALGAE: RESULTS FROM IN-SITU INCUBATION EXPERIMENTS

Introduction

Increased urbanization and excessive use of agricultural fertilizers over the last decades led to high nitrogen loads in many rivers and the resulting eutrophication in coastal areas became a serious, worldwide problem. High nitrogen loads into river-systems are often associated to elevated $\delta^{15}\text{N}$ values ($> 8 \text{ ‰}$) in dissolved inorganic nitrogen (DIN), particulate organic matter (POM) and macroalgae (Heaton, 1986). Nitrate and ammonium deriving from human and animal waste usually show $\delta^{15}\text{N}$ values $>10 \text{ ‰}$ (Wassenaar, 1995; Kendall, 1998) because of fractionation processes during transformations from one N-species to another. Rivers with low anthropogenic N loading usually show $\delta^{15}\text{N}$ values in DIN, POM and macroalgae $< 8 \text{ ‰}$ (McClelland & Valiela, 1998; Mayer *et al.*, 2002), which reflects nitrate and ammonium sources from atmospheric deposition or nitrate from nitrification in natural soils.

A common method to estimate and track these anthropogenic N inputs in rivers is direct measurement of $\delta^{15}\text{N}$ in nitrate and ammonium, which requires long-lasting preparation procedures. An alternative method is the measurement of $\delta^{15}\text{N}$ values in macroalgae' tissues grown in polluted rivers or estuaries (Costanzo *et al.*, 2000; Gartner *et al.*, 2002; Savage & Elmgren, 2004). Macroalgae only show small or no fractionation during uptake of nitrogen (Högberg, 1997), and thus directly reflect the $\delta^{15}\text{N}$ value of the riverine DIN.

In this study, $\delta^{15}\text{N}$ values in macroalgae from an unpolluted brackish site were compared to algae grown in a nitrogen rich estuary. With the additional determination of $\delta^{15}\text{N}\text{-NO}_3$ values, the hypothesis was tested if $\delta^{15}\text{N}$ values in macroalgae tissue directly reflect the $\delta^{15}\text{N}$ value of the river nitrate. Incubations were carried out in February/March and May, incubation times

were 14 and 10 days, respectively. Additional determinations of $\delta^{15}\text{N}$ values of naturally grown macroalgae in the estuary were assumed to provide further information on the natural ^{15}N -variation in the estuary.

Material and methods

Study site

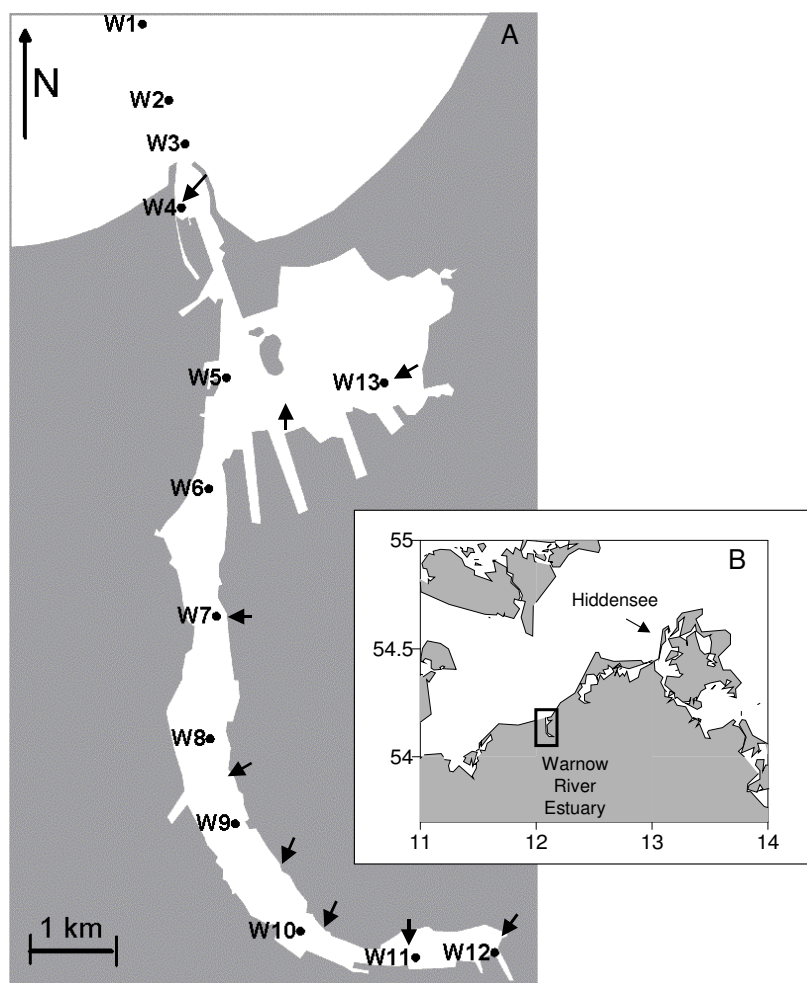


Figure VIII.1: Location of the Warnow River Estuary and the island of Hiddensee in the southern Baltic Sea (B). Figure A shows the map of the sampled stations (W1-W13). The arrows indicate the stations where macroalgae were sampled.

channel passes through the whole estuary (Fig. VIII.1A).

The Warnow river-estuary has a length of 15 km, flows through the city of Rostock and is separated from the river with a dam. Mixing of the river water with the Baltic Sea in the estuary strongly depends on wind strength and direction. Because of high N inputs into the Warnow river the inner part of the estuary shows nitrate concentrations $> 250 \mu\text{M NO}_3^-$. The port of Rostock is located in the north eastern part and a shipping

Collection of macroalgae

Macroalgae were collected near shore in 0.5-1m water depth at the west coast of the island of Hiddensee (Fig VIII.1B) in the southern Baltic Sea four days prior to incubation. These Macroalgae grew at salinities >7 PSU and nitrate concentrations between 0-4 μM . For the first incubation experiment, the brown macroalga *Fucus vesiculosus* and the red macroalga *Polysiphonia sp.* were collected, for the second incubation *Fucus vesiculosus* and the red macroalga *Ceramium rubrum*. Within several hours algae were transported to the Baltic Sea

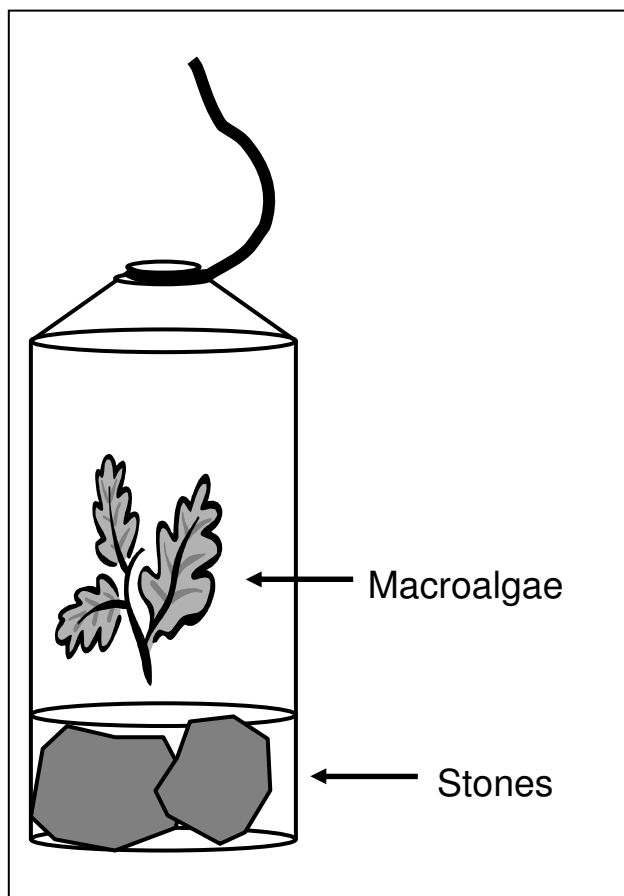


Figure VIII.2: Gauze bags used for incubation of macroalgae.

Research Institute and stored in the cold room at 10°C in transparent and ventilated buckets. Red algae were partly covered with epiphytes, which could not be removed.

Additional collection of macroalgae in the estuary

During an additional cruise carried out in July 2004, macroalgae were collected along the estuary. They were sampled at 9 stations (Fig. VIII.1A) by scraping them off from ships landing places or shipping signs. Collected species were *Ulva sp.*, *Enteromorpha sp.*, and the red alga *Ceramium rubrum*.

Incubation experiments

Prior to the incubation macroalgae were subsampled to determine the natural $\delta^{15}\text{N}$ value of the tissue. During the experiment macroalgae were incubated in 500 μm gauze bags which were weighted with stones (Fig. VIII.2). Both algae species were put together in one bag. Bags were fixed in replicates to 13 shipping signs along the estuary (Fig.1A) in 30 and 100 cm depth. The depth was chosen according to water turbidity. Twelve stations plus one station near the transshipment pear for mineral fertilizers in the port area (Station W13) were selected. The first experiment was carried out from Feb 26 to March 11, 2004 (14 days), second experiment in the period from May 4 to 14, 2004 (10 days). Water samples were taken at every shipping sign when experiments started and ended; samples were stored in preacidified 1L-PE bottles and prepared using the method of Sigman *et al.* (1997).

Results*First incubation experiment*

At the start of the first experiment (Feb. 26) salinity ranged from 9.9 to 0.3 PSU (Fig. VIII.3A), with lowest salinities in the inner part of the estuary. A regression ($r^2 = 0.95$, $p < 0.001$) indicated a linear mixture of seawater with river water. Nitrate also

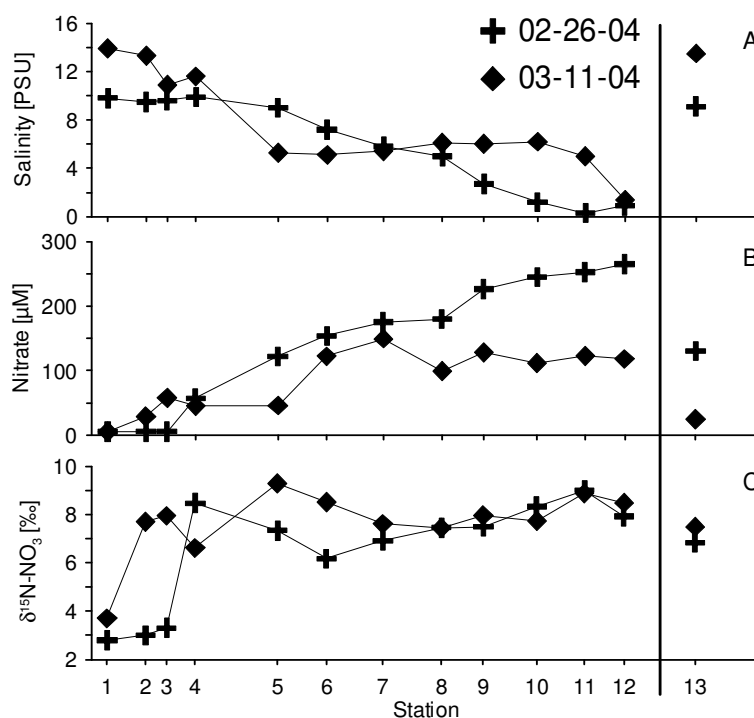


Figure VIII.3: Salinity (A), nitrate concentration (B), and $\delta^{15}\text{N-NO}_3$ values (C) measured at the start and end of the first experiment.

linearly increased from 5.3 μM at station W1 to 265.4 μM at station W12 ($r^2 = 0.96$, $p < 0.001$; Fig. VIII.3B). The $\delta^{15}\text{N-NO}_3$ values were lowest at the outer stations W1-W3 (2.8 – 3.3 ‰) and jumped up to values between 6.2 and 9.0 ‰ in the estuary (Fig. VIII.3C). Station W13

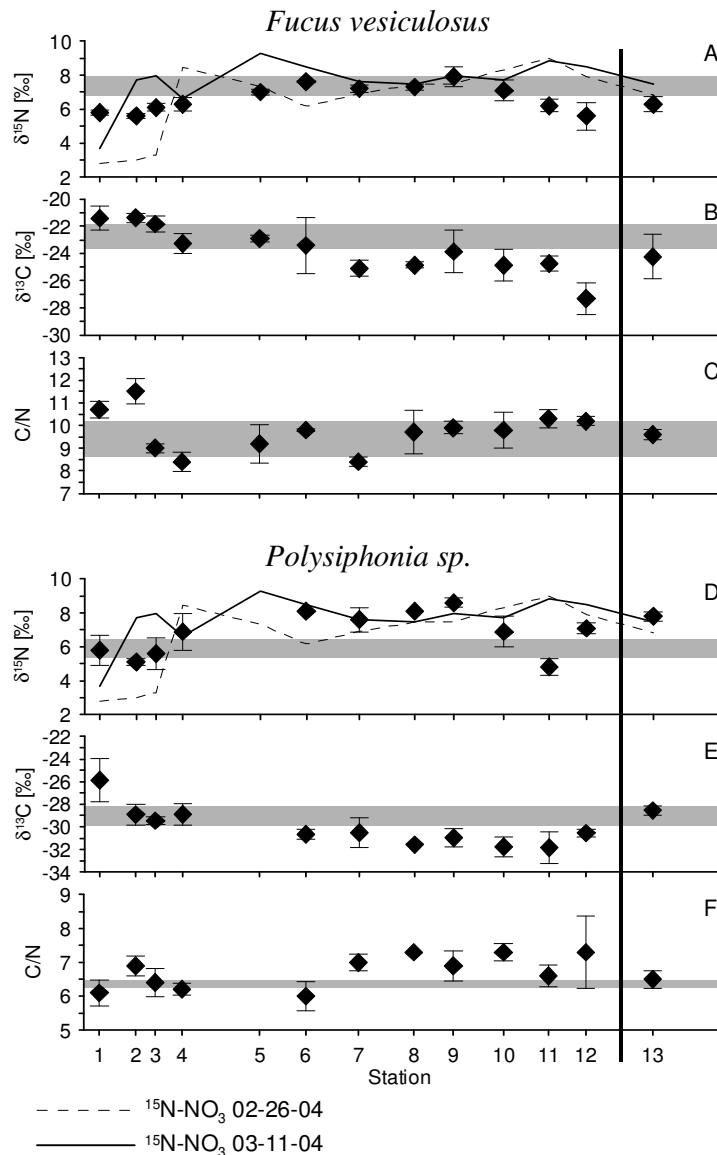


Figure VIII.4: $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C/N ratios of *Fucus vesiculosus* (A-C) and *Polysiphonia sp.* (D-F) with standard deviations after the first experiment. The grey areas show the range of the initial values. Dashed and solid lines in figures A and D show the $\delta^{15}\text{N-NO}_3$ values measured at the start and end of the first experiment.

μM (Fig. VIII.3B) with a linear regression of $r^2=0.67$ ($p < 0.01$). $\delta^{15}\text{N-NO}_3$ values were

which is located outside the main shipping channel had a salinity of 9.0 PSU, NO_3^- concentration of 130.2 μM and $\delta^{15}\text{N-NO}_3$ of 6.8 ‰. At the end of the first experiment (Mar. 11) salinity was higher at the stations W1 to W4 (13.9 - 10.9 PSU; Fig VIII.3A) compared to the start date. Salinity decreased and remained nearly constant between stations 5 to 8. Only station W12 showed a lower value of 1.4 PSU. Linear regression was again significant ($r^2 = 0,75$, $p < 0,001$). Nitrate concentrations were lower in the inner part of the estuary compared to the start of the experiment and ranged between 122.5 and 5.3

comparable with the start values and showed a range between 3.7 and 9.0 ‰ (Fig. VIII.3C). Station W13 had a salinity of 13.5 PSU and a nitrate concentration of 24.1 μM . $\delta^{15}\text{N}\text{-NO}_3$ value was 7.5 ‰.

Fucus vesiculosus initially had $\delta^{15}\text{N}$ values between 6.8 and 7.8 ‰ (mean 7.7 ± 0.43 ‰; $n=5$) that were close to the $\delta^{15}\text{N}\text{-NO}_3$ values in the estuary. After the experiment $\delta^{15}\text{N}$ values of *Fucus*' tissue ranged between 5.6 and 7.9 ‰ (Fig. VIII.4A). At stations W1 to W 4, W11, W12 and W13 $\delta^{15}\text{N}$ values were lower compared to the initial value. Initial $\delta^{13}\text{C}$ values were between -23.2 and -21.9 ‰ (mean -22.9 ± 0.70 ‰). At the end of the experiment $\delta^{13}\text{C}$ values showed a gradient with higher values outside of the estuary, and lower values in the inner parts. Values were higher than the initial values at stations W1 and W2, and below at stations W7 to W13. The starting C/N ratios of *Fucus vesiculosus* showed a wide range between 8.7 and 10.3 (mean 9.6 ± 0.56). End values ranged between 8.4 and 11.5 (Fig. VIII.4C).

Polysiphonia sp. had lower initial $\delta^{15}\text{N}$ values than *Fucus vesiculosus* of 5.2 to 6.1 ‰ (mean 6.0 ± 0.35 ‰; $n=5$). After the experiment values were higher at most stations (6.9 – 8.6 ‰, Fig VIII.4D), except W2 (5.1 ‰) and W11 (4.8 ‰). Initial $\delta^{13}\text{C}$ values of *Polysiphonia sp.* were lower than those of *Fucus vesiculosus*. They ranged between -29.9 and -28.0 ‰ (mean -28.6 ± 0.70 ‰). After 14 days of incubation value was higher at station W1 (-25.9 ‰) and lower at stations W6-W12 (-31.9 – 30.5 ‰, Fig. VIII.4E). C/N ratios showed scattered around the initial ratio (6.6 ± 0.28) and developed a trend towards higher ratios in the inner estuary (Fig. VIII.4F).

Second incubation experiment

During the second experiment (May 04) we again found a linear decrease in the salinity from 12.8 PSU at station W1 to 1.2 PSU at station W12 ($r^2 = 0.90$, $p < 0.001$; Fig. VIII.5A). Nitrate concentrations were lower compared to the first experiment, and highest concentrations were

measured at the innermost station W12 (25.2 μM , Fig. VIII.5B). Towards the Baltic Sea concentrations decreased and were below detection limit at stations W1 to W4 with only station 7 having slightly elevated concentrations. The $\delta^{15}\text{N-NO}_3$ was not measurable at stations W1 to W4, at all other stations values ranged between 6.7 and 9.7 ‰ (Fig.

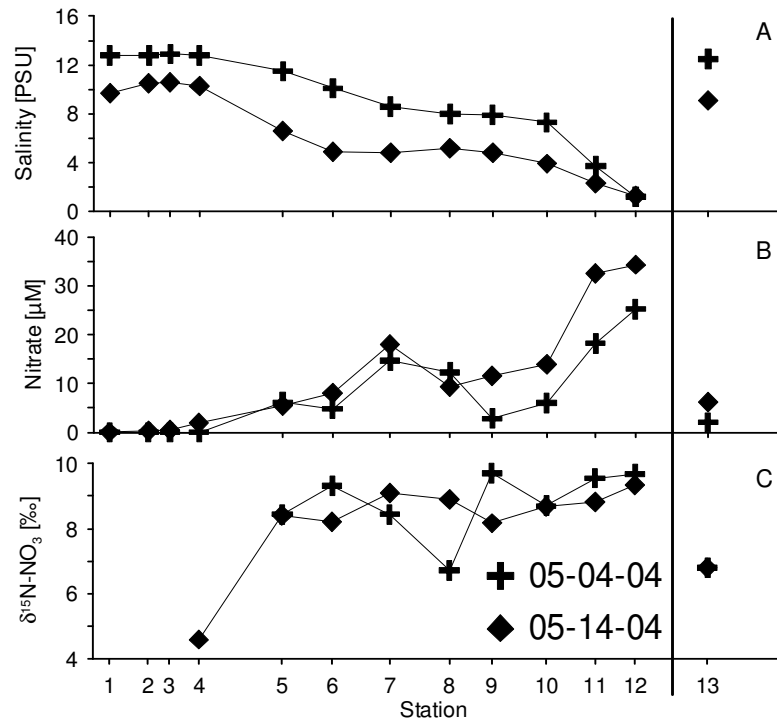


Figure VIII.5: Salinity (A), nitrate concentration (B), and $\delta^{15}\text{N-NO}_3$ values (C) measured at the start and end of the second experiment.

VIII.5C). Highest $\delta^{15}\text{N-NO}_3$ values were found at stations W9, W11, and W12, the lowest value at station W8. Station W13 again showed a higher salinity (12.5 PSU) than the stations along the shipping channel. NO_3^- was low with a $\delta^{15}\text{N-NO}_3$ value of 6.8 ‰. At the end of the experiment (May 14) salinities had dropped by 2-6 PSU compared to the start date (Fig. VIII.5A). They linearly decreased from 10.6 PSU (station W3) to 1.3 PSU at station W12 ($r^2 = 0.91$, $p < 0.001$). Nitrate concentrations were rather higher at station W12 (34.3 μM) and a second nitrate peak was found at station W7 (17.9 μM ; Fig. VIII.5B). At stations W5 to W12 $\delta^{15}\text{N-NO}_3$ values were in a range from 8.4 to 9.4 ‰ (Fig. VIII.5C). Only at station W4 close to the Baltic Sea the value was remarkable low (4.6 ‰). Station W13 only showed small changes.

Fucus vesiculosus had initial $\delta^{15}\text{N}$ values between 6.1 and 6.9 ‰ (mean 6.4 ± 0.32 ‰, $n=5$), around 1 ‰ lower than those of the first experiment. After the experiment, values had increased at stations W5 to W12 but stayed close to the $\delta^{15}\text{N}$ values of nitrate. The increase

compared to the mean initial value was $> 0.9 ‰$. The highest value could be found at station W6 (8.4 ‰, Fig. VIII.6A). At the stations in the Baltic results were lower compared to the starting values. At station W13 no change in $\delta^{15}\text{N}$ value of *F. vesiculosus* was observed.

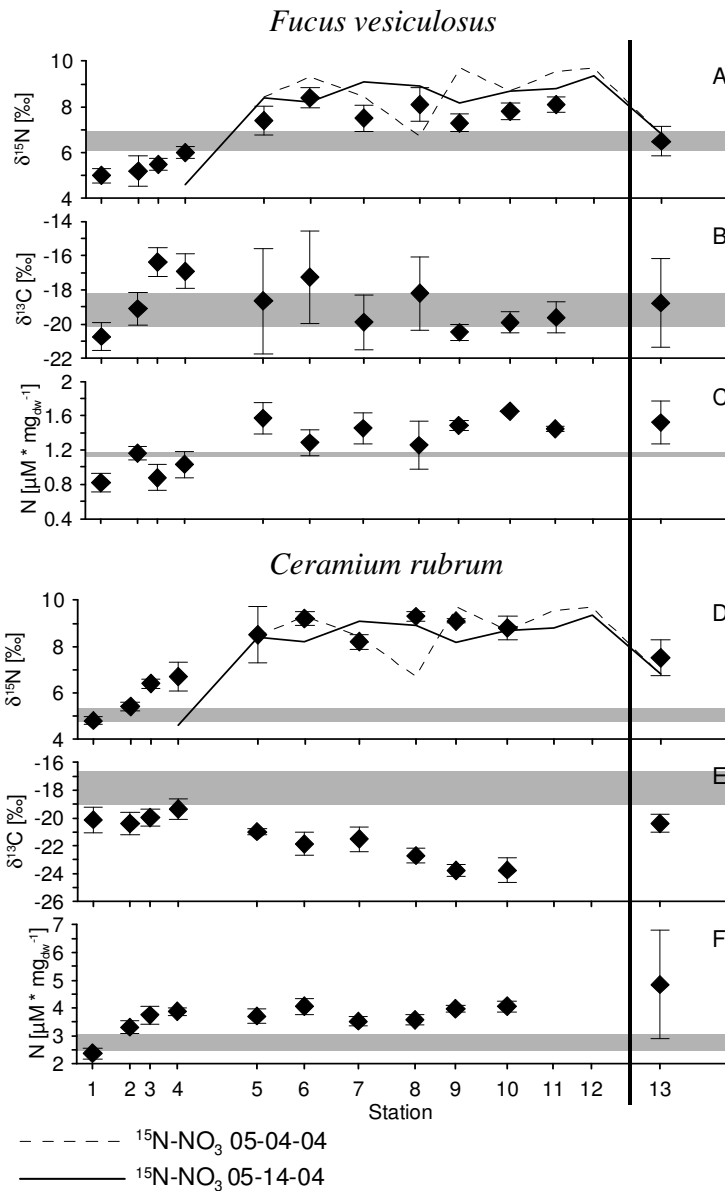


Fig. VIII.6: $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and N contents of *Fucus vesiculosus* (A-C) and *Polysiphonia sp.* (D-F) with standard deviations after the first experiment. The grey areas show the range of the initial values. Dashed and solid lines in figures A and D show the $\delta^{15}\text{N-NO}_3$ values measured at the start and end of the first experiment.

covered with sludge and macroalgae had begun to decompose.

Initial $\delta^{13}\text{C}$ was highly variable between -20.1 and -18.2 ‰ (mean $-18.8 \pm 0.67 ‰$, $n=5$). A gradient as found after the first experiment was not visible after the second experiment (Fig. VIII.6B). For the second experiment, N contents of the macroalgae were calculated from the weights and concentrations. *F. vesiculosus* initially had $1.25 \mu\text{M} \cdot \text{mg}_{\text{dw}}^{-1}$ ($n=2$) with some higher contents at stations W5 to W11 and W13 ($1.26 - 1.65 \mu\text{M} \cdot \text{mg}_{\text{dw}}^{-1}$; Fig. VIII.6C) and lower N contents at stations W1, W3, and W4. For the station W12 no data are available because the gauze bags were

Ceramium rubrum started with $\delta^{15}\text{N}$ values between 4.7 and 5.3 ‰ (mean 5.1 ± 0.22 ‰, $n = 5$). After the experiment $\delta^{15}\text{N}$ values were higher than the initial ones except W1 (Fig. III.6D). From stations W5 to W10 the $\delta^{15}\text{N}$ values were 3.2 to 4.2 ‰ higher than the initial mean value and close to the $\delta^{15}\text{N}$ values of the nitrate. At all stations $\delta^{13}\text{C}$ values of *C. rubrum* were lower compared to the starting values (-19.0 to -16.6 ‰; mean -17.5 ± 0.93 ‰, $n = 5$; Fig. VIII.6E). Values decreased from station W4 to station W10, where $\delta^{13}\text{C}$ was 6.3 ‰ below the initial mean value. Similar to the course of the $\delta^{15}\text{N}$ values the N contents of *C. rubrum* developed. A nitrogen content below the initial mean of $2.75 \mu\text{M}\cdot\text{mg}_{\text{dw}}^{-1}$ ($n=2$) was only observed at station W1. All other stations had higher N contents between $0.56 \mu\text{M}\cdot\text{mg}_{\text{dw}}^{-1}$ (W2) and 2.09

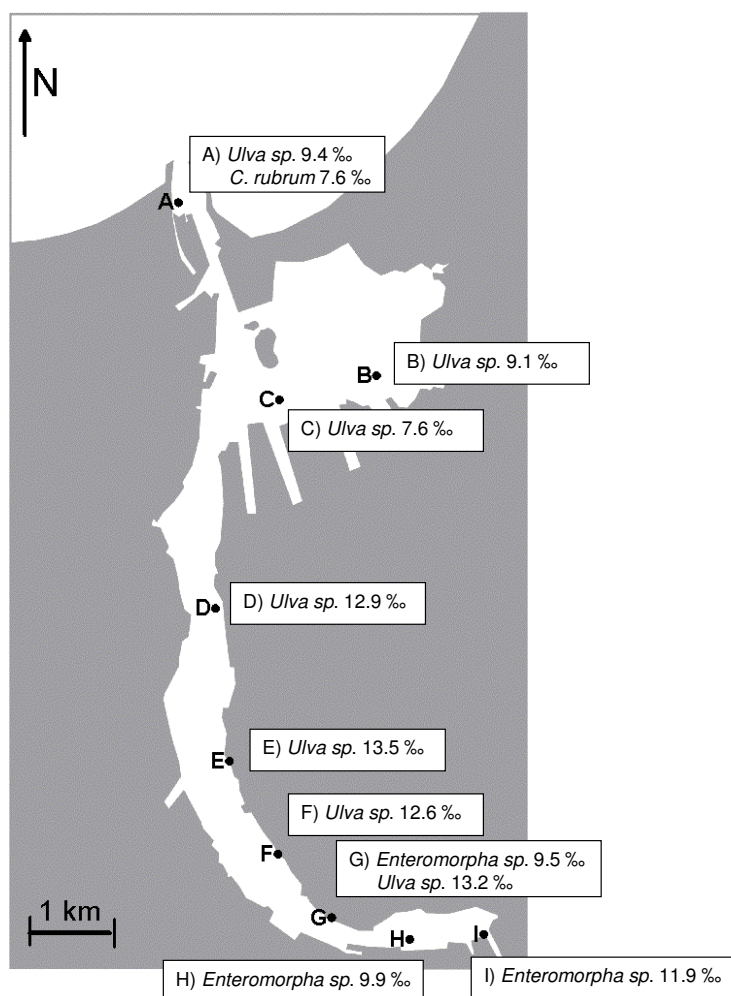


Figure VIII.7: $\delta^{15}\text{N}$ values of naturally occurring macroalgae in the Warnow River Estuary collected in July 2004

$\mu\text{M}\cdot\text{mg}_{\text{dw}}^{-1}$ (W13) above initial mean (Fig. VIII.6F). No measurements were performed from stations W11 and W12, because macroalgae had decomposed.

$\delta^{15}\text{N}$ values of naturally occurring macroalgae

The $\delta^{15}\text{N}$ values of the macroalgae collected along the estuary were 7.6 to 13.5 ‰ (Fig. VIII.7). *Enteromorpha sp.* was only found in the almost inner part of the estuary and showed a gradient in the $\delta^{15}\text{N}$

values between 11.9 ‰ at the inner station, and 9.5 ‰ at the station towards the Baltic Sea. *Ulva sp.*, which was found at every sampled station except stations H and I had $\delta^{15}\text{N}$ values between 13.5 ‰ (station E) and 7.6 ‰ at station C. *C. rubrum* only found at station A had a $\delta^{15}\text{N}$ value of 7.6 ‰.

Discussion

Estuarine Mixing

The salinities indicate linear mixing of Baltic Sea waters with the fresh water from the Warnow River during the sampling events. The innermost station W12 seemed to be unaffected by salinity fluctuations, while some stations in the middle part of the estuary showed changes in salinity of up to 6.2 PSU. The prevailing winds in the area are

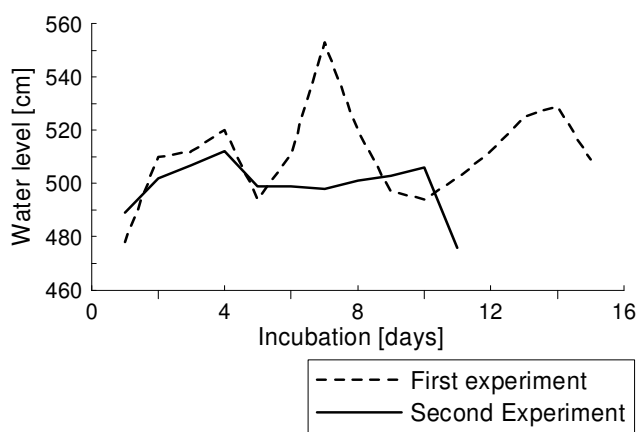


Figure VIII.8: Water level in the outer part of the estuary during the incubation experiments.

responsible for these patterns. Generally, strong northerly winds push seawater into the estuary, resulting in increased salinities inside, and winds from southerly directions push the river-water out of the estuary, which leads to reduced salinities. The water levels in the outer part of the estuary during the incubation experiments are shown in Figure VIII.8. During the first experiment, a strong increase in the water level was observed after 7 days of incubation, which indicates a strong inflow of water from the Baltic Sea, whereas the conditions seemed nearly constant during the second experiment. Despite this influence of wind conditions in the outer part of the estuary, there was a homogeneous mixture of river-water with seawater inside the estuary at every sampling date, which resulted in linear decreased salinities along

the estuary. This mixture was also visible in the nitrate concentrations measured at the start and end date of the first experiment. Nitrate concentrations generally followed the salinity changes and were higher in the inner part of the estuary, where we found $265 \mu\text{M NO}_3^-$ on Feb 26. The lower concentrations on March 11 are assumed to result from increased NO_3^- uptake due to primary production in March. The start of the spring bloom at this latitude in the Baltic is in March (Wasmund *et al.*, 1998). During the second experiment nitrate concentrations were even lower than during the first experiment, and at detection limit in the Baltic Sea water, reflecting the post bloom period. Moreover, nitrate did not decrease linearly but varied in concentration along the transect. A possible explanation for this nonlinear nitrate mixing could be an additional input of nitrate, which may be located near station W7, where the nitrate concentrations were higher than at the surrounding stations. Since two small streams draining urban areas discharge near this station they might be a source of nitrate.

When high nitrate concentrations with high $\delta^{15}\text{N}$ values mix with waters of lower concentration and low $\delta^{15}\text{N}$, a logarithmic decrease occurs (Fry, 2002). However, the $\delta^{15}\text{N-NO}_3$ values did not show this trend of linear mixing. Values remained at a nearly constant level throughout the whole estuary. The elevated values of up to 9.7 ‰ indicate a high anthropogenic influence presumably from sewage or manure that is described to have such $\delta^{15}\text{N}$ signatures (Kendall, 1998). Mayer *et al.* (2002) found in sixteen rivers in the northeastern U.S. a positive linear correlation between the percentage of agricultural and urban land in the catchments and the $\delta^{15}\text{N-NO}_3$ values. They reported a maximum $\delta^{15}\text{N-NO}_3$ value of 8.4 ‰ for a river with 38.4 % agricultural area. The high $\delta^{15}\text{N-NO}_3$ values despite of decreased nitrate concentrations might be a result of fractionation processes of nitrate inside the estuary. These can be NO_3^- uptake or denitrification and would enrich the $\delta^{15}\text{N-NO}_3$ values (Mariotti *et al.*, 1988; Kendall, 1998). Outside the estuary the $\delta^{15}\text{N-NO}_3$ values decreased rapidly. In February we found values below the range given for marine nitrate

($\delta^{15}\text{N-NO}_3 \sim 5 \text{‰}$; Liu & Kaplan, 1989). This may reflect the value typical for nitrate in the Baltic Sea, that is heavily influenced by cyanobacterial nitrogen fixation (Voss *et al.*; in press)

The macroalgae naturally growing along the estuary generally reflected the gradient of higher $\delta^{15}\text{N-NO}_3$ values in the inner and middle part of the estuary (Stations D-I), and lower values in the outer part (Stations A-C). However, among sampled species, there were large differences in the $\delta^{15}\text{N}$ values even at the same stations. At station G the differences between the $\delta^{15}\text{N}$ value of *Ulva sp.* (13.2 ‰) and *Enteromorpha sp.* (9.5 ‰) was 3.7 ‰. Since no $\delta^{15}\text{N-NO}_3$ values were measured, we do not know which species directly reflects the nitrate

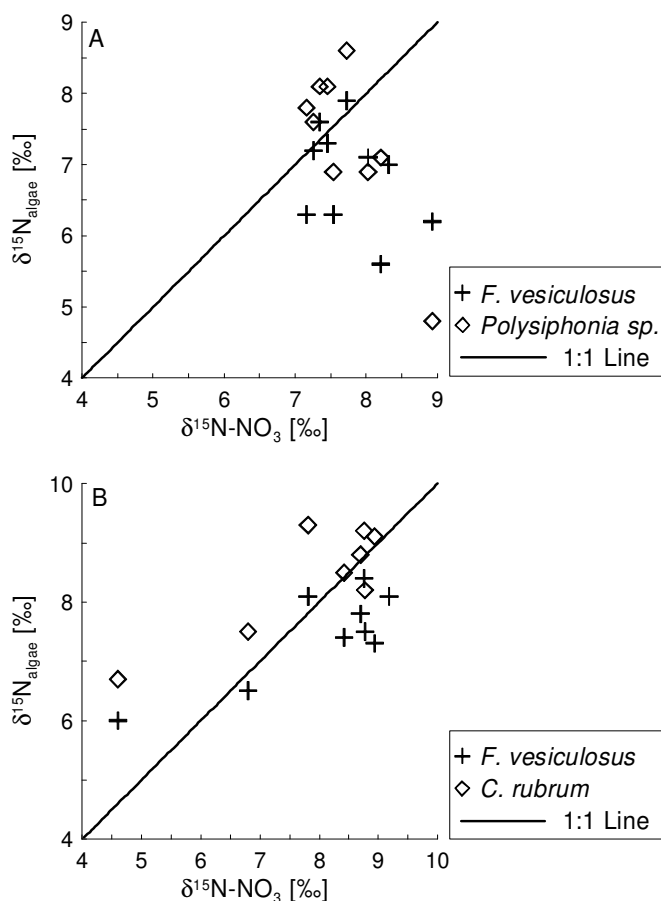


Figure VIII.9: $\delta^{15}\text{N-NO}_3$ values vs. $\delta^{15}\text{N}$ values of macroalgae after experiments 1 (A) and 2 (B) for stations W4-W13. The 1:1 line indicates the theoretical $\delta^{15}\text{N}$ value of the macroalgae, if the $\delta^{15}\text{N}$ value of the nitrate was mirrored exactly.

signature, however, we assume that the algae integrate their values over a longer time periods that may be different for different species. Since the Warnow River Estuary shows a typical anthropogenic loading with a gradient in $\delta^{15}\text{N}$ values this is reflected in the macroalgae. McClelland & Valiela (1998) had similar results in a comparative study of three estuaries, deriving different amounts of anthropogenic nitrogen loads. They reported the highest $\delta^{15}\text{N}$ values in macroalgae (8.4 ‰) from sites with the highest nitrogen loads.

The same was described in a study by

Costanzo *et al.* (2000) in Australian coastal waters of different anthropogenic N-loading.

Macroalgae as bio-indicator for anthropogenic derived N

Figure VIII.9 shows the relationship between $\delta^{15}\text{N-NO}_3$ values and the $\delta^{15}\text{N}$ values of the macroalgae after the first (A) and second experiment (B) for the stations W4-W13. At most stations, the $\delta^{15}\text{N-NO}_3$ values were better mirrored by the red algae species *Polysiphonia sp.* and *Ceramium rubrum*. These findings agree with results from Gartner *et al.* (2002), where macroalgae assimilated sewage-derived DIN and displayed higher $\delta^{15}\text{N}$ values in their tissue after 7 days of incubation.

The results from the experiments with the brown alga *Fucus vesiculosus* were not that clear, mainly because of higher initial $\delta^{15}\text{N}$ values. *F. vesiculosus* collected in February for the first experiment had an initial mean $\delta^{15}\text{N}$ value of 7.7 ‰ which was close to the $\delta^{15}\text{N}$ values of the river nitrate (inside the estuary; stations W4-W13; 6.2-9.0 ‰). Consequently, no change in the $\delta^{15}\text{N}$ value of *F. vesiculosus* could be observed. Nevertheless, adaptation towards lower values was visible outside the estuary at stations W1-W3. Here $\delta^{15}\text{N-NO}_3$ values were lower (2.8-7.9 ‰) and *F. vesiculosus* showed lower $\delta^{15}\text{N}$ values than the initial. Similar low values were also observed at stations W11 and W12 in spite of high $\delta^{15}\text{N-NO}_3$ values. It seems that growth conditions were not favourable for *F. vesiculosus* at these stations. In the Baltic Sea *F. vesiculosus* is found in waters with a salinity >4 PSU. Salinity at stations W11 and W12 ranged between 0.3 and 5 PSU, which might have been too low for algal growth. Also *Polysiphonia sp.* had lower $\delta^{15}\text{N}$ values in spite of high $\delta^{15}\text{N-NO}_3$ values in the almost inner part of the estuary (stations W10-W12), which might be as well a result of low salinity.

F. vesiculosus collected for the second experiment in May had a mean $\delta^{15}\text{N}$ value 1.3 ‰ lower than those used in the first experiment. These differences in the $\delta^{15}\text{N}$ values can be a result of the nitrogen storage of *F. vesiculosus* during the winter months, when nutrient

availability is high but light conditions are not sufficient for photosynthesis (Lehvo *et al.*, 2001). The conversion of the stored nitrogen into biomass could lead to fractionation, where light ^{14}N is preferential converted and the stored nitrogen gets enriched in ^{15}N . This would result in lower $\delta^{15}\text{N}$ values of the new built tissue until all stored nitrogen is used up. The second experiment revealed an increase in the $\delta^{15}\text{N}$ values of the macrophytes in the inner part of the estuary (stations W5-W11), and a decrease outside the estuary (stations W1-W3). The $\delta^{15}\text{N}$ values were close to $\delta^{15}\text{N}\text{-NO}_3$ values in the inner part of the estuary, but remained slightly below at most stations. This may be explained by fractionation during N-uptake, which would result in lower $\delta^{15}\text{N}$ values of the macroalgae tissue compared to the $\delta^{15}\text{N}\text{-NO}_3$ values (Högberg, 1997). But the elevated $\delta^{15}\text{N}$ values of *F. vesiculosus* at station W4 (second experiment) relative to the $\delta^{15}\text{N}\text{-NO}_3$ value argue against this fractionation theory. Another reason for differences between $\delta^{15}\text{N}$ value of macroalgae and $\delta^{15}\text{N}\text{-NO}_3$ values could be the slow N uptake- and growth rate of *F. vesiculosus*. In that case the $\delta^{15}\text{N}$ signal of the river

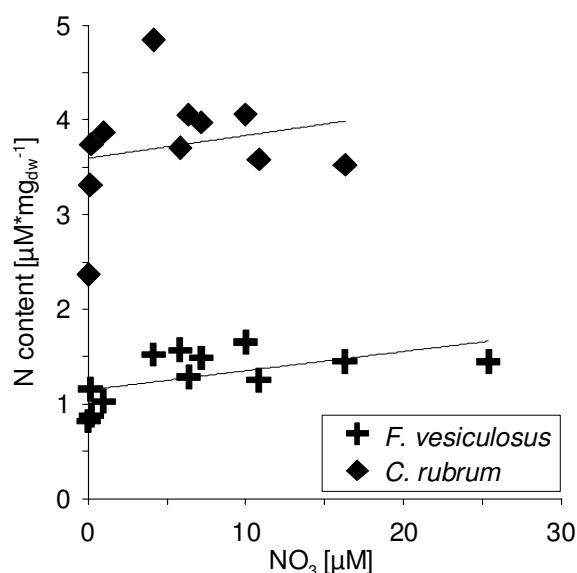


Figure VIII.10: Nitrate concentration versus N content in the macroalgae' tissue for *F. vesiculosus* and *C. rubrum* after the second experiment.

nitrate may not be mirrored during the short incubation period. These slow N uptake rates were also observed by Gartner *et al.* (2002) in experiments with the kelp *Ecklonia radiata* (Phaeophyta). On the other hand the measured $\delta^{15}\text{N}\text{-NO}_3$ values only represent the start and end points of the experiment and we are not certain how $\delta^{15}\text{N}\text{-NO}_3$ developed between these two samplings.

The maximum $\delta^{15}\text{N}$ value *C. rubrum* showed was 9.3 ‰, 4.2 ‰ above the initial

mean. These both species seemed to have a shorter growth rate and N-uptake rate than *F. vesiculosus*, what makes them more suitable for these experiments.

The measurements of the N-content in the dry weight of the algae tissue from the second experiment showed that *F. vesiculosus* and *C. rubrum* store nitrogen when nitrate is available in excess. While *F. vesiculosus* needed a nitrate concentration above 5 μM to increase the N content (station W5), *C. rubrum* started to store N at nitrate concentrations below 1 μM (station W2). A significant positive linear correlation between N content of tissue to nitrate concentration was only observed for *F. vesiculosus*: $r^2 = 0.57$, $p < 0.02$, Fig. VIII.10). A positive correlation between N content and N availability is reported for *F. vesiculosus* as well as *C. rubrum* from other studies (Lyngby, 1990; Savage & Elmgren, 2004). This increase in N content of macroalgae tissue could not be observed by means of C/N ratios during the first experiment.

The $\delta^{13}\text{C}$ values of the red algae after the incubation well reflect the mixture of seawater and riverwater along the estuary. DIC in fresh water usually has lower $\delta^{13}\text{C}$ values than in sea water (Boutton, 1991). Assuming a constant fractionation during the assimilation of DIC along the estuary, a trend is visible, with lower $\delta^{13}\text{C}$ values of the macroalgae and consequently of DIC inside the estuary, and an increase towards the Baltic Sea. This gradient in the $\delta^{13}\text{C}$ values of DIC is also reported from other estuaries (Hellings *et al.*, 2001; Atekwana *et al.*, 2003). *F. vesiculosus* showed this trend only after the first experiment. After the second experiment no trend in $\delta^{13}\text{C}$ was visible. Besides these fluctuations in the $\delta^{13}\text{C}$ values along the estuary, *F. vesiculosus* showed a large variability especially at the stations W5-W8 and W13, indicated by large standard deviations. The reason for these fluctuations remained unclear, but it seems that the DIC assimilation and consequently photosynthesis of *F. vesiculosus* was disturbed during the second experiment.

Station W13

Station W13 was additionally sampled because of the loading and unloading of mineral fertilizers in the port area. We assumed that mineral fertilizers pollute the water, which would result in lower $\delta^{15}\text{N-NO}_3$ values and consequently lower $\delta^{15}\text{N}$ values in macroalgae. Mineral fertilizers usually have $\delta^{15}\text{N}$ values around 0 ‰, because of their production from atmospheric N (Bedard-Haughn *et al.*, 2003). However, an impact of mineral fertilizers could not be observed at this station. $\delta^{15}\text{N-NO}_3$ values ranged between 6.8-7.5 ‰, which was in the range of the $\delta^{15}\text{N-NO}_3$ values inside the estuary. All macroalgae species well reflected this signal, so we exclude pollution by fertilizers at this site. Nevertheless, an additional input of nitrogen into the waters the near port area is assumed. Salinities at station W13 were at all sampling dates comparable to salinities found outside the estuary, but nitrate concentrations as well as $\delta^{15}\text{N-NO}_3$ values were always higher than outside the estuary. This indicates an input of a small volumes of water with high nitrate concentrations and elevated $\delta^{15}\text{N-NO}_3$ values. There are some small streams in the almost easterly part of the estuary, which might be responsible for additional N input..

Conclusions

The results of the incubation experiments show, that macroalgae reliably reflect $\delta^{15}\text{N-NO}_3$ values of the surrounding waters that indicate the degree of anthropogenic nitrogen in estuaries. These findings agree with results from similar incubation experiments carried out by Costanzo *et al.* (2000) in Moreton-Bay (Australia), and with results from Gartner *et al.* (2002) and Savage & Elmgren (2004) who investigated $\delta^{15}\text{N}$ values in naturally occurring macroalgae along a sewage N gradient. The additional measurement of $\delta^{15}\text{N-NO}_3$ values showed whether the macroalgae fractionated during NO_3^- uptake. The comparison between

$\delta^{15}\text{N}$ values of red and brown algae showed that the former (*Polysiphonia sp.* and *C. rubrum*) were more suitable for these experiments than *F. vesiculosus*. Furthermore we showed that the $\delta^{15}\text{N}$ was most precisely reflected in those parts of the estuary with low variation in $\delta^{15}\text{N-NO}_3$. Outside the estuary, where $\delta^{15}\text{N-NO}_3$ was very variable, the macroalgae did not mirror the nitrate signal adequately. In contrast to that, $\delta^{13}\text{C}$ values of macroalgae reflected the salinity changes from the fresh water source to the Baltic Sea. A combination of stable isotopes data can thus deliver information on the impact of anthropogenic N as well as on the prevailing salinities along an estuarine gradient.

IX. FINAL CONCLUSIONS AND FUTURE OUTLOOK

The results of this PhD-thesis close a gap between agricultural and marine research. By means of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in nitrate it could be shown that nitrification is the dominant process responsible for high nitrate losses from agricultural soils.

The combination of the isotope data with the mixing-model well documented the importance of drainage water nitrate for the Warnow river and, consequently, for the coastal areas of the southern Baltic Sea. These findings were supported by the results of the second study which identified drainage water nitrate as the dominant N source for the adjacent surface waters. Finally the method was successfully applied to trace anthropogenic N on its way to the coastal waters.

As suggested by the study of Grimvall & Stalnacke (2001), a reduction in the anthropogenic nitrogen loads to the coastal waters of the Baltic Sea can only be achieved by reducing diffuse agricultural inputs. With application of the dual-isotope approach diffuse nitrate sources can be identified, which is a prerequisite to set suitable measures to reduce diffuse N inputs. Furthermore, the mixing-model approach confirms results from nutrient emission models, which is of particular interest if model results are ambiguous.

A major point not taken into account in this PhD thesis is the retention of nitrogen within the river-system and the export into coastal waters. Model estimations from 16 river-systems in the north-eastern United States showed that up to 76 % of the N inputs are denitrified or stored in the riverine sediments (Seitzinger *et al.*, 2002). How much N really reaches coastal waters and how much is denitrified, assimilated or stored in coastal sediments remains unclear as yet.

The “nitrogen isotope-pairing method” is a suitable tool to estimate denitrification rates (Nielsen, 1992), and is successfully applied in the Norsminde Fjord (Denmark; Nielsen *et al.*, 1995). The “denitrifier method” developed by Sigman *et al.* (2001) allows the determination

of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate in the marine environment at very low NO_3^- concentrations and with small sample volumes. Thus it can be used for stable isotope measurements in pore-water, in atmospheric deposition, and in waters with low nitrate concentrations, e.g. when primary productivity is high.

With a combination of both methods and the integrative agricultural and marine approach as carried out in this thesis it will be possible to calculate N budgets for coastal areas and river-systems which can be more precise than model estimates.

X. REFERENCES

- Richtlinie 2000/60/EG des Europäischen Parlaments und des Rates vom 23. Oktober 2000 zur Schaffung eines Ordnungsrahmens für Maßnahmen der Gemeinschaft im Bereich Wasserpolitik (WRRL). In *Amtsblatt der EG Nr. I*. 327/1.
- Addiscott, T. M., Whitmore, A. P. & Powlson, D. S. (1992). *Farming, Fertilizers and the Nitrate Problem*. CAB International, Wallingford.
- Amberger, A. & Schmidt, H.-L. (1987). Natürliche Isotopengehalte von Nitrat als Indikatoren für dessen Herkunft. *Geochimica et Cosmochimica Acta* **51**, 2699-2705.
- Andersson, K. K. & Hooper, A. B. (1983). O₂ and H₂O are each the source of one O in NO₂⁻ produced from NH₃ by Nitrosomonas: ¹⁵N-NMR evidence. *FEBS* **164**, 236-240.
- Atekwana, E. A., P., T. L. & Jackson, L. R. (2003). Dissolved Inorganic Carbon (DIC) and Hydrologic Mixing in a Subtropical River Estuary, Southwest Florida, USA. *Estuaries* **26**, 1391-1400.
- Atxotegi, U., Iqbal, M. Z. & Czarnetzki, A. C. (2003). A preliminary assessment of nitrate degradation in simulated soil environments. *Environ. Geology* **45**, 161-170.
- Baden, S., Gullström, M., Lunden, B., Pihl, L. & Rosenberg, R. (2003). Vanishing Seagrass (*Zostera marina*, L.) in Swedish Coastal Waters. *Ambio* **32**, 374-377.
- Bedard-Haughn, A., Groenigen, J. W. v. & Kessel, C. v. (2003). Tracing ¹⁵N through landscapes: potential uses and precautions. *Journal of Hydrology* **272**, 175-190.
- Behrendt, H., Huber, P., Kornmilch, M., Opitz, D., Schmoll, O., Scholz, G. & Uebe, R. (1999). *Nährstoffbilanzierung der Flussgebiete Deutschlands*, vol. 75-99. Umweltbundesamt, Berlin.
- Beilke, S. & Uhse, K. (2002). *Jahresbericht 2001 aus dem Messnetz des Umweltbundesamtes*, vol. 69-02. Umweltbundesamt, Berlin.

- Benckiser, G. (1996). *In situ Bestimmung von Denitrifikationsverlusten auf unterschiedlichen landwirtschaftlichen Nutzflächen mit der Acetylen-Inhibierungstechnik*, vol. 41/1996. VDLUFA-Verlag, Darmstadt.
- Bergström, L. (1987). Leaching of ¹⁵N-labeled Nitrate Fertilizer Applied to Barley and Grass Ley. *Acta Agric. Scand.* **37**, 199-206.
- Bergström, L. & Kirchmann, H. (1999). Leaching of Total Nitrogen from Nitrogen-15-labeled Poultry Manure and Inorganic Nitrogen Fertiliser. *J. Environ. Qual* **28**, 1283-1290.
- Bockholt, R. & Kappes, K. (1994). Stoffaustrag über Entwässerungssysteme - ein aktueller Niedermoorgrünland - Ackerland - Vergleich. In *Ökologische Untersuchungen im Warnow-Einzugsgebiet II*, vol. 2/1994, pp. 136-146. LAUN M-V.
- Bockholt, R., Koch, G., Ebert, W. & Fedderwitz, E. (1992). Nährstoffgehalte von Drän- und Grabenwasser landwirtschaftlich genutzter Flächen im Trinkwassereinzugsgebiet der Kösterbeck. *Z. f. Kulturtechnik und Landentwicklung* **33**, 178-185.
- Boesch, D. F. (2001). Science and Integrated Drainage Basin Coastal Management: Chesapeake Bay and Mississippi Delta. In *Science and Integrated Coastal Management*. ed. Bodungen, B. v. & Turner, R. K., pp. 37-50. Dahlem University Press, Berlin.
- Börner, R., Bönsch, R., Fadschild, K., Gosselck, F., Hübener, T., Klinkenberg, G., Kolbow, D., Lill, D., Merkel, G., Neumann, C., Randow, F. F. E., Schlungbaum, G., Selig, U. & Winkler, H. (1994). Ein Beitrag zur Biologie der Warnow, eines norddeutschen nacheiszeitlichen Tieflandflusses. In *Ökologische Untersuchungen im Warnow-Einzugsgebiet II*, vol. Heft 2, pp. 56-92. Landesamt für Umwelt und Natur Mecklenburg-Vorpommern.
- Borum, J. (1996). Shallow Waters and land/sea Boundaries. In *Eutrophication in Coastal Marine Ecosystems*. ed. Jorgensen, B. B. & Richardson, K., pp. 179-203. AGU, Washington D.C.

- Böttcher, J., Strebel, O., Voerkelius, S. & Schmidt, H.-L. (1990). Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. *Journal of Hydrology* **114**, 413-424.
- Boutton, T. W. (1991). Stable Isotope Ratios of Natural Materials: II. Atmospheric, Terrestrial, Marine, and Freshwater Environments. In *Carbon Isotope Techniques*. ed. Coleman, D. C. & Fry, B., pp. 173-185. Academic Press, San Diego.
- Bouwman, A. F. & Boumans, L. J. M. (2002). Estimation of Global NH₃ Volatilization Loss From Synthetic Fertilizers and Animal Manure Applied to Arable Lands and Grasslands. *Global Biogeochem. Cycles* **16**, 8-1 - 8-10.
- Brandes, J. A., Yoshinari, T., Jayakumar, D. A. & 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.* **42**, 1680-1689.
- Bremner, J. M. & Tabatabai, M. A. (1973). Nitrogen-15 enrichment of soils and soil-derived nitrate. *J. Environ. Quality* **2**, 363-365.
- Burns, D. A. & Kendall, C. (2002). Analysis of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York. *Water Resources Research* **38**, 9-1 - 9-11.
- Campbell, D. H., Kendall, C., Chang, C. C. Y., Silva, S. R. & Tonnessen, K. A. (2002). Pathways for nitrate release from an alpine watershed: determination using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. *Water Resources Research* **38**, 10-11 - 10-19.
- Carpenter, E. J. & Romans, K. (1991). Major role of the Cyanobacterium *Trichodesmium* in Nutrient Cycling in the North Atlantic Ocean. *Science* **254**, 1356-1358.
- Chang, C. C. Y., Kendall, C., Silva, S. R., Battaglin, W. A. & Campbell, D. H. (2002). Nitrate stable isotopes: Tools for determining nitrate sources among different land uses in the Mississippi River Basin. *Can. J. Fish. Aquat. Sci.* **59**, 1874-1885.

- Cloern, J. E. (1982). Does the Benthos Control Phytoplankton Biomass in South San Francisco Bay (USA)? *Mar. Ecol. Prog. Ser.* **9**, 191-202.
- Cloern, J. E. (2001). Our Evolving Conceptual Model of the Coastal Eutrophication Problem. *Mar. Ecol. Prog. Ser.* **210**, 223-253.
- Costanzo, S. D., O'Donohue, M. J., Dennison, W. C., Loneragan, N. R. & Thomas, M. (2000). A new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin.*
- Craig, H. (1957). Isotopic Standards for Carbon and Oxygen and Correction Factors for Mass-spectrometric Analysis of Carbon Dioxide. *Geochimica et Cosmochimica Acta* **12**, 133-149.
- Craig, H. (1961). Standard for Reporting Concentrations of Deuterium and Oxygen-18 in Natural Waters. *Science* **133**, 1833-1834.
- de Jonge, V. N. (1990). Responses of the Dutch Wadden Sea Ecosystem to Phosphorous Discharges from the Rhine River. *Hydrobiologia* **195**, 49-62.
- de Jonge, V. N. & Elliott, M. (2001). Eutrophication. In *Encyclopedia of Ocean Sciences*. ed. Steele, J. H., Turekian, K. K. & Thorpe, S. A., pp. 852-870. Academic Press, San Diego.
- Dortch, Q., Robichaux, R., Pool, S., Milsted, D., Mire, G., Rabalais, N. N., Soniat, T. M., Fryxell, G. A., Turner, R. E. & Parsons, M. L. (1997). Abundance and Vertical flux of *Pseudo-nitzschia* in the Northern Gulf of Mexico. *Mar. Ecol. Prog. Ser.* **146**, 249-264.
- Durka, W., Schulze, E.-D., Gebauer, G. & Voerkelius, S. (1994). Effects of forest decline on uptake and leaching of deposited nitrate determined from ¹⁵N and ¹⁸O measurements. *Nature* **372**, 765-767.
- Egmond, K. v., Bresser, T. & Bouwman, L. (2002). The European Nitrogen Case. *Ambio* **31**, 72-78.

- Feige, W. & Röthlingshöfer, R. (1990). Nitratauswaschung aus zwei unterschiedlich bewirtschafteten Ackerböden. *Z. f. Kulturtechnik und Landentwicklung* **31**, 89-95.
- Feigin, A., Shearer, G., Kohl, D. H. & Commoner, B. (1974). The Amount and Nitrogen-15 Content of Nitrate in Soil Profiles from two Central Illinois Fields in a Corn-Soybean Rotation. *Soil Sci. Soc. Amer. Proc.* **38**, 465-471.
- Fixen, P. E. & West, F. B. (2002). Nitrogen Fertilizers: Meeting Contemporary Challenges. *Ambio* **31**, 169-176.
- Flipse, W. J. & Bonner, F. T. (1985). Nitrogen-isotope ratios of nitrate in ground water under fertilized fields, Long Island, New York. *Groundwater* **23**, 59-67.
- Fogg, G. E., Rolston, D. E., Decker, D. L., Louie, D. T. & Grismer, M. E. (1998). Spatial variation in nitrogen isotope values beneath nitrate contamination sources. *Groundwater* **36**, 418-427.
- Freyer, H. D. (1991). Seasonal variation of $^{15}\text{N}/^{14}\text{N}$ ratios in atmospheric nitrate species. *Tellus* **43**, 30-44.
- Freyer, H. D. & Aly, A. I. M. (1974). Nitrogen-15 variations in fertilizer nitrogen. *Journal Environmental Quality* **3**, 405-406.
- 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**, 264-271.
- Galloway, J. N. & Cowling, E. B. (2002). Reactive Nitrogen and the World: 200 Years of Change. *Ambio* **31**, 64-70.
- Gartner, A., Lavery, P. & Smit, A. J. (2002). Use of $\delta^{15}\text{N}$ Signatures of Different Functional Forms of Macroalgae and Filter-feeders to Reveal Temporal and Spatial Patterns in Sewage Disposal. *Mar. Ecol. Prog. Ser.* **235**, 63-73.

- Göbel, B. (2000). Stickstoff-Austragsverhalten einer gedrännten Fläche in Schleswig-Holstein. *Wasser und Boden* **52**, 33-36.
- Gonfiantini, R., Fröhlich, K., Araguas-Araguas, L. & Rozanski, K. (1998). Isotopes in Groundwater Hydrology. In *Isotope Tracers in Catchment Hydrology*. ed. Kendall, C. & McDonnell, J. J., pp. 203-246. Elsevier, Amsterdam.
- Grimvall, A. & Stalnacke, P. (2001). Riverine Inputs of Nutrients to the Baltic Sea. In *A System Analysis of the Baltic Sea*. ed. Wulff, F. V., Rahm, L. A. & Larsson, P., pp. 113-131. Springer, Berlin.
- Heaton, T. H. E. (1986). Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. *Chemical Geology* **59**, 87-102.
- Heaton, T. H. E. (1990). $^{15}\text{N}/^{14}\text{N}$ Ratios of NO_x from Vehicle Engines and Coal-fired Power Stations. *Tellus* **42B**, 304-307.
- HELCOM. (2003). Executive Summary of the Fourth Baltic Sea Pollution Load Compilation PLC-4, pp. 1-12. HELCOM, Helsinki.
- Hellings, L., Dehairs, F. & van Damme, S. (2001). Dissolved Inorganic Carbon in a Highly Polluted Estuary (the Scheldt). *Limnol. Oceanogr.* **46**, 1406-1414.
- Hoefs, J. (2004). *Stable Isotopes Geochemistry*. Springer Verlag, Berlin.
- Hofman, G. & Cleemput, O. V. (2004). *Soil and Plant Nitrogen*. International Fertilizer Industry Association, Paris.
- Högberg, P. (1997). ^{15}N natural abundance in soil-plant systems. *New Phytologist* **137**, 179-203.
- Hollocher, T. C., Tate, M. E. & Nicholas, D. J. D. (1981). Oxidation of ammonia by *Nitrosomonas europaea*. *J. Biol. Chem* **256**, 10834-10836.

- Howarth, R. W., Boyer, E. W., Pabich, W. & Galloway, J. N. (2002). Nitrogen use in the United States from 1961-2000, and Estimates of Potential Future Trends. *Ambio* **31**.
- Hsieh, J. C. C., Chadwick, O. A., Kelly, E. F. & Savin, S. M. (1998). Oxygen Isotopic Composition of Soil Water: Quantifying Evaporation and Transpiration. *Geoderma* **82**, 269-293.
- IAEA. (1981). Deuterium and Oxygen-18 in the Water Cycle. In *Stable Isotope Hydrology*. ed. Gat, J. R. & Gonfiantini, R.
- IAEA. (2001). GNIP Maps and Animations. International Atomic Energy Agency, Vienna.
- IFA/FAO. (2001). *Global Estimates of Gaseous Emissions of NH₃, NO and N₂O from Agricultural Land*, Rome.
- Ingraham, N. L. (1998). Isotopic Variations in Precipitation. In *Isotope Tracers in Catchment Hydrology*. ed. McDonnell, J. J., pp. 87-118. Elsevier.
- Iqbal, M. Z., Krothe, N. C. & Spalding, R. F. (1997). Nitrogen isotope indicators of seasonal source variability to groundwater. *Environmental Geology* **32**, 210-218.
- Jansson, B.-O. & Dahlberg, K. (1999). The Environmental Status of the Baltic Sea in the 1940s, Today, and in the Future. *Ambio* **28**, 312-319.
- Jansson, M. (2001). Role of Sediments in the Nutrient Dynamics of the Baltic. In *A System Analysis of the Baltic Sea*. ed. Wulff, F. V., Rahm, L. A. & Larsson, P., pp. 229-255. Springer, Berlin.
- Kahle, P., Tiemeyer, B. & Lennartz, B. (2005). Nutrient Export from Agricultural Areas by Tile Drainage (in german). *in press*.
- Karr, J. D., Showers, W. J. & Hinson, T. H. (2002). Nitrate source identification using ¹⁵N in a ground water plume near an intensive swine operation. *GWMMR* **22**, 68-75.

- Kautsky, N., Kautsky, H., Kautsky, U. & Waern, M. (1986). Decreased Depth Penetration of *Fucus vesiculosus* (L.) Since the 1940's Indicates Eutrophication of the Baltic Sea. *Mar. Ecol. Prog. Ser.* **28**, 1-8.
- Kendall, C. (1998). Tracing nitrogen sources and cycles in catchments. In *Isotope Tracers in Catchment Hydrology*. ed. Kendall, C. & McDonnell, J. J., pp. 519-576. Elsevier, Amsterdam.
- Kendall, C. & Aravena, R. (1999). Nitrate Isotopes in groundwater systems. In *Environmental Tracers in Subsurface Hydrology*. ed. Cook, P. G. & Herzeg, A. L., pp. 261-297. Kluwer Academic, Boston.
- Kendall, C. & Caldwell, E. A. (1998). Fundamentals of Isotope Geochemistry. In *Isotope Tracers in Catchment Hydrology*. ed. Kendall, C. & McDonnell, J. J., pp. 51-86. Elsevier, Amsterdam.
- Kirchmann, H., Johnston, A. E. J. & Bergström, L. F. (2002). Possibilities for Reducing Nitrate Leaching from Agricultural Land. *Ambio* **31**, 404-408.
- Krauss, W. (2001). Baltic Sea Circulation. In *Encyclopedia of Ocean Sciences*. ed. Steele, J. H., Turekian, K. K. & Thorpe, S. A., pp. 236-244. Academic Press, San Diego.
- Kreitler, C. W., Ragone, S. E. & Katz, B. G. (1978). N^{15} / N^{14} ratios of ground-water nitrate, Long Island, New York. *Groundwater* **16**, 404-409.
- Kroopnick, P. M. & Craig, H. (1972). Atmospheric oxygen: Isotopic composition and solubility fractionation. *Science* **175**, 54-55.
- Lam, C. W. Y. & Ho, K. C. (1989). Red Tides in Tolo Harbour, Hong Kong. In *Red Tides: Biology, Environmental Science and Toxicology*. ed. Okaichi, T., Anderson, D. M. & Nemoto, T., pp. 49-52. Elsevier, New York.
- Lammel, J. (1990). *Nährstoffausträge durch Vorfluter und Dräne*, vol. 386. Landwirtschaftsverlag GmbH, Münster-Hiltrup.

- Lehvo, A., Bäck, S. & Kiirikki, M. (2001). Growth of *Fucus vesiculosus* L. (Phaeophyta) in the Northern Baltic Proper: Energy and Nitrogen Storage in Seasonal Environment. *Botanica Marina* **44**, 345-350.
- Lennartz, B., Michaelsen, J., Wichtmann, W. & Widmoser, P. (1999). Time variance analysis of preferential solute movement at a tile-drained field site. *Soil Sci. Soc. Am. J.* **63**, 39-47.
- Lewan, E. (1994). Effects of a Catch Crop on Leaching of Nitrogen from a Sandy soil: Simulations and Measurements. *Plant Soil* **166**, 137-152.
- Liu, K.-K. & Kaplan, I. R. (1989). The Eastern Tropical Pacific as a source of ^{15}N -enriched Nitrate in Seawater off Southern California. *Limnol. Oceanogr.* **34**, 820-830.
- Lyngby, J. E. (1990). Monitoring of Nutrient Availability and Limitation Using the Marine Macroalga *Ceramium rubrum* (Huds.) C. Ag. *Aquat. Bot.* **38**, 151-161.
- MacDonald, A. J., Powlson, D. S., Poulton, P. R. & Jenkinson, D. S. (1989). Unused Fertilizer Nitrogen in Arable Soils - Its Contribution to Nitrate Leaching. *J. Sci. Food Agric.* **49**, 407-419.
- Mariotti, A. (1983). Atmospheric Nitrogen is a Reliable Standard for Natural ^{15}N Abundance Measurements. *Nature* **303**, 685-687.
- Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A. & Tardieux, P. (1981). Experimental Determination of Nitrogen Kinetic Isotope Fractionation: Some Principles; Illustration for the Denitrification and Nitrification Process. *Plant Soil.* **62**, 413-430.
- Mariotti, A., Landreau, A. & Simon, B. (1988). ^{15}N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. *Geochimica et Cosmochimica Acta* **52**, 1869-1878.

- Matthäus, W. (1995). Temperatur, Salzgehalt und Dichte. In *Meereskunde der Ostsee*. ed. Rheinheimer, G., pp. 75-81. Springer, Berlin.
- Mayer, B., Bollwerk, S. M., Mansfeldt, T., Hütter, B. & Veizer, J. (2001). The oxygen isotope composition of nitrate generated by nitrification in acid forest floors. *Geochimica et Cosmochimica Acta* **65**, 2743-2756.
- Mayer, B., Boyer, E. W., Goodale, C., Jaworski, N. A., Breemen, N. v., Howarth, R. W., Seitzinger, S., Billen, G., Lajtha, K., Nadelhoffer, K., Dam, D. v., Hetling, L. J., Nosal, M. & Paustian, K. (2002). Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: Isotopic constraints. *Biogeochemistry* **57/58**, 171-197.
- McClelland, J. W. & Valiela, I. (1997). Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology and Oceanography* **42**, 930-937.
- McClelland, J. W. & Valiela, I. (1998). Linking Nitrogen in Estuarine Producers to Land-derived Sources. *Limnol. Oceanogr.* **43**, 577-585.
- Mee, L. D. (2001). Eutrophication in the Black Sea and a Basin-wide Approach to its Control. In *Science and Integrated Coastal Management*. ed. Bodungen, B. v. & Turner, R. K., pp. 71-91. Dahlem University Press, Berlin.
- Mengis, M., Schiff, S. L., Harris, M., English, M. C., Aravena, R., Elgood, R. J. & MacLean, A. (1999). Multiple geochemical and isotopic approaches for assessing groundwater NO_3^- elimination in a riparian zone. *Groundwater* **37**, 448-457.
- Mengis, M., Walther, U., Bernasconi, S. M. & Wehrli, B. (2001). Limitations of using $\text{d}18\text{O}$ for the source identification of nitrate in agricultural soils. *Environ. Sci. Technol.* **35**, 1840-1844.
- Meybeck, M. (1982). Carbon, Nitrogen, and Phosphorous Transport by World Rivers. *Am. J. Sci.* **282**, 401-450.

- Monbet, Y. (1992). Control of Phytoplankton Biomass in Estuaries: A Comparative Analysis of Microtidal and Macrotidal Estuaries. *Estuaries* **15**, 563-571.
- Mulvaney, R. L. (1993). Mass Spectrometry. In *Nitrogen Isotope Techniques*. ed. Knowles, R. & Blackburn, T. H., pp. 11-54. Academic Press.
- Nausch, G., Matthäus, W. & Feistel, R. (2003). Hydrographic and Hydrochemical Conditions in the Gotland Deep Area Between 1992 and 2003. *Oceanologia* **45**, 557-569.
- Neumann, T., Fennel, W. & Kremp, C. (2002). Experimental Simulations with an Ecosystem Model of the Baltic Sea: A Nutrient Load Reduction Experiment. *Global Biogeochem. Cycles* **16**, 7-1 - 7-19.
- Nielsen, K., Nielsen, L. P. & Rasmussen, P. (1995). Estuarine Nitrogen Retention Independently Estimated by Denitrification rate and mass Balance Methods: A study of Norsminde Fjord, Denmark. *Marine Ecology Progress Series* **119**, 275-283.
- Nielsen, L. P. (1992). Denitrification in Sediment Determined from Nitrogen Isotope Pairing. *FEMS Microbiol Ecol* **86**, 357-362.
- Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia* **41**, 199-219.
- Nixon, S. W., Ammerman, J. W., Atkinson, L. P., Berounsky, V. M., Billen, G., Boicourt, W. C., Boynton, W. R., Church, T. M., Ditoro, D. M., Elmgren, R., Garber, J. H., Giblin, A. E., Jahnke, R. A., Owens, N. J. P., Pilson, M. E. Q. & Seitzinger, S. P. (1996). The fate of Nitrogen and Phosphorous at the land-sea Margin of the North Atlantic Ocean. *Biogeochemistry* **35**, 141-180.
- Paerl, H. W. (1995). Coastal eutrophication in relation to atmospheric nitrogen deposition : current perspectives. *Ophelia* **41**, 237-259.
- Pagenkopf, W. (2001). Aktuelle Nährstoffbilanzierung für Teilgebiete des Einzugsgebiets der Warnow. Geodaten Integration & Analyse, Berlin.

- Pfennig, K. S. & McMahon, P. B. (1996). Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *Journal of Hydrology* **187**, 283-295.
- Phillips, D. L. & Koch, P. L. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia* **130**, 114-125.
- Rabalais, N. N. (2002). Nitrogen in Aquatic Ecosystems. *Ambio* **31**, 102-112.
- Rabalais, N. N., Smith, L. E., Jr., D. E. H. & Justic, D. (2001). Effects of Seasonal Hypoxia on Continental Shelf Benthos. In *Coastal Hypoxia: Consequences for Living Resources and Ecosystems*. ed. Rabalais, N. N. & Turner, R. E., pp. 211-240. American Geophysical Union, Washington.
- Rennie, D. A., Paul, E. A. & Johns, L. E. (1976). Natural nitrogen-15 abundance of soil and plant samples. *Canadian Journal Soil Science* **59**, 43-50.
- Robinson, D. (2001). $\delta^{15}\text{N}$ as an Integrator of the Nitrogen Cycle. *Trends in Ecology & Evolution* **16**, 153-162.
- Rydberg, L., Edler, L., Floderus, S. & Graneli, W. (1990). Interaction Between Supply of Nutrients, Primary Production, Sedimentation and Oxygen Consumption in SE Kattegat. *Ambio* **19**, 134-141.
- Sanden, P. & Hakansson, B. (1996). Long-term Trends in Secchi Depth in the Baltic Sea. *Limnol. Oceanogr.* **41**, 346-351.
- Savage, C. & Elmgren, R. (2004). Macroalgal (*Fucus vesiculosus*) $\delta^{15}\text{N}$ values trace decrease in sewage influence. *Ecological application* **14**, 517-526.
- Schinke, H. & Matthäus, W. (1998). On the Causes of Major Baltic Inflows and Analysis of long time Series. *Cont. Shelf. Res.* **18**, 67-97.

- Schneider, B., Nausch, G., Nagel, K. & Wasmund, N. (2003). The Surface Water CO₂ Budget for the Baltic Proper: A new way to Determine Nitrogen Fixation. *Journal of Marine Systems* **42**, 53-64.
- Schramm, W. (1996). The Baltic Sea and its Transition Zones. In *Marine Benthic Vegetation - Recent Changes and the Effects of Eutrophication*. ed. Schramm, W. & Nienhuis, P. H., pp. 131-163. Springer, Berlin.
- Seitzinger, S. P., Styles, R. V., Boyer, E. W., Alexander, R. B., Billen, G., Howarth, R. W., Mayer, B. & van Breemen, N. (2002). Nitrogen Retention in Rivers: Model Development and Application to Watersheds in the Northeastern U.S.A. *Biogeochemistry* **57/58**, 199-237.
- Sigman, D. M., Altabet, M. A., Michener, R., McCorkle, D. C., Fry, B. & Holmes, R. M. (1997). Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaption of the ammonia diffusion method. *Marine Chemistry* **57**, 227-243.
- Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M. & Böhlke, J. K. (2001). A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* **73**, 4145-4153.
- Sigman, D. M., Robinson, R., Knapp, A. N., Geen, A. v., McCorkle, D. C., Brandes, J. A. & Thunell, R. C. (2003). Distinguishing Between Water Column and Sedimentary Denitrification in the Santa Barbara Basin Using the Stable Isotopes of Nitrate. *Geochemistry Geophysics Geosystems* **4**.
- Silva, S. R., Kendall, C., Wilkison, D. H., Ziegler, A. C., Chang, C. C. Y. & Avanzino, R. J. (2000). A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. *Journal of Hydrology* **228**, 22-36.
- Smil, V. (1999). Nitrogen in Crop Production: An Account of Global Flows. *Global Biogeochemical Cycles* **13**, 647-662.

- Smil, V. (2001). *Enriching the Earth*. The MIT Press, Cambridge, MA.
- Solic, M., Krstulovic, N., Marasovic, I., Baranovic, A., Pucher-Petkovic, T. & Vucetic, T. (1997). Analysis of time Series of Planktonic Communities in the Adriatic Sea: Distinguishing Between Natural and Man-induced Changes. *Oceanologica Acta* **20**, 131-143.
- Spoelstra, J., Schiff, S. L., Elgood, R. J., Semkin, R. G. & Jeffries, D. S. (2001). Tracing the sources of exported nitrate in the Turkey Lakes Watershed using $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ isotopic ratios. *Ecosystems* **4**, 536-544.
- Stalnacke, P., Grimvall, A., Sundblad, K. & Tonderski, A. (1999). Estimation of riverine loads of nitrogen and phosphorous to the Baltic Sea, 1970-1993. *Environmental Monitoring and Assessment* **58**, 173-200.
- Stedman, D. H. & Shetter, R. (1983). The Global Budget of Atmospheric Nitrogen Species. In *Trace Atmospheric Constituents: Properties, Transformations and Fates*. ed. Schwartz, S. S., pp. 411-454. John Wiley, New York.
- Stepanauskas, R. & Leonardson, L. (1999). Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. *Limnology and Oceanography* **44**, 1477-1485.
- Sweitzer, J., Langaas, S. & Folke, C. (1996). Land cover and population density in the Baltic Sea drainage basin: A GIS database. *Ambio* **25**, 191-198.
- Thiele, V. & Mehl, D. (1995). *Ein ökologisch begründetes Sanierungskonzept für das Gewässereinzugsgebiet der Warnow (Mecklenburg-Vorpommern)*, vol. Heft 2. Landesamt für Umwelt und Natur Mecklenburg-Vorpommern, Güstrow.
- Tomer, M. D., Meek, D. W., Jaynes, D. B. & Hatfield, J. L. (2003). Evaluation of nitrate nitrogen fluxes from a tile-drained watershed in Central Iowa. *J. Environ. Qual* **32**, 642-653.

- Vallin, L. & Nissling, A. (2000). Maternal Effects on egg size and egg Buoyancy of Baltic cod, *Gadus morhua* Implications for Stock Structure Effects on Recruitment. *Fish. Res.* **49**, 21-37.
- van Breemen, N., Boyer, E. W., Goodale, C. L., Jaworski, N. A., Paustian, K., Seitzinger, S. P., Lajtha, K., Mayer, B., van Dam, D., Howarth, R. W., Nadelhoffer, K. J., Eve, M. & Billen, G. (2002). Where did all the Nitrogen go? Fate of Nitrogen Inputs to Large Watersheds in the Northeastern U.S.A. *Biogeochemistry* **57/58**, 267-293.
- Voss, M., Dippner, J. W. & Montoya, J. P. (2001). Nitrogen Isotope Patterns in the Oxygen Deficient Waters of the Eastern Tropical Pacific Ocean (ETNP). *Deep-Sea Research I* **48**, 1905-1921.
- Voss, M., Emeis, K. C., Neumann, T., Hille, S. & Dippner, J. W. ((in press)). The Nitrogen Cycle of the Baltic Sea from and Isotopic Perspective. *Global Biogeochem. Cycles*.
- Wasmund, N., Nausch, G. & Matthäus, W. (1998). Phytoplankton Spring Blooms in the Southern Baltic Sea - Spatio-temporal Development and Long-term Trends. *Journal of Plankton Research* **20**, 1099-1118.
- Wassenaar, L. I. (1995). Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^- . *Applied Geochemistry* **10**, 391-405.
- Wulff, F. V., Rahm, L. & Larsson, P. (2001). Introduction. In *A System Analysis of the Baltic Sea*. ed. Wulff, F. V., Rahm, L. A. & Larsson, P., pp. 1-17. Springer, Berlin.
- Zaman, M., Di, H. J., Cameron, K. C. & Frampton, C. M. (1999). Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. *Biol. Fertil. Soils* **29**, 178-186.

XI. DANKE

Mein Dank gilt allen, die zum Gelingen dieser Arbeit beigetragen haben!

Ganz besonders möchte ich mich bei Maren Voß bedanken, die mich während der ganzen Zeit hervorragend betreute. Vielen Dank für die Unterstützung, die vielen aufmunternden Worte und die abendlichen Diskussionen über stabile Isotope und die WRRL im Kurhaus und im Casablanca.

Prof. von Bodungen danke ich für die Unterstützung und für die hilfreichen Diskussionen beim Anfertigen der Arbeit.

Natalie Loick und Deniz Bombar danke ich ganz besonders, dass sie sich soviel Zeit genommen haben um mir bei den Korrekturen und bei der Formatierung der Arbeit zu helfen.

Bei Iris Liskow möchte ich mich dafür bedanken, dass sie mich so hervorragend in die Geheimnisse der IR-Massenspektrometrie eingeweiht hat. Ausserdem nochmals vielen Dank für das Lesen der ersten Versionen.

Rolf Peinert danke ich ganz herzlich für die sprachlichen Korrekturen und die aufmunternden Smileys.

Vielen Dank auch an Joachim Dippner für die Hilfe bei statistischen Problemen und die Unterstützung besonders bei der Anwendung der Gauß'schen Fehlerfortpflanzung!!!

Bei der DBU bedanke ich mich für das Stipendium. Ein besonderer Dank geht an Frau Dr. Schlegel-Starmann die mich hervorragend betreut hat. Vielen Dank auch an alle Ostsee-Schwerpunktlern für die vielen Diskussionen während unserer gemeinsamen Zeit.

Ich bedanke mich bei meinen Eltern, meiner Schwester, meinem Schwager und bei meinen Freunden für die Unterstützung!

XII. ERKLÄRUNG

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe, keine ausser den von mir angegebenen Hilfsmitteln und Quellen dazu verwendet habe und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen als solche kenntlich gemacht habe.

Rostock, den

XIII. LEBENS LAUF

- Geboren am 26.09.1973 in Marl
- 1994 Abitur am Gymnasium Besigheim
- 1995-1998 Grundstudium Biologie an der Johannes-Gutenberg-Universität Mainz
- 1998-2001 Hauptstudium Biologie an der Universität Rostock
- 2001 Abschluß des Biologiestudiums (Hauptfach: biologische Meereskunde; Nebenfächer: Fischereibiologie und Zoologie). Diplomarbeit am Institut für Ostseeforschung (Titel: Ermittlung von Stickstoffquellen anhand von Isotopenmessungen im hypertrophen Mariager-Fjord, Dänemark)
- 2001-2005 Promotionsstipendium bei der Deutschen Bundesstiftung Umwelt
- 2003 zweimonatige Beurlaubung des Stipendiums zur Teilnahme an einer sechswöchigen Expedition nach Vietnam im Rahmen des DFG-Projektes „Pelagic Processes and Biogeochemical Fluxes in the South China Sea off Southern Central Vietnam.“