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HANNA SILVENNOINEN

Nitrogen and Greenhouse Gas Dynamics in Rivers and Estuaries of the Bothnian Bay (Northern Baltic Sea)

Doctoral dissertation

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ABSTRACT

Denitrification (microbial reduction of nitrate to molecular nitrogen and nitrous oxide) can diminish the anthropogenic nitrogen (N) load in aquatic ecosystems. This thesis focuses on denitrification rates and associated nitrous oxide (N_2O) effluxes in boreal rivers discharging into the Bothnian Bay, in the northern Baltic Sea. The capacity of river sediment denitrification to diminish N loading to the Baltic Sea and the contribution of N_2O to the gaseous end-products of denitrification were evaluated. Additionally, emissions of the greenhouse gases carbon dioxide (CO_2), methane (CH_4) and N_2O were measured from a boreal eutrophic river-estuary-bay continuum. Production of N_2 and N_2O in denitrification and the environmental factors regulating the process were studied with undisturbed sediments in a continuous water flow laboratory microcosm, which allowed control of the temperature, oxygen status and nutrient load of the sediments. Greenhouse gas emissions in the Temmesjoki River and its estuary were also studied *in situ*.

The results showed that denitrification rates in high latitude river sediments were low (330-905 µmol N m^{-2} d^{-1}) and denitrification had minor importance in reducing NO_3^- loading in the river water. At the nitrate concentrations currently prevailing in the Temmesjoki River (10 µM) no more than 8 % of the added nitrate was removed via denitrification. However, increased NO_3^- loading and temperature, and decreased near-bottom oxygen concentrations, would increase the denitrification rates. The N_2O production in sediments also increased with increased NO_3^- load, but decreased with increasing temperature. As the NO_3^- concentration in high latitude rivers is highest in winter when the temperature is low, the N_2O/N_2 ratio in denitrification is highest in winter. However, as the N_2O/N_2 ratio in denitrification in high latitude rivers always remains low (< 4%), denitrification in river sediments does not substantially increase N_2O effluxes to the atmosphere. The Temmesjoki River was a source of greenhouse gases to the atmosphere, CO_2 (70-14,300 mg CO_2-C m^{-2} d^{-1}) and CH_4 (3.2-120 mg CH_4-C m^{-2} d^{-1}) emissions being in the range reported for boreal and temperate rivers, whereas the N_2O (180-650 µg N_2O-N m^{-2} d^{-1}) emissions were lower than emissions from highly N loaded rivers but similar to emissions from pristine temperate rivers. The Temmesjoki River estuary at Liminganlahti Bay was a minor source of or sink for atmospheric greenhouse gases. Greenhouse gases emitted from the Temmesjoki River were mainly leached from the catchment. The upstream sites, surrounded by forests and peatlands, exhibited the highest CO_2 and CH_4 emissions, whereas the N_2O emissions increased towards the estuary concurrently with the appearance of agricultural fields in the downstream areas of the catchment. This shows that the greenhouse gas emissions from boreal rivers can be attributed to the land use and associated greenhouse gas production in the catchments. The greenhouse gas emissions from boreal rivers showed substantial seasonal variation. The wintertime emissions from unfrozen parts of the river were shown to be important in the annual greenhouse gas budget of the river.

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CAB Thesaurus: eutrophication; climatic change; Baltic Sea; rivers; estuaries; nitrogen; nitrate; greenhouse gases; nitrous oxide; carbon dioxide; methane; denitrification; seasonal variation

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Kuopio, December 2008

Hanna Silvennoinen

ABBREVIATIONS

Anammox	Anaerobic ammonium oxidation
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
Dn	Denitrification originating from the NO_3^- of sediment nitrification
DNRA	Dissimilatory NO_3^- reduction to ammonium
DON	Dissolved inorganic nitrogen
Dw	Denitrification originating from the NO_3^- in the overlying water
Fe^{3+}	Ferric ion
ΔG^0	Gibb's free energy change, describes the energy yield in microbiological processes
K_m	Kinetic factor, describes the concentration of substrate where the process reaches half of its maximum reaction rate according to Michaelis-Menten kinetics
Mn^{4+}	Manganese ion
N	Nitrogen
N_2	Molecular nitrogen
N_2O	Nitrous oxide
NH_2OH	Hydroxylamine
NH_4	Ammonium ion
NO	Nitric oxide
NO_2	Nitrite ion
NO_3	Nitrate ion
PON	Particulate organic nitrogen
ppm	Parts per million (10^{-6}l/l)
ppb	Parts per billion (10^{-9}l/l)
SO_4^{2-}	Sulfate ion
Q_{10}	Factor describing the relative change in reaction rate with temperature change of 10 °C

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred in the text by their chapter numbers.

Chapter II Hanna Silvennoinen, Susanna Hietanen, Anu Liikanen, C. Florian Stange, Rolf Russow, Jorma Kuparinen and Pertti J. Martikainen. 2007. Denitrification in the river estuaries of the northern Baltic Sea. Ambio 36, 134–140.

Chapter III Hanna Silvennoinen, Anu Liikanen, Jouni Torssonen, C. Florian Stange and Pertti J. Martikainen. Denitrification and nitrous oxide effluxes in boreal, eutrophic river sediments under increasing nitrate load - A laboratory microcosm study. Biogeochemistry, in press.

Chapter IV Hanna Silvennoinen, Anu Liikanen, Jouni Torssonen, C. Florian Stange and Pertti J. Martikainen. 2008. Denitrification and N_2O effluxes in the Bothnian Bay (northern Baltic Sea) river sediments as affected by temperature under different oxygen concentrations. Biogeochemistry 88, 63–72.

Chapter V Hanna Silvennoinen, Anu Liikanen, Jaana Rintala and Pertti J. Martikainen. 2008. Greenhouse gas fluxes from the eutrophic Temmesjoki River and its estuary at the Liminganlahti Bay (the Baltic Sea). Biogeochemistry 90, 193-208

CONTENTS

CHAPTER I:

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1 Background

Nutrient loading to the waterbodies has produced one of the greatest environmental challenges in northern Europe today: how to prevent eutrophication of the Baltic Sea (Kauppila & Bäck 2001, Kononen 2001). The Baltic Sea suffers from extensive loading of nitrogen (N) and phosphorus (P), originating from non-point sources that are difficult to manage, such as agriculture, forestry and peat mining. The largest single source of nutrients discharging into the Baltic Sea is agriculture (Rekolainen 1995, Rekolainen et al. 1993, Vuorenmaa et al. 2002, HELCOM 2004). The Baltic Sea can be divided into various parts that have different characteristics, the Bothnian Bay being the northernmost section, a part of which is in near-pristine condition. The major fraction of dissolved inorganic nitrogen (DIN) leaching from the catchments to the river is nitrate (NO_3^-) derived from inorganic fertilizers (Kronholm et al. 2005). Increasing concentrations of organic N have recently been reported from boreal streams (Lepistö et al. 2008). Presently scientists debate the importance of internal and external loading especially in the case of phosphorus. However, external loading not only influences the Baltic Sea but also the intermediate ecosystems between it and the terrestrial ecosystems. Lakes, rivers and estuaries are simultaneously affected by the increased loading.

Microbial processes in aquatic ecosystems can remove significant amounts of the harmful inorganic nitrogen that causes eutrophication. Denitrification has often been referred to as the most important of these processes. In some aquatic ecosystems, it has been estimated to remove even up to 40-50 % of the external N loading (e.g. Seitzinger 1988, Laursen & Seitzinger 2004). In denitrification, DIN in aquatic ecosystems is reduced to gaseous forms of N, molecular nitrogen (N_2) and nitrous oxide (N_2O), and thereby is removed from aquatic ecosystems and enters the atmosphere. Molecular nitrogen is an abundant (78%), inert gas in the atmosphere, but N_2O is an important greenhouse gas, accounting for nearly 6% of the anthropogenic greenhouse effect (IPCC, 2007). Therefore, denitrification, while diminishing anthropogenic N loads in aquatic ecosystems, might transform this water protection problem into an atmospheric one by increasing emissions of N_2O to the atmosphere (Law & Owens 1990, Middelburg 1996, Kroeze & Seitzinger 1998, Seitzinger 1998).

Several environmental factors affect the amount of N processed in denitrification and the relative amounts of the two gases, N_2 and N_2O, produced in the process. Understanding these regulatory factors is important in denitrification studies (Piña-Ochoa & Álvarez-Cobelas 2006). Over the past decades studies on denitrification have been challenging mainly due to high atmospheric background concentrations of the end product (N_2) and due to the variety of factors controlling denitrification and causing high spatial and temporal variation in denitrification rates. Although much research has been done on denitrification during recent years in marine, estuarine and riverine ecosystems, neither denitrification rates nor N_2O production have been studied in high latitude rivers and estuaries.

Eutrophication of watercourses has additionally had an impact on production of carbon dioxide (CO_2), the most abundant greenhouse gas, and methane (CH_4). It is known that rivers and estuaries contribute to the global budgets of these gases, but there is a lack of data on greenhouse gas (CO_2, CH_4 and N_2O) dynamics from high latitude rivers and estuaries. Boreal rivers and estuaries have special characteristics compared to more southerly aquatic ecosystems, where data on emissions are available. Boreal rivers and estuaries, in contrast to those in more southerly regions, are annually subjected to approximately five months of cold.

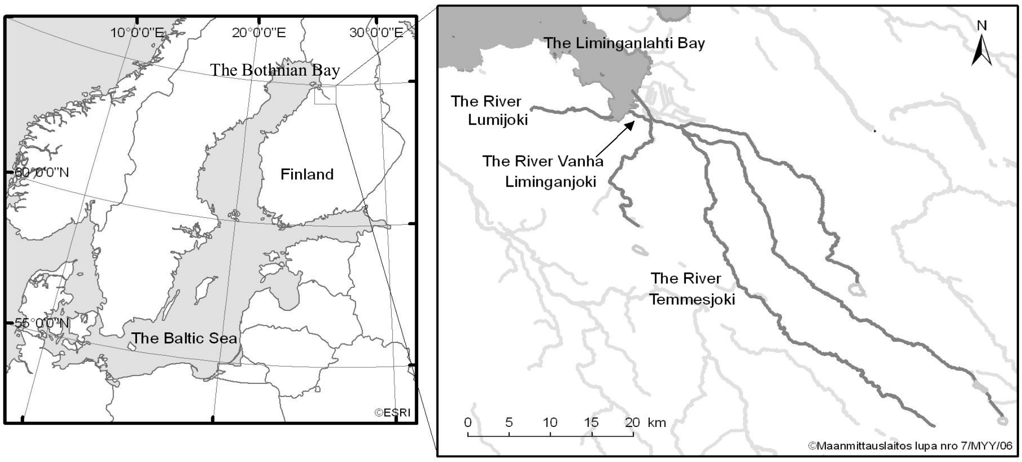

Figure 1. The Temmesjoki River and the Liminganlahti Bay at the Bothnian Bay

1.2. The Bothnian Bay and the rivers discharging to it

The Bothnian Bay is the northernmost part of the Baltic Sea, receiving an increasing nutrient and organic carbon load primarily via Finnish rivers (HELCOM 1998). The mean annual N load from the largest 24 rivers to the Bothnian Bay during the years 1995-2000 was 47,200 tons. This accounts for >90 % of the total annual N load, the remaining part originating from sewage treatment and industrial plants (Kronholm et al. 2005). The Bothnian Bay differs substantially from other parts of the Baltic Sea: The area is less saline due to physical separation from the Baltic Proper by shallow sills at the Archipelago Sea and large freshwater inflow, the open sea area of Bothnian Bay is in a near-pristine state and harmful cyanobacterial blooms are rarely observed and the primary production in the open sea area is generally limited by phosphorus (P) rather than nitrogen (N) (Tamminen et al 2007).

Rivers are links between terrestrial and marine ecosystems, receiving, transporting and processing nutrients and organic matter (Raymond & Bauer 2001, Wetzel 2001). They also act as important conduits for gas exchange between terrestrial ecosystems and the atmosphere (Kling et al. 1991, Cole & Caraco 2001, Hope et al. 2001, Richey et al. 2002, Mayorga et al. 2005, Cole et al. 2007).

The rivers entering the Bothnian Bay and the coastal waters are eutrophic and receive increasing loads of NO_3^- (Kronholm et al. 2005). Dissolved inorganic nitrogen (DIN) leaches from catchments. Anthropogenic diffuse sources (e.g. agriculture and forestry) have a great importance in the total N load.

1.2.1 The Temmesjoki River

Most of the research in this thesis was done at the Temmesjoki River and its estuary at Liminganlahti Bay (Fig. 1). The Temmesjoki River represents one of the most eutrophic rivers discharging to the Bothnian Bay. It is classified as polytrophic for total P and eupolytrophic for total N (classification according to Vollenweider 1968), with NO_3^- concentrations having seasonal variations from 10 to 100 μmol N l^{-1}. It has a relatively small annual mean flow (11 $m^3 s^{-1}$) and the drainage basin is small in size (1190 km^2). Therefore, the annual N load leaching via the Temmesjoki River to the Bothnian Bay is rather low (520 tons) despite the high N concentrations. As the Temmesjoki River catchment has low lake coverage it represents an ideal site for studying the effects of river channel processes on the N cycle.

1.3 Microbiological processes related to N and C cycles in sediments

The Earth's most abundant form of N is molecular nitrogen (N_2, oxidation state of N = 0, 78 % in the air). Other important gaseous N species are nitrous oxide (N_2O, oxidation state of N = +1) and nitric oxide (NO, oxidation state of N = +2), which contribute to global climate change (see chapter 1.4). Dissolved species of N are nitrate (NO_3^-, oxidation state of N = +5) nitrite (NO_2^-, oxidation state of N = +3) and ammonium (NH_4^+, oxidation state of N = -3), which are important nutrients for plants and microbes. In boreal river water N is also present in particulate (PON) and dissolved organic forms (DON) (Stepanauskas et al. 2002).

Microbial oxidation/degradation of organic matter in sediments is vertically distributed according to the prevailing electron acceptors (Fig 2). As an electron acceptor oxygen (O_2) is thermodynamically most favourable, leading to the highest energy yield. Oxygen is followed by NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} and CO_2^-, respectively (Capone & Kiene 1988). Thus, respiration and other O_2 requiring processes (e.g. nitrification) dominate in the uppermost sediment layers, where O_2 is available. Microbial processes are dependent on the vertical diffusion and availability of the electron acceptors.

NO_3^- has a key role in the nitrogen cycle of the river sediments: Due to its high solubility and application as a fertilizer, it is the most abundant N species leaching into rivers. For microbes, NO_3^- acts both as a nitrogen source and an electron acceptor, which affects the degradation and degradation mechanisms of organic matter.

1.3.1 Autotrophic nitrification

Nitrification is an autotrophic (chemolithotrophic), aerobic process in which NH_4^+ is oxidized to NO_2^- and further to NO_3^- (Fig. 2). In contrast to the denitrifying bacteria (see next paragraph) nitrifying bacteria are not dependent on the supply of organic carbon because they use CO_2 as their carbon source. Nitrifiers use either NH_4^+ (NH_4^+ oxidizers) or NO_2^- (NO_2^- oxidizers) as energy sources. As a process requiring O_2, nitrification proceeds in the upper layers of sediment, above the denitrifying layer, and thus supplies denitrification with NO_3^-. In terms of N removal from aquatic ecosystems, nitrification is important as a supplier of NO_3^- for denitrification, especially if the external N load consists of NH_4^+, or of organic matter mineralized to NH_4^+ in the recipient. In some cases, e.g. in sea floors and lake sediments, O_2 is depleted and limits or prevents nitrification (Jenkins & Kemp 1985). Nitrification has been reported to liberate N_2O as a side product in terrestrial ecosystems (Tortoso & Hutchinson 1990).

1.3.2 Denitrification and its regulatory factors

Denitrification is a microbial process whereby NO_3^- is reduced via NO_2^- , NO and N_2O to N_2 (Fig. 2). In aquatic ecosystems, where external N loading is low, denitrification is limited by the availability of NO_3^- and is therefore dependent on other microbial processes, i.e. coupled mineralization/ ammonification and nitrification, in the sediment supplying denitrification with NO_3^-The total denitrification in sediments can be divided into two types, *uncoupled* and *coupled* denitrification. The denitrification supplied by NO_3^- originating from the overlying water is uncoupled denitrification (often referred to as Dw) and is regulated by the vertical diffusion of the NO_3^- through the top sediment layers.

Dw has been found to dominate in aquatic ecosystems where NO_3^- concentrations in the water are high (Pind et al. 1993, Rysgaard et al. 1995). Denitrification supplied by NO_3^- originating from nitrification in the top sediment layers is called coupled denitrification (or coupled nitrification denitrification, referred to as Dn) (Jenkins & Kemp 1985).

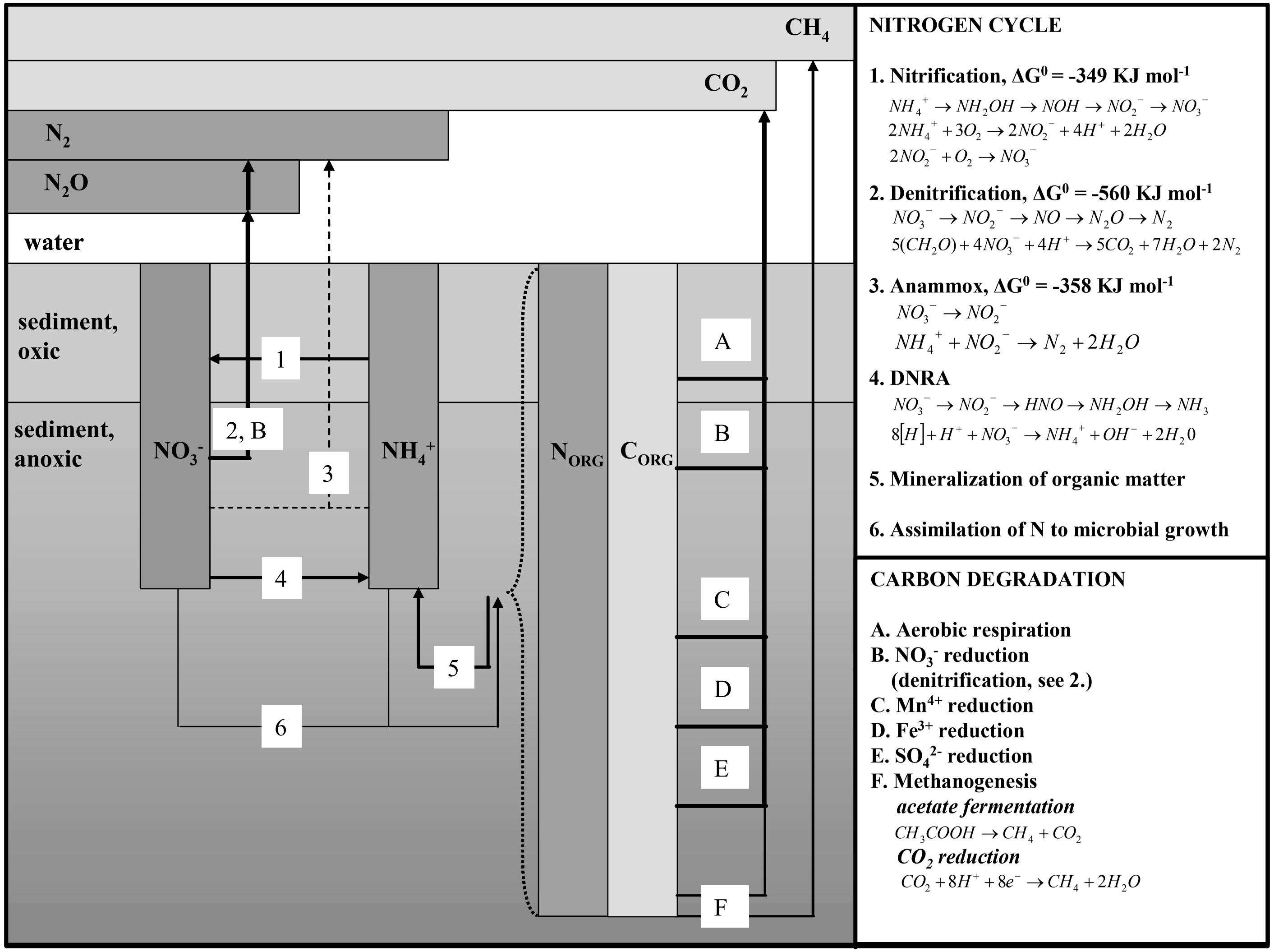

Figure 2. Processes related to N and C cycling.

The most important environmental factors regulating denitrification include temperature, availabilities of NO_3^- and O_2 and energy sources. Oxygen availability regulates denitrification, because denitrifying bacteria, as facultative anaerobes, replace oxygen with NO_3^- as the electron acceptor only when O_2 has been consumed. Therefore, vertical diffusion of O_2 and NO_3^- in the sediments highly regulates denitrification. As a majority of denitrifying bacteria are chemo-organotrophs, availability of labile organic carbon regulates denitrification. Depending on the sediment quality and the type of ecosystem, denitrification might be well supplied by the internal carbon mineralization of the sediment or it might be dependent on the external transportation of organic carbon into the sediment from overlying water.

The ratio of N_2O to N_2 produced in denitrification is regulated by several environmental factors. The last step of denitrification (reduction of N_2O to N_2) is catalyzed by the N_2O reductase enzyme. Studies conducted in soil ecosystems reveal a reduction in the activity of this enzyme as temperature decreases, increasing the N_2O/N_2 ratio (Mellin & Nõmmik 1983, Maag & Vinther 1996). The increased NO_3^- concentrations have been reported to increase the N_2O/N_2 ratio in both terrestrial and aquatic ecosystems (Koch et al. 1992, Oremland 1984, Oren & Blackburn 1979). Of these gases, N_2O is an effective greenhouse gas (see chapter 1.4) whereas production of inert N_2 is more beneficial in terms of atmospheric impact.

1.3.3 Anammox, DNRA and assimilation of N to the microbial biomass

In addition to denitrification, there are other processes in sediments that can transform NO_3^-. Anaerobic ammonium oxidation (anammox) is a chemolithotrophic oxidation of NH_4^+ with NO_2^- to N_2 (Fig. 2). In order to be consumed in anammox, NO_3^- must first be reduced to NO_2^-. Dissimilatory nitrate reduction to ammonium (DNRA) reduces NO_3^- to NH_4^+ (Fig. 2) in strictly anaerobic conditions. It has also been referred to as “nitrate fermentation”. DNRA has been reported to produce N_2O as a side product in terrestrial ecosystems (Smith & Zimmerman 1981). NO_3^- and NH_4^+ can be assimilated by microbes to their biomass. This assimilation is, particularly in soil sciences, also called immobilisation. Assimilation is the reverse process to mineralization, which liberates NH_4^+ when organic matter is decomposed.

1.3.4 Ecological importance of the processes in removing NO_3^-

Denitrification and anammox are both equally important in removing NO_3^- from water: they both reduce NO_3^- to gaseous N species, thus eliminating NO_3^- permanently from the water phase. Thermodynamically, anammox is similar to nitrification (oxidation of NH_4^+ with O_2), leading to an approximately similar energy yield (Strous and Jetten 2004). Both of these chemolithotrophic processes are thermodynamically less favorable than heterotrophic denitrification. Aerobic nitrifying bacteria as well as anammox bacteria have very slow growth rates, the latter being the most slow growing. Anammox, discovered rather recently, is important at least in marine sediments (e.g. Arrigo, 2005; Hulth et al., 2005, Risgaard-Petersen et al. 2003, Thamdrup & Dalsgaard 2002, Trimmer et al. 2006), and it has also been reported to occur in the sediments of the Gulf of Finland (Hietanen et al. 2008).

In contrast to denitrification and anammox, the end product of DNRA (NH_4^+) is easily available to primary producers and heterotrophic microbes and can, like NO_3^-, be assimilated. Nitrate is therefore only temporarily removed in DNRA. Generally denitrification has been recognized as the most important process for removing NO_3^-, but several studies have reported high levels of DNRA especially in marine sediments (Table 1).

Table 1. The percentage of N removed in different processes in riverine and coastal ecosystems

Ecosystem	Denitrification (%)	DNRA (%)	Assimilation (%)	Source
Bothnian Bay	23	-	-	Stockenberg & Johnstone 1998
Baltic Sea	74	-	-	Seitzinger & Giblin 1996
coastal marine	17	-	-	Giblin et al. 1997
coastal marine	2-54	-	-	Nixon et al. 1996
coastal marine	63	32	4	Goyens et al. 1987
coastal marine	70 -95	-	-	Kaspar 1983
coastal marine	38 -90	7 -3	-	Koike & Hattori, 1978
coastal marine	32 -54	56	-	Sørensen, 1978
coastal marine	3	-	-	Kim et al. 1997
coastal marine	0-43	18-100	-	Bonin et al. 1998
coastal marine	-	80	-	Bonin 1996
coastal marine	-	1.6-10	-	Enoksson & Samuelsson 1987
coastal marine	82-100	-	-	Kaspar et al. 1985
coastal marine	27-57	-	-	Nishio et al. 1982
Estuary	13 -51	4 -21	<5	Jørgensen 1989
Estuary	29	15-75	-	An & Gardner 2002
Estuary	40	-	-	Nixon et al. 1996
marsh estuary	-	0-23	-	Ma & Aelion 2005
Riparian wetland	29	49	22	Matheson et al. 2002
river	-	30	-	Brunet & Garcia- Gill 1996
river	15-30	-	-	Pfenning & McMahon 1996

DNRA was widely studied in marine sediments, especially in the 70's and 80's, and in some studies it was found to be an important pathway for NO_3^- reduction in coastal marine and estuarine sediments (e.g. Blackmer & Bremner 1978, Enoksson 1987, Goyens 1987, Jørgensen 1989). DNRA has also been found in anaerobic sediments of rivers (Kelso et al. 1997). Brunet and Garcia-Gill (1996) reported that DNRA was responsible for as much as 30 % of the NO_3^- loss in temperate river sediments. Several studies report the coexistence of DNRA and denitrification (Table 1). Long term laboratory incubation experiments run the risk of misinterpreting increased NH_4^+ liberation as enhanced DNRA, although the NH_4^+ could as well originate from sediment mineralization. Assimilation of NO_3^- to the organic nitrogen pool, related to the growth of microbes, has been reported in only a few studies. It was found from an estuary (Jørgensen 1989) and a riparian wetland (Matheson et al. 2002) and from coastal marine sediments (Goyens et al. 1987) (Table 1).

Prevailing environmental conditions regulate these processes and their importance in NO_3^- removal (e.g. Kaspar et al. 1985, Jørgensen & Sørensen 1988, Jørgensen 1989, Bonin et al. 1998). Regarding denitrification and DNRA, several theories have been presented regarding the factors favoring one process over the other: Low availability of electron donors (carbon), promotes denitrification and low availability of electron acceptors (NO_3^-) promotes DNRA (Brunet & Garcia-Gill 1996, Laverman et al. 2006, Jørgensen 1989). In estuarine sediments, denitrification was favoured only in a narrow temperature range, from 14 to 17°C, whereas DNRA prevailed at temperatures below and above this range (Kelly-Gerreyn et al. 2001). The simultaneous presence of sulfide has been found to induce DNRA via inhibition of NO- and N_2O -reductases (Brunet & Garcia-Gil 1996, An & Gardner 2002). Low redox conditions stimulate DNRA at the expense of denitrification in estuarine sediments (Cole 1996), and thus DNRA prevails in deeper sediment layers (Enoksson & Samuelsson 1987).

1.4. Greenhouse gas dynamics in rivers and estuaries

Carbon dioxide (CO_2) is the second most abundant greenhouse gas in the atmosphere after water vapor. The atmospheric concentration of CO_2 has increased due to anthropogenic activity, being currently 379 ppm (IPCC 2007). CO_2 is produced in both aerobic and anaerobic decomposition of organic matter (Fig. 2) and is consumed mainly in photosynthesis.

Methane (CH_4) is 23 times more powerful as a greenhouse gas than CO_2, with a time horizon of 100 years. Its current concentration in the atmosphere is 1.7 ppm (IPCC 2007). Methane is formed by methanogenetic microbes in methanogenesis (either in acetate fermentation or in CO_2 reduction), which is one of the terminal processes in anaerobic carbon degradation (Fig. 2). Aquatic ecosystems and water saturated terrestrial ecosystems, like wetlands and riparian zones, that exhibit anaerobic conditions have high CH_4 production and emissions (Cicerone & Oremland 1988). In aerobic conditions methanotrophic bacteria consume a fraction of the CH_4 in aerobic methane oxidation to CO_2 (Hanson & Hanson 1996). Methane oxidation therefore reduces CH_4 emissions to the atmosphere. From non-vegetated sediments, CH_4 can be liberated to the water and further to the atmosphere by diffusion and, as it is poorly soluble in water, by ebullition (Chanton 1989).

Nitrous oxide is 298 times more powerful as a greenhouse gas than CO_2, with a time horizon of 100 years, and its current concentration in the atmosphere is 319 ppb (IPCC 2007). In sediments, N_2O is formed as an intermediate in denitrification (Fig. 2) and to some extent as a side-product of nitrification and DNRA (see Chapter 1.3)

Gases emitted from rivers can be produced *in situ* from the organic matter and inorganic nitrogen species leached from the catchments, or the gases produced in terrestrial ecosystems can be leached and degassed from the river. Surface runoff and groundwater discharging to the rivers have been found to contain high amounts of greenhouse gases originally produced in the terrestrial ecosystems of the catchment (Jones & Mulholland 1998a and b, Hasegawa et al. 2000; Hope et al. 2001). Therefore, most of the gases emitted from rivers are associated with the processes and the nutrients/carbon of terrestrial ecosystems. (de Angelis & Lilley 1987; Jones & Mulholland 1998a; Reay et al. 2003; Mayorga et al. 2005). By processing nutrients and organic matter to gases, river ecosystems are able to remove part of the C and N load of marine ecosystems, but simultaneously they are sources of greenhouse gases to the atmosphere. However, a great part of the nutrients, organic matter and greenhouse gases discharging to rivers ends up in estuaries (Bange et al. 1994, Bange et al. 1996, Frankignoulle et al. 1998, Seitzinger & Kroeze 1998, Borges 2005)

Estuaries and coastal areas are considered to be important sources of greenhouse gases. They are net sources of CO_2, whereas marginal seas are net sinks for this gas (Borges 2005). Coastal areas contribute up to 75 % and 35-60 % of the total marine CH_4 (Bange et al. 1996) and N_2O (Bange et al. 1996, Seitzinger et al. 2000) emissions, respectively.

1.5 Methods of the study

In prior studies denitrification activity has been determined either directly by measuring the production rate of the end products or indirectly from the fluxes of DIN species, O_2 and DIC. Measuring denitrification directly from the end products is difficult, because of the high background concentration of N_2 in the atmosphere.

Denitrification has been measured by the acetylene inhibition technique. Acetylene inhibits N_2O reductase, the enzyme catalysing the last reduction step in denitrification. Therefore, only the easily measurable N_2O should accumulate with acetylene treatment. However, this technique has problems such as simultaneous inhibition of nitrification (Seitzinger et al. 1993), reversal blockage by sulfide (Sørensen et al. 1987), incomplete blockage by acetylene (Christensen et al. 1989) and scavenging of intermediate NO (Bollman & Conrad 1997). Therefore, use of the acetylene inhibition technique can underestimate denitrification rates. Acetylene inhibition, however, is informative, particularly in agricultural soils that are non-watersaturated and in which the supply of NO_3^- is not limited (Groffman et al. 2006).

Benthic fluxes of N_2 have been measured directly with GC by incubating the sediments in air tight cores (Seitzinger 1987, Nowicki 1994, Devol 1991, Devol & Christensen 1993, LaMontagne & Valiela 1995). Direct mass spectrometric measurements based on the altered N_2/Ar ratio are reported (Kana et al. 1998, Cornwell et al. 1999).

With the development of the mass spectrometers, various stable isotope techniques ($^{15}N/^{14}N$) are now applied in denitrification studies (Nishio et al. 1983, Jenkins & Kemp 1985). Nielsen et al. (1992) introduced an isotope pairing technique that allows determination of Dn and Dw. There has been some criticism against this technique concerning the possible incomplete mixing of the labelled and non-labelled N pools (Middelburg et al. 1996). Additionally, when added in-correctly to N limited systems, ^{15}N has a fertilizing effect that may bias the results (Groffman et al 2006). However, this technique is commonly used and considered to produce reliable denitrification estimates (Cornwell et al. 1999). ^{15}N techniques have an additional benefit, as the amended ^{15}N can be traced back from various N pools, and thus the technique allows the study of the overall N cycling in the sediment.

Riverine denitrification can be estimated from mass balance approaches (e.g. Pribyl et al. 2005, David & Gentry 2000, Rekolainen et al. 1995). Mass balance technique provides an insight into the potential importance of denitrification, but is limited in giving quantitative measurements of denitrification (Groffman 2006). The fate of removed nitrogen cannot be unambiguously determined with mass balance technique. The ^{15}N technique used in this study allows process separation. However, extrapolating these results to the ecosystem scale (e.g. nitrate removal in the course of the entire river) is challenging and would require modelling.

In this thesis, the experiments related to the denitrification rates and N_2O/N_2 ratios were done with undisturbed sediment cores in a continuous flow laboratory microcosm, which allows regulation of ion concentration loading of the experimental sediment, O_2 status and temperature of the system (Liikanen et al. 2002) (Chapters II- IV). Water amended with

^{15}N –labelled NO_3^- (60-98 at.%) was allowed to flow over the sediments (Chapters II-IV). Concentrations of N_2, N_2O and DIC and the ^{15}N abundances of N_2 and N_2O, were measured from the effluent waters. The NO_3^- removal in denitrification was estimated from the benthic fluxes of ^{15}N and $^{15}N_2O$ in relation to the isotopic dilution of effluent $^{15}NO_3^-$.

Greenhouse gas dynamics were measured *in situ* at the Temmesjoki River and at Liminganlahti Bay (Chapter V). Atmospheric fluxes were calculated from the measured surface water gas concentrations, wind speeds and water current velocities (Borges et al. 2004, Kremer et al. 2003, Wannikof et al. 1992, O'Connor & Dobbins 1958) for the Temmesjoki River and Liminganlahti Bay and additionally with floating static chambers (Huttunen et al. 2002) for the bay (Chapter V). The degree of saturation of gases in water was calculated by comparing the measured gas concentrations in the water samples to that in equilibrium with the atmosphere (Chapter V).

1.6 Objectives and hypothesis

The overall objectives of this thesis were to estimate NO_3^- retention in boreal river sediments and greenhouse gas emissions from them. The focus was also on regulatory factors for denitrification. More specific objectives of thesis were to:

Study denitrification rates in sediments of the rivers discharging to the Bothnian Bay were studied. Denitrification rates have been measured from lower latitude aquatic rivers and also from boreal lakes, but there is a lack of data on denitrification rates of high latitude rivers. High latitude rivers differ substantially from those at lower latitude, showing more profound seasonal variation and often lower nutrient concentrations.

Estimate the importance of denitrification in diminishing NO_3^- load to the Bothnian Bay. Denitrification may diminish anthropogenic N loading from eutrophic high latitude rivers and thus have a positive impact on water quality.

Estimate the magnitude of N_2O production in denitrification. High amounts of N_2O can be produced by denitrification. If this were the case, the water protection problem (high NO_3^- concentration) would be turned into an atmospheric problem (increased emissions of N_2O).

Evaluate the importance of main environmental factors in regulation of denitrification. The main factors controlling denitrification are NO_3^- and O_2 concentrations and temperature. The regulation of these processes, both denitrification and the N_2O/N_2 ratio of denitrification, is based on the variation in these driving factors.

Measure the greenhouse gas (CO_2, CH_4, N_2O) emissions, and their spatial and seasonal variation in boreal rivers and estuaries. There are only a few studies on CH_4 and CO_2 emissions from boreal rivers and estuaries, and their N_2O emission estimates are based on N input to watersheds (Seitzinger & Kroeze 1998, Seitzinger et al. 2000), not on gas flux studies *in situ*. Recent reviews have pointed out that data from river environments at high latitudes are needed for the producing global greenhouse gas budgets (Uppstill-Goddard et al. 2000, Borges 2005).

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CHAPTER II:

DENITRIFICATION IN THE RIVER ESTUARIES OF THE NORTHERN BALTIC SEA.

Hanna Silvennoinen, Susanna Hietanen, Anu Liikanen, C. Florian Stange, Rolf Russow, Jorma Kuparinen and Pertti J. Martikainen. 2007. *Ambio* 36, 134-140.

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Hanna Silvennoinen, Susanna Hietanen, Anu Liikanen, C. Florian Stange, Rolf Russow, Jorma Kuparinen and Pertti J. Martikainen

Denitrification in the River Estuaries of the Northern Baltic Sea

Estuaries have been suggested to have an important role in reducing the nitrogen load transported to the sea. We measured denitrification rates in six estuaries of the northern Baltic Sea. Four of them were river mouths in the Bothnian Bay (northern Gulf of Bothnia), and two were estuary bays, one in the Archipelago Sea (southern Gulf of Bothnia) and the other in the Gulf of Finland. Denitrification rates in the four river mouths varied between 330 and 905 μmol N m^{-2} d^{-1}. The estuary bays at the Archipelago Sea and the Gulf of Bothnia had denitrification rates from 90 μmol N m^{-2} d^{-1} to 910 μmol N m^{-2} d^{-1} and from 230 μmol N m^{-2} d^{-1} to 320 μmol N m^{-2} d^{-1}, respectively. Denitrification removed 3.6–9.0% of the total nitrogen loading in the river mouths and in the estuary bay in the Gulf of Finland, where the residence times were short. In the estuary bay with a long residence time, in the Archipelago Sea, up to 4.5% of nitrate loading and 19% of nitrogen loading were removed before entering the sea. According to our results, the sediments of the fast-flowing rivers and the estuary areas with short residence times have a limited capacity to reduce the nitrogen load to the Baltic Sea.

INTRODUCTION

The Baltic Sea is a large brackish water basin suffering from eutrophication. The drainage area of the sea is four times larger than the sea itself, and populated by 85 million people. The Gulf of Bothnia is the northernmost basin of the Baltic Sea. Shallow sills at the Archipelago Sea prevent the inflow of the more saline water. This isolation from the rest of the Baltic Sea, together with the large freshwater inflow, makes the area less saline than the northern Baltic Sea in general. Primary production in the Gulf of Bothnia is phosphorus (P) limited and, unlike the rest of the Baltic Sea, the gulf is in an ecologically good state. Annual nitrogen (N) loading to the gulf was 74 100 000 kg in 2000 (1). Since the 1990s, increasing nitrate concentrations have been measured especially in Finnish coastal-water rivers entering the Gulf of Bothnia (2).

The Gulf of Finland is an estuary-like area, directly connected to the Baltic Proper at its western end and under the influence of the river Neva at the eastern end. In contrast to the Gulf of Bothnia, it is heavily eutrophied. Nutrient loading into the Gulf of Finland has been decreasing in the last decades because of the active protection of the Gulf of Finland and economic depression in the surrounding states of Russia and Estonia (3). However, 120 000 tonnes of N still enter the Gulf of Finland every year (4).

The fate of N entering the aquatic ecosystem depends on the prevailing conditions. Organic N compounds are decomposed by microbes to ammonium (NH_4^+), which can be taken up by primary producers, or nitrified by bacteria to nitrate (NO_3^-). NO_3^- can be taken up or processed further in denitrification, anaerobic ammonium oxidation (anammox), or dissimilatory NO_3^- reduction to NH_4^+ (DNRA). Of these processes, DNRA reduces NO_3^- back to NH_4^+, still available to other organisms. N taken up by primary producers is bound to the biomass and later released back to the water ecosystem. Denitrification (the sequential reduction of NO_3^- to nitrogen gas [N_2]) and anammox (oxidizing of NH_4^+ with nitrite [NO_2^-] to N_2) are the only processes that remove N permanently from the system. Denitrification, but not anammox, may have an effect on global warming via the gaseous intermediate, nitrous oxide (N_2O). N_2, the end product of denitrification, is abundant (78%) in the atmosphere, whereas the intermediate N_2O is an effective greenhouse gas. The ratio of these gases produced by denitrification in aquatic ecosystems is affected by several environmental factors, e.g., temperature, oxygen concentration, and NO_3^- availability (5–7). In sediments, usually less than 5% of the gases produced in denitrification is N_2O (8). Nevertheless, it has been suggested that in areas with high NO_3^- load, such as river mouths and estuaries, N_2O production may be enhanced (8).

Denitrification has been estimated to remove 30% of the annual N loading in the Gulf of Finland (9), and 23% in the Bothnian Bay (10). Both of these estimates are based on results from the depositional areas of the open sea. Estuaries have been suggested to be effective sinks of N loading, reducing the load transported to the sea (8, 11, 12). The few estimates of the filtering capacity published so far from the northern Baltic Sea seem to challenge this view (Hietanen, S. and Kuparinen, J. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea, submitted for publication) (13, 14). More data is needed to understand the capacity of these ecosystems to reduce the N load entering the sea.

We measured denitrification rates in six estuaries of the northern Baltic Sea. Four of them were river mouths in the Bothnian Bay (northern Gulf of Bothnia), and two were estuary bays, one in the Archipelago Sea (southern Gulf of Bothnia) and the other in the Gulf of Finland (Fig. 1). Two projects, both part of the Baltic Sea Research Programme (BIREME) of the Academy of Finland (2003–2006) contributed to this work. Two ^{15}N methods were applied in the studies. The CoastGas project (University of Kuopio, Finland, and Hemholtz Centre for Environmental Research–Umweltforschungzentrum [UFZ] Leipzig-Halle, Germany) measured NO_3^- removal in the river mouths using a flow-through method, and the SEGUE-N project (searching efficient protection strategies for the eutrophied Gulf of Finland: the integrated use of experimental and modelling tools; N: subproject 2: nitrogen fluxes at the sediment-water interface; University of Helsinki, Finland) measured denitrification in the two estuary bays using the isotope pairing technique (15). The measured rates were compared to the local N loading to estimate the filtering capacity of the estuaries.

MATERIALS AND METHODS

Area Descriptions

In the Bothnian Bay, the study sites were at river mouths of Temmesjoki, Siikajoki, Pyhäjoki and Kalajoki (Fig. 1A). The

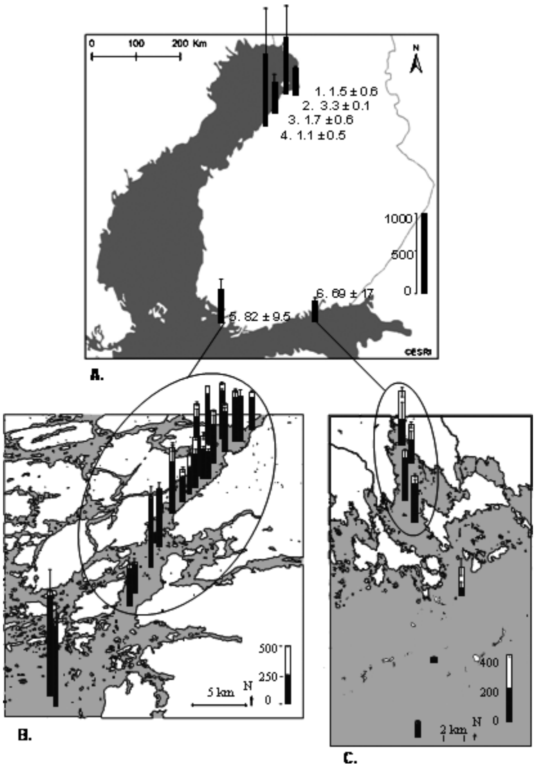

Figure 1. (A) Denitrification in the rivers Temmesjoki (1), Siikajoki (2), Pyhäjoki (3), and Kalajoki (4), in the Paimionlahti Bay (5) and in the Ahvenkoskenlahti Bay (6). Black columns = total denitrification (Dtot). Numbers = the percentage of coupled nitrification-denitrification (Dn) of Dtot. (B) Black columns = Dn, white columns = denitrification based on the NO_3^- in the overlying water. in the Paimionlahti Bay and (C) in the Ahvenkoskenlahti Bay. Averages and standard deviations shown. The circled sites are inside the estuary.

catchments of these rivers contain mainly peatlands and forests, and water systems contain only few lakes. Therefore, the river water is rich in organic matter. The N load to the rivers is at its highest in April–May, diminishes rapidly toward summer months, and peaks again slightly in late autumn. In the Archipelago Sea the study site was at the Paimionlahti Bay, an estuary of the river Paimionjoki (Fig. 1A). The discharges of both freshwater and N compounds to the bay are remarkably pulsed, with half of the N loading reaching the estuary in April–May and most of the other half late in the autumn. The flow diminishes substantially for the summer months. In the Gulf of Finland, the study site was at the Ahvenkoskenlahti Bay, which is a semi-enclosed estuary receiving loading from the rivers Kymijoki and Taasianjoki (Fig. 1A). The discharge and the N loading fluctuate only modestly from season to season and are usually highest in April–May. In contrast to the rivers in the Bothnian Bay, the catchments of rivers Kymijoki and Taasianjoki have a low coverage of peatlands, but are significantly affected by agriculture. The Paimionlahti Bay has a catchment with low lake content, whereas the catchment of the Ahvenkoskenlahti Bay contains several lakes.

Denitrification Measurements in the Laboratory Experiments (Rivers Temmesjoki, Siikajoki, Pyhäjoki, and Kalajoki)

Intact sediment cores were taken from three sampling sites in the river Temmesjoki and four sites in the rivers Siikajoki, Pyhäjoki, and Kalajoki in August 2004. The sediment cores were incubated in a laboratory microcosm for 3 weeks. Samples for the determination of denitrification were collected six times from each core during the last 2 weeks.

Sediments were taken directly to transparent acrylic cores (ø 94 mm, height 650 mm). The height of the intact sediments in the cores was ≤200 mm. The sediment cores were placed into a laboratory microcosm equipped with a continuous water flow (16). The microcosm was situated in a dark, temperature-controlled room at 15°C, similar to the *in situ* temperatures in river waters during sampling (13–20°C, Table 1). Water was pumped from an 80-L water reservoir over the cores by a peristaltic pump (Ismatec, BVK-MS/CA8–6, Glattbrugg-Zürich, Switzerland) at the rate of 50 mL h^{-1}. Overlying water in the cores was gently stirred with a rotating magnet to prevent stratification (16).

The water reservoir was vacuumed three times to remove N_2 from the gas phase of the water and flushed each time with a gas mixture consisting of Ar/O_2 80/20 (v/v) (AGA, Finland) to improve the sensitivity of the concentration analyses of N_2 formed in denitrification. Distilled water, amended with *in situ* concentrations of sulfate (0.2 mM) and chloride (0.5 mM) (added as $MgSO_4$ and $CaCl_2$), was used as inflow water. The sediments were incubated at 30 μM of $^{15}NO_3^-$ (60 at %). The fluxes of NO_3^- and gases were measured from the difference between concentrations in the in- and outflowing water. During the experiment, the NO_3^- concentrations and the isotopic composition ($^{15}N/^{14}N$) of the NO_3 of the in- and outflowing water were determined three times in each week (fourth, fifth, and sixth incubation days). Samples were stored at −20°C before analyses. NO_3^- and SO_4^{2-} concentrations were measured with ion chromatography (Dionex DX-130, Sunnyvale, CA,

Table 1. Description of the study areas.

						Annual		During sampling				
	Drainage basin (km^2)	**Cultivated field (%)**	**Peatlands (%)**	**Forests (%)**	**Lakes (%)**	**Mean flow (m^3s^{-1})**	**N load (tons)**	**NO_3-N (μM)**	**DIN/N_{tot} (%)**	**NO_3/DIN (%)**	**Temp (°C)**	**O_2 (μM)**
River Temmesjoki Estuary	1 184	15	2	82	0.5	11	520	80	48	88	14–18	ND
River Siikajoki Estuary	4 318	8	3	87	2	60	1 700	20	26	92	15–20	ND
River Pyhajoki Estuary	3 724	10	0	85	5	40	1 620	25	23	95	15–19	ND
River Kalajoki Estuary	4 247	16	0	82	2	50	4 000	70	44	98	13–20	ND
Paimionlahti Bay	1 088	42	4	45	2.7	7	950	2–24	100	99	8–11	65–250
Ahvenkoskenlahti Bay	37 158	9	11	56	19	175	3 540	3–10	29	88	8–18	170–280

ND = not determined; DIN = dissolved inorganic nitrogen.

Table 2. Oxygen demand (mmol m^{-2} d^{-1})and fluxes of nitrous oxide (μmol N m^{-2} d^{-1}), methane, carbon dioxide, and inorganic nitrogen species from sediment to the water (mmol m^{-2} d^{-1}) and pH in the manipulation experiments (average and standard deviation).

	N	O_2 demand	N_2O-N	CH_4-C	DIC	NO_3-N	NH_4-N	pH
River Temmesjoki Estuary	3	19 ± 2.0	15.1 ± 3.4	0.8 ± 0.7	37 ± 4.5	−0.3 ± 0.2	3.7 ± 1.5	5.4 ± 0.1
River Siikajoki Estuary	4	20 ± 2.5	11.4 ± 5.6	0.02 ± 0.01	16 ± 2.0	−0.3 ± 0.2	3.0 ± 0.8	5.3 ± 0.1
River Pyhajoki Estuary	4	24 ± 4.1	13.6 ± 4.3	0.6 ± 0.5	20 ± 4.11	−0.4 ± 0.2	0.9 ± 0.1	5.6 ± 0.2
River Kalajoki Estuary	4	27 ± 5.8	15.6 ± 6.7	2.3 ± 2.2	21 ± 5.7	−0.4 ± 0.2	1.2 ± 0.4	5.5 ± 0.1

DIC = dissolved inorganic carbon. N = number of replicates.

US) with an anion column A59-HC, 129-mM Na_2CO_3 as an eluent. The N isotopic composition (at %) of the NO_3^- was determined with a reaction continuous flow quadrupole mass spectrometer (17).

The fluxes of dissolved inorganic carbon (DIC) and dissolved gases CH_4, N_2O, and N_2 were determined from the concentration differences between the in- and outflowing water and by taking into account the flow rates and sediment surface area (69 cm^2). The water samples for DIC, CH_4, and N_2O were balanced for 1 day with an argon headspace, and then analyzed with a gas chromatograph (GC) (Agilent 6890N, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) equipped with a peristaltic pump (Minipuls 3, Gilson Inc., Middleton, WI, US) and an autosampler (Gilson autosampler 222XL, Gilson Inc., Middleton, WI, US). The gas concentrations in the original sample were calculated according to Henry's law (modified from McAuliffe et al. 1971 [18]). The N_2 samples were stored in vacuumed 12-mL exetainers (Labco, Co., UK) in concentrated salt solution (NaCl). Concentration and isotopic composition ($^{15}N/^{14}N$) of N_2 were measured by a specifically configurated gas chromatography quadrupole mass spectrometer coupling (QP 2000, Schimadzu Corp., [19]) The masses 28, 29, and 30 were measured, and the peaks were calibrated against normal air (78% N_2) for concentration measurements. The contamination of samples by N_2 in the laboratory atmosphere was prevented by flushing the injection system and the sample loop of the GC with He flow before injection of the sample. The amount of N_2 derived from denitrification was calculated according to nonrandom distribution of the masses 28, 29, and 30 (20–22).

At the end of each incubation week the oxygen concentrations of overlying water (1cm above the sediment surface) were measured with an oxygen electrode (Dissolved Oxygen Meter Oxi 330 with Dissolved oxygen Probe CellOx 325, WTW, Weilheim, Germany), and pH (0.5 cm below the sediment surface) was measured with an electrode (Microprocessor pH meter pH 320, WTW, Germany, with Hamilton pH electrode, Bonaduz, Switzerland).

Field Measurements (Ahvenkoskenlahti Bay and Paimionlahti Bay)

Sediment was sampled either with a single or twin gravity corer, both having an inner diameter of 80 mm. Oxygen and NO_3^- concentrations in the overlying water were measured about 2 cm above the sediment surface. Denitrification was measured using the isotope pairing technique (15). Three replicate subsamples were taken in clear plastic (acrylic) cores (ø 2.6 cm, height 9 cm) so that about half of the core was filled with the sediment and half with the overlying water. Samples were enriched with $K^{15}NO_3$ (98% labeling, Cambridge Isotope Laboratories, MA, US) to a final concentration of 100 μM of $^{15}NO_3^-$ in the water phase and incubated, with a magnetic stirrer on the lid of the cores, at *in situ* temperature in darkness for 3 hours. The biological activity was terminated with $ZnCl_2$, and the samples were mixed. Subsamples of sediment–water slurry were sent in gas-tight 12-mL vials (Exetainer, Labco, UK) to the National Environmental Research Institute, Silkeborg, Denmark, for the analysis of the N_2 isotopic composition.

Data Processing and Statistical Analyses

In the laboratory experiments, the total denitrification (Dtot) was calculated as a sum of measured N_2O and N_2. Calculation of Dn (denitrification from coupled nitrification-denitrification) was based on the differences in the $^{15}NO_3^-$ content in the output water (i.e., overlying water) and in the output N_2 (and N_2O). Denitrification based on the NO_3^- in the overlying water (Dw) was calculated as the remaining part of the total denitrification.

In the field measurements, the share of Dw was calculated from the ratio of $^{14}NO_3^-$ and $^{15}NO_3^-$ concentrations at the nitrate reducing zone and the Dtot (15). Dn was then calculated as the difference between Dtot and Dw.

Statistical analyses were done with SPSS statistical package (SPSS Inc. US). The normal distribution of the variables was tested with the Kolmogorov–Smirnov test and correlations of N_2 effluxes to environmental variables with Spearman correlation coefficients. The statistical significance of differences in denitrification and N_2O/N_2 between rivers were tested with Kruskall-Wallis post hoc-test, suitable for nonparametric data.

RESULTS

Denitrification Rates in the Rivers in the Bothnian Bay

The total denitrification rates in the four river mouths varied between 330 μmol N m^{-2} d^{-1} and 905 μmol N m^{-2} d^{-1} (Fig. 1A). Denitrification was mainly based on the nitrate diffusing from the overlying water into the sediments (Dw), with only a minor share based on the coupled nitrification–denitrification (Dn) (Fig. 1A). The variation in denitrification rates within a site was high (e.g., the river Kalajoki, 906 ± 590 μmol N m^{-2} d^{-1}, distance between replicate samples 500-1000 m), and the denitrification rates between the rivers did not differ statistically significantly. The denitrification rate correlated positively (0.876, $p = 0.05$) with the CH_4 efflux, which varied from 0.02 mmol m^{-2} d^{-1} to 2.3 mmol m^{-2} d^{-1} (Table 2), and with oxygen consumption (0.550, $p = 0.01$), which varied from 19 mmol m^{-2} d^{-1} to 27 mmol m^{-2} d^{-1} (Table 2). It did not correlate with pH or with the fluxes of CO_2 or NH_4^+ (Table 2). The N_2O effluxes varied from 11 μmol N m^{-2} d^{-1} to 16 μmol N m^{-2} d^{-1}(Table 2). The percentage of N_2O in the gaseous end products of denitrification did not exceed 5%. Differences in the N_2O effluxes between the rivers were not statistically significant.

Denitrification in the Bays in the Archipelago Sea and the Gulf of Finland

In Paimionlahti Bay, the denitrification rate varied 10-fold within the estuary, from 90 μmol N m^{-2} d^{-1} in one station in the middle to 910 μmol N m^{-2} d^{-1} in the outer end of the estuary. The bulk of denitrification was coupled to nitrification in the whole estuary, and the proportion of denitrification that was dependent on the NO_3^- in the water column (Dw) was at its highest in the middle estuary (Fig. 1B), where the NO_3^- concentration was highest. Dw was positively correlated with the NO_3^- concentration (0.917, $p = 0.00$) and negatively with the oxygen concentration (–0.871, $p = 0.000$). The rates of coupled nitrification–denitrification (Dn) and total denitrification did not correlate with any of the environmental factors measured.

The denitrification rate in the Ahvenkoskenlahti Bay varied from 230 μmol N m^{-2} d^{-1} to 320 μmol N m^{-2} d^{-1}. No clear gradient in the total denitrification could be seen within the basin, but Dn increased from about 50% at the innermost station to about 80% toward the outer end of the basin (Fig. 1C). Outside the basin, however, denitrification was lower than in the basin itself, and the share of Dn was lower, reflecting the changes in the sediment quality (higher water content and lower concentration of total carbon, nitrogen, and sulfur per sediment volume, data not shown).

The denitrification rates in the estuaries of the Gulf of Finland and Archipelago Sea were in the same range in the rivers (Fig. 1). The possible statistical differences were not tested because of the differences in the methodology.

DISCUSSION

Denitrification Rates

Denitrification rates reported from the rivers range up to 18 000 μmol N m^{-2} d^{-1} (23). The studies made in northern latitudes are few, but Garcia-Ruiz et al. (24) reported rates of 0–13 800 μmol N m^{-2} d^{-1} from the Swale-Ouse river system in UK. In this study, the denitrification rates in the rivers were lower (330–910 μmol N m^{-2} d^{-1}) than those measured from other river ecosystems, but were higher than the values measured in the open sea area of the Bothnian Bay (120–160 μmol N m^{-2} d^{-1}) (10). They fall into same range with the values measured from the open Gulf of Bothnia (0–940 μmol N m^{-2} d^{-1}) (10). In those studies, denitrification was measured using the acetylene blockage method, now known to have some serious flaws (inhibition of nitrification [25], reversal of blockage by sulfide [26], incomplete blockage by acetylene [27], scavenging of intermediate NO [28]). Therefore, the earlier denitrification estimates from the open Gulf of Bothnia (10) may be too low. If that is the case, the rates measured in the river estuaries are, in fact, lower than those measured in the open sea. In the Bothnian Sea, the denitrification rate, measured using the isotope pairing technique (15) varied between 250 μmol N m^{-2} d^{-1} and 300 μmol N m^{-2} d^{-1} (9), which is lower than the rates measured in the river sediments in this study, and is also lower than the rates measured in the Paimionlahti Bay (90–910 μmol N m^{-2} d^{-1}, average 460 μmol N m^{-2} d^{-1}), southern Gulf of Bothnia.

In the Gulf of Finland, denitrification measurements have been done using the isotope pairing technique since the mid-1990s. Gran and Pitkänen (14) found a gradient in the denitrification rates from the eastern Gulf of Finland, inner Neva estuary, toward the open gulf. The denitrification rates were lowest (<10 μmol N m^{-2} d^{-1}) in the inner Neva estuary, higher in the outer estuary, and the highest, up to 1260 μmol N m^{-2} d^{-1}, in the open Gulf of Finland. Tuominen et al. (9) measured the highest denitrification rates of 150–650 μmol N m^{-2} d^{-1} from the open sea area of the Gulf of Finland, with lower rates in the eastern and western ends of the gulf (100–400 μmol N m^{-2} d^{-1}). Thus, the denitrification rates in the Ahvenkoskenlahti Bay (Gulf of Finland) (230–320 μmol N m^{-2} d^{-1}, average 280 μmol N m^{-2} d^{-1}) are in the lower end reported for the gulf. However, there can be some overestimation in the rates reported for the open Gulf of Finland, resulting from the potential effect of anammox in the estimates, whereas no anammox was detected in the Ahvenkoskenlahti Bay . The denitrification rates in the Ahvenkoskenlahti Bay were much higher than the rates outside the bay, toward the open Gulf of Finland (Fig. 1C). The stations at the estuary were located on accumulation bottoms, with high carbon and nitrogen content per sediment volume, whereas the stations on the transportation/transient accumulation bottoms outside the estuary had very low dry matter and low carbon and nitrogen content per sediment volume. In the Paimionlahti Bay, the inner estuary rates were slightly (but significantly, $p = 0.01$) lower than those immediately outside the estuary (Fig. 1B). As the highly variable denitrification rate (260–620 μmol N m^{-2} d^{-1}, with a single station showing a rate of 90 μmol N m^{-2} d^{-1}) did not correlate with any of the environmental factors measured (depth, temperature, salinity, oxygen and nitrite/nitrate concentration, total carbon, nitrogen, and loss on ignition in the sediment) likewise showing high variation, it is unclear why the rates outside the estuary basin were higher (720–910 μmol N m^{-2} d^{-1}).

Although the total denitrification rates fall into the same range in the laboratory experiments and in the field measurements, the ratio of Dw/Dn was remarkably different. In the laboratory experiments, the share of Dn was always less than 10%, whereas in the field measurements the share was 50–85% in Ahvenkoskenlahti and 65–95% in Paimionlahti. Similar results—small share of Dn—have been reported earlier from flow-through systems as well (29,23). In the laboratory experiments, the sediment surface is continuously provided with NO_3^-, mimicking the natural conditions in river ecosystems. Dw correlates with the NO_3^- concentration, and the high NO_3^- concentrations in river waters (Table 1) are likely to sustain high Dw in the river sediments. The NO_3^- concentrations were much higher in the rivers than in the two estuaries (Table 1) and probably explain the lower share of Dn in the river sediments. Low share of Dn has been reported from river and estuary systems using the isotope pairing technique, too (e.g., 30, 31). In river sediments, NO_3^- is easily available in the denitrification zone because of more efficient penetration and thus the importance of nitrification as NO_3^- source is diminished. Another factor affecting the share of Dn is the oxygen concentration in the overlying water of the sediments. Low oxygen concentration lowers the oxygen penetration depth, thereby enhancing Dw by shortening the distance NO_3^- needs to diffuse into the denitrifying zone in the sediment. Oxygen deficiency also lowers nitrification rate and thereby Dn rate (32). In the laboratory experiments the oxygen demand was high, but no anoxia developed because of the continuous feeding of the system with oxic water. Also in the field measurements oxygen was not limiting nitrification and Dn, as the rates were high and did not correlate with oxygen concentration.

N_2O Production in Denitrification

The N_2O effluxes measured in the river sediments (manipulation experiments) were lower than the rates reported for rivers in the literature (33–35). In the river Swale-Ouse, NE England, the lowest effluxes measured at the highest upstream sites (36), were more than 10 times the effluxes measured in this study. The N_2O effluxes have not been measured previously from the rivers of the northern Baltic Sea. In shallow profundal sediments of freshwater lake of the same latitude, the effluxes in aerobic conditions were of the same magnitude (up to 17 μmol N_2O-N m^{-2} d^{-1}) (37) as measured from the rivers in this study. Seitzinger et al. (8) reported that in eutrophic water ecosystems, up to 5% of the gases produced in denitrification are released as N_2O. Of the rivers studied in the manipulation experiment, none showed such a high ratio. The N_2O production rates measured were low, and therefore the N removal by denitrification did not, so far, have a significant climatic impact. Increasing NO_3^- concentrations in the river water would, however, probably enhance N_2O over N_2. Ratios as high as 80% have been measured from very eutrophic rivers in northeast England (38) and very high N_2O concentrations exist in estuaries around the world (5, 39) and also in the southern Baltic Sea (40).

Could Anammox Cause Inaccuracies in the N_2 Production Rate Estimates?

Until recently, denitrification was seen as the only process removing fixed nitrogen from the water ecosystem. However, a decade ago, another nitrogen removing process, anammox (anaerobic ammonium oxidation), was discovered in wastewater treatment plants (41, 42), and later also in marine sediments (43–46). In a recent study, anammox was found in a coastal station of the northern Gulf of Finland, (Hietanen, S. and Kuparinen, J. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea, submitted for publication). The discovery of the anammox process in these sediments challenges the previous measurements made in the area, as the coexistence of anammox and denitrification compromises the central assumptions behind the method used in denitrification measurements and causes overestimates in the N_2 production. Therefore, the true N_2 production rates cannot be reliably calculated without knowing the share of anammox in the total N_2 production. In the coastal Gulf of Finland, anammox contributed 10–15% to the total N_2 production, with the effect that the N_2 production was overestimated by 80–150% (Hietanen, S. and Kuparinen, J. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea, submitted for publication). It has been studied also the Ahvenkoskenlahti Bay and found to be negligible (<1%) (Hietanen, S. Anammox in the sediments of the Gulf of Finland, submitted for publication). Thus, there is no bias in the N_2 production rates presented here for that estuary. Anammox was not measured at the other field measurement area, the Paimionlahti Bay, where much higher denitrification rates were found, nor in the river sediments used in the laboratory experiments. Therefore, it is possible that the denitrification rates presented here for these areas are overestimates. Anammox has so far been measured only in two different river ecosystems, the temperate Thames estuary (45) and a subtropical Logan and Albert river system in Australia (47). In both of these locations, the highest contribution of anammox to the overall N_2 production (8–9%), as well as the highest rates, were measured upstream, with decreasing rates toward the river mouth and open sea, where anammox was found to be negligible. In addition, the relative contribution of anammox to the overall nitrogen reduction is minor in coastal environments and increases with depth as the rate of denitrification decreases (43, 48, 49). Based on these published findings and those measured from Ahvenkoskenlahti Bay and the coastal station at the Gulf of Finland, we have assumed that in the Paimionlahti Bay and the river sediments, the anammox activity is negligible, and our denitrification estimates are therefore valid.

Importance of Nitrogen Removal by Denitrification

We evaluated the efficiency of the nitrogen removal in the studied estuaries. In the laboratory experiments, the calculations of N removal are based on Dw because of the small share of Dn. The NO_3^- removal by denitrification was calculated as a ratio of output labeled gaseous nitrogen species to the input of labeled NO_3^-. Dw accounted for 17–22% total NO_3^- removal. The overall NO_3^- removal, which includes DNRA and assimilation in addition to denitrification, was 21–27%. According to these results, denitrification was always the most important process removing nitrate, if the assumption of negligible anammox is correct. When estimating the role of denitrification in reducing the total nitrogen load *in situ*, one has to bear in mind that only a share of total nitrogen is in the form of nitrate in the river waters (Table 1). No information exists yet about the seasonal variation of denitrification in these ecosystems nor about the possible differences in the rates between accumulation and transportation bottoms. Stockenberg and Johnstone (23) have suggested that the denitrification rate on transportation and erosion areas is only 30% of that on the accumulation areas. The studied sediments were collected from accumulation bottoms only, and the share of accumulation bottoms has not been mapped in these rivers. Therefore, the estimate given only applies for the environmental conditions prevailing during the experiment.

The two estuaries studied differed greatly from each other in their capacity to remove nitrogen entering the bay. In Ahvenkoskenlahti Bay in August 2004, assuming that the denitrification rate was similar throughout the basin, the average rate of 280 μmol N m^{-2} d^{-1} (Dw 86 μmol N m^{-2} d^{-1}) removed 1.7% of NO_3^- loading (by Dw) and 3.6% of the total N loading (by Dtot) reaching the bay. The share of accumulation bottom in the study area is 58% (Heikki Pitkänen, SYKE, unpubl. data). Using the lower rates for the transportation and erosion areas (23) gives only 1.2% reduction to the nitrate and 1% reduction to the total nitrogen loading in August 2004. In Paimionlahti Bay the average denitrification rate of 460 μmol N m^{-2} d^{-1} (Dw 70 μmol N m^{-2} d^{-1}) was high enough to remove all of the nitrogen loading reaching the bay in September 2003. The year 2003 was exceptionally dry, so the nitrogen removal was calculated also using the loading data of more typical conditions in September 2004. If the denitrification rate was similar in 2004, 4.5% of nitrate loading and 19% of total nitrogen loading were removed from the water in the estuary in 2004. The amount of accumulation areas has not been mapped in the Paimionlahti Bay, and the given value is likely to be an overestimate.

The residence time has been indicated as having a major effect in the estuarine retention capacity in several different estuaries (30, 50–53). In Ahvenkoskenlahti Bay, the discharge is high year round, and the estimated residence time fluctuates between 10 and 14 days (average 12 days). In the larger and deeper Paimionlahti Bay, the flow is 20 times lower and more pulsed, and the calculated residence time varies from 2 to 19 years (average 7 years). Clearly, more nitrogen is removed as it stays longer in the estuary, and, therefore, Paimionlahti Bay is more efficient in removing nitrogen than Ahvenkoskenlahti Bay.

Estuaries and River Mouths as N Filters in Northern Baltic

In 1988, Seitzinger (8) introduced a “rule of thumb” of 40–50% N removal by denitrification in estuaries, indicating them as important filters for the N loading transported toward the sea and therefore having a considerable role in slowing down eutrophication of seas. Since then, various studies have either supported (e.g., 12) or opposed (51, 53–56) this claim. Only a few studies have been made in the Baltic Sea area. A lowland stream entering the Kattegat was found to remove $<1\%$ of the annual loading, although during low discharge in summer, the removal was temporarily up to 60% of the loading (30). Similarly, a small, shallow estuary opening to the Kattegat denitrified only 2% of the annual loading (50). In the Swedish east coast, a coastal embayment was found to remove 5–11% of wastewater N input, with denitrification being the most important mechanism for removal (11). A study of several rivers entering the Gulf of Bothnia revealed them to be ineffective sinks of N (13). All of these studies, as well as the results presented here, confirm the pattern of increasing efficiency with decreasing discharge, suggested by Nixon (53). In conclusion, according to our results, the sediments of the fast flowing rivers and the estuary areas with short residence times of both the Gulf of Bothnia and the Gulf of Finland are inefficient filters of N load. This emphasizes the role of reduction of anthropogenic N loading to the high latitude rivers and estuaries to avoid further eutrophication of susceptible sea areas.

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CHAPTER III:

DENITRIFICATION AND NITROUS OXIDE EFFLUXES IN BOREAL, EUTROPHIC RIVER SEDIMENTS UNDER INCREASING NITRATE LOAD: A LABORATORY MICROCOSM STUDY

Hanna Silvennoinen, Anu Liikanen, Jouni Torssonen, C. Florian Stange and Pertti J. Martikainen. *Biogeochemistry,* in press.

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DENITRIFICATION AND NITROUS OXIDE EFFLUXES IN BOREAL, EUTROPHIC RIVER SEDIMENTS UNDER INCREASING NITRATE LOAD - A LABORATORY MICROCOSM STUDY

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Abstract

Intact sediment cores from rivers of the Bothnian Bay (Baltic Sea) were studied for denitrification based on benthic fluxes of molecular nitrogen (N_2) and nitrous oxide (N_2O) in a temperature controlled continuous water flow laboratory microcosm under 10, 30, 100 and 300 µM of ^{15}N enriched nitrate (NO_3^-, ~98 at. %). Effluxes of both N_2 and N_2O from sediment to the overlying water increased with increasing NO_3^- load. Although the ratio of N_2O to N_2 increased with increasing NO_3^- load, it remained below 0.04, N_2 always being the main product. At the NO_3^- concentrations most frequently found in the studied river water (10-100 µM), up to 8% of the NO_3^- was removed in denitrification, whereas with the highest concentration (300 µM), the removal by denitrification was less than 2%. However, overall up to 42% of the NO_3^- was removed by mechanisms other than denitrification. As the microbial activity was simultaneously enhanced by the NO_3^- load, shown as increased oxygen consumption and dissolved inorganic carbon efflux, it is likely that a majority of the NO_3^- was assimilated by microbes during their growth. The ^{15}N content in ammonium (NH_4^+) in the efflux was low, suggesting that reduction of NO_3^- to NH_4^+ was not the reason for the NO_3^- removal. This study provides the first published information on denitrification and N_2O fluxes and their regulation by NO_3^- load in eutrophic high latitude rivers.

Keywords: stable isotopes; eutrophication; NO_3^- removal; N_2O/N_2 ratio; Bothnian Bay; river sediment

Introduction

The Bothnian Bay is the northernmost part of the Baltic Sea, which is receiving an increasing nutrient and organic carbon load primarily via Finnish rivers. During the years 1995-2000, the largest 24 rivers brought an annual average of 47,200 tons of N to the Bothnian Bay, which accounts for > 90% of the total annual N load (Kronholm et al. 2005). The Bothnian Bay differs substantially from other parts of the Baltic Sea: (i) The area is less saline due to physical separation from the Baltic Proper by shallow sills at the Archipelago Sea and a large freshwater inflow, (ii) the open sea area of the Bothnian Bay is in a near-pristine state and harmful cyanobacterial blooms are rarely observed and (iii) primary production in the open sea area is limited by phosphorus (P) instead of nitrogen (N).

Microbial processes in estuarine sediments have been estimated to remove up to 90 % of the external N input and can thus have importance in

controlling anthropogenic N loading to seas (Seitzinger 1988). N removal capacity of watersheds and sediments has received growing interest during recent decades due to increased anthropogenic N loading and its effects on the recipient waterbodies (Jenkins and Kemp 1985; Bange et al. 1996; Middelburg et al. 1996). In non-vegetated sediments, NO_3^- can be removed from overlying water by four different microbiological processes: denitrification, anaerobic ammonium oxidation, dissimilatory nitrate reduction to ammonium and assimilation to microbial biomass.

The most studied of the four processes, and the one that has been found to be most important, is denitrification, which is reduction of NO_3^- via nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) to dinitrogen (N_2) (Eq. 1). In denitrification, two moles of NO_3^- are reduced to one mole of N_2 (Eq. 2).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (1)$$

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O \quad (2)$$

Denitrifying bacteria are facultative aerobes using NO_3^- as an electron acceptor when oxygen is limited. As a form of heterotrophic metabolism, denitrification is dependent on the supply of labile organic carbon. In freshwater, estuarine and coastal sediments, denitrification occurs in the suboxic layer a few millimeters to centimeters below the sediment-water interface, and directly below the oxic layer where nitrification (i.e. the aerobic oxidation of NH_4^+ to NO_3^-) occurs. Denitrification is thus regulated by the transport of NO_3^- and O_2, principally by molecular diffusion along concentration gradients to the site of biological reaction within the sediments. In sediments, denitrification is fuelled by NO_3^- diffusing from the overlying water (Dw, uncoupled denitrification) or by NO_3^- derived from mineralization and nitrification processes in the oxic sediment layers (Dn, coupled nitrification denitrification). High denitrification rates have been measured in eutrophic aquatic ecosystems and denitrification has also been found to be highly efficient in reducing NO_3^- loading in temperate rivers (15-30 %, Pfenning and McMahon 1996) and coastal ecosystems (70 -100%, Kaspar 1983; Kaspar et al. 1985). Few data are available from high latitude aquatic ecosystems, but denitrification was found to remove 23 % of the annual N load in the open sea area of the Bothnian Bay (Stockenberg and Johnstone 1997). N_2O, an effective greenhouse gas, is produced as an intermediate of denitrification. In water-saturated ecosystems the ratio of N_2O to N_2 has been found to vary from 0.002 to 0.05 (Seitzinger 1988). High saturations of N_2O have been measured from estuarine and open sea waters (e.g. Kroeze and Seitzinger 1998). N_2O/N_2 has been reported to exhibit a high positive correlation with NO_3^- concentrations (Oren and Blackburn 1979; Oremland et al. 1984; Koch et al. 1992). Therefore, especially in watercourses affected by agricultural runoff, there is a risk for high N_2O emissions as N loads increase.

Anaerobic ammonium oxidation, i.e. oxidation of NH_4^+ by nitrite (NO_2^-) to N_2, (anammox) (Kuypers et al. 2003) (Eq. 3) has been reported to contribute to N_2 fluxes along with denitrification. Anammox has not been extensively described in fresh waters, but has been reported to bias measured denitrification rates in marine ecosystems (e.g. Hulth et al. 2005, Hietanen et al. 2007).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (3)$$

The remaining two processes, DNRA and assimilation to microbial biomass, do not exhaust NO_3^- from water, but convert it into forms of N that are available to primary producers directly (NH_4^+) or after mineralization processes (microbial N). The regulation and magnitude of DNRA (dissimilatory NO_3^- reduction to NH_4^+) is still poorly understood, but co-occurrence with denitrification has been reported in aquatic ecosystems with high NO_3^- concentrations. High NO_3^- reduction rates via DNRA have been reported in lower latitude rivers (30 %, Brunet and Garcia-Gill 1996) and coastal marine ecosystems (18-100 %, Bonin et al. 1998). Few studies report signs of NO_3^- uptake and assimilation into microbial biomass in aquatic ecosystems. It has been reported from a riparian wetland (22 % of NO_3^-, Matheson et al. 2002) and at low levels from estuarine and coastal ecosystems (<5 % of NO_3^-, Goyens et al. 1987; Jørgenssen 1989).

The processes involved in N cycling in river and estuarine ecosystems are driven by a range of environmental factors, with availability of NO_3^-, carbon and oxygen together with temperature being the most important ones. As a result of the changes in these driving factors, reported seasonal patterns vary remarkably (e.g. Christensen and Sørensen 1986; Jørgensen and Sørensen 1988; Koch et al. 1992; Nielsen et al. 1995; Ogilvie et al. 1997; Pind et al. 1997; Trimmer et al. 1998). In addition to temperature, the availability of NO_3^- fluctuates during the year. During summer, when the temperature is highest, the availability of NO_3^- is low due to low discharges and high uptake by primary producers in river water, thus limiting denitrification. NO_3^- concentration is an important factor regulating the N cycle in sediments. The nitrogen leaching from fertilized agricultural soils into rivers is mainly in the form of NO_3^- (Kronholm et al. 2005). Additionally, NO_3^- has a dual role in sediment as a source of N for growth and as an electron acceptor in organic carbon oxidation.

There are few studies on denitrification and N_2O effluxes from high latitude rivers. In the eutrophic rivers discharging into the Baltic Sea and receiving increasing amounts of NO_3^- from terrestrial ecosystems, denitrification could be important in diminishing NO_3^- concentrations. Furthermore, high NO_3^- loading can lead to enhanced N_2O production during denitrification. We report here our determinations of denitrification rates in the laboratory as benthic fluxes of N_2 and N_2O from intact riverine sediments from a boreal eutrophic river, under different external NO_3^- concentrations. We use these results to evaluate the potential for denitrification to regulate the riverine NO_3^- load.

Material and methods

Site description

The sediment samples were collected 17 May 2003 from the mouth of the Temmesjoki River (64° 84'N, 25° 37'E) (Fig.1A). The Temmesjoki River is characterized as a eupolytrophic river for total N concentrations and a polytrophic river for total P. N generally limits primary production in the Temmesjoki River, but occasionally there is a shortage of P. The drainage basin of the river consists of different and clearly separate catchments including forested areas, wetlands and agriculture. Dissolved inorganic nitrogen (DIN) leaches from catchments, where diffuse anthropogenic sources (e.g. agriculture and forestry) have a great importance to the total N load, nitrate (NO_3^-) being the major fraction of DIN. The Temmesjoki River has a drainage basin which, in comparison to most rivers of the Bothnian Bay, is small in size (1190 km^2) and has a high coverage with agricultural fields (15 %). The river has a low annual mean flow (11 $m^3 s^{-1}$). Thus, the annual N load to the Bothnian Bay from the Temmesjoki River is rather low (520 tons), despite the high NO_3^- concentrations in the river water. The NO_3^- concentration in the main channel of the river varies greatly (~1-100 μM), being generally highest during winter and lowest in summer (Fig. 1B) (Data from the Environmental Information System, HERTTA). The NO_3^- concentration range investigated in this study varied from 10 to 300 μM NO_3^- , which covers well the current concentration range in the main channel. The highest concentration also allows estimation of the impact of greatly increased NO_3^- concentrations on denitrification.

Sampling and experimental set-up

Sediments were collected directly into transparent acrylic tubes (ø 94 mm, height 650 mm). The height of the collected intact sediment was ≤ 200 mm. The sediments were placed in a laboratory microcosm equipped with continuous water flow (Liikanen et al. 2002a). The microcosm was situated in a dark, temperature controlled room (15 °C). Water was pumped from an 80-liter water reservoir over the cores by a peristaltic pump (IPC-24,Ismatec, Glattbrugg-Zürich, Switzerland) at a rate of 50 ml h^{-1}. Water overlying the cores was gently stirred with a rotating magnet to prevent stratification (Liikanen et al. 2002a). The water reservoir was flushed continuously with a gas mixture consisting of Ar/O_2 80/20 (v/v) (AGA, Finland) to allow the use of the isotope pairing technique in distinguishing between coupled and uncoupled denitrification. The sediments (five replicate intact sediment samples) were incubated under 10, 30, 100 and 300 µM $^{15}NO_3^-$ (98 at. %) for the 1st, 2nd, 3rd and 4th incubation weeks, respectively. Incubation for each NO_3^- concentration lasted one week starting with the lowest concentration. The concentrations of N_2, N_2O, NO_3^- and NH_4^+ in water were measured and the effluxes were calculated from the difference between concentrations in the in- and outflowing waters and by taking into account the flow rates and sediment surface area (69 cm^2).

Analyses of N_2 and N_2O

For determining the N_2, N_2O and DIC effluxes, the effluent water samples were preserved with sulfuric acid (1 ml H_2SO_4 20 % v/v) and equilibrated for 1 day with Ar headspace, which was then measured for gas concentrations and isotopic composition of N_2. The gas concentrations in the original water sample were calculated according to Henry's law (McAuliffe 1971). Due to sulfuric acid preservation, all the inorganic carbon was liberated to the syringe headspace as CO_2 and therefore the values presented here represent the dissolved inorganic carbon (DIC) fluxes. Nitrous oxide and DIC concentrations were analyzed with a Gas Chromatograph (GC) (Hewlett Packard Series II, Palo Alto, US) equipped with two two-meter long packed columns (Hayesep Q (80/100 mesh), Porapak S (80/100 mesh)) and an electron capture detector for N_2O analyses (see Nykänen et al. 1995 for details). N_2 concentrations and isotopic compositions were measured by Gas Chromatography - Quadrupole Mass Spectrometer coupling (GC-QMS) (QP 2000, Shimadzu Corp., Japan) (see Russow and Förstel 1993 for details). The masses 28, 29 and 30 were measured and the peaks were calibrated against normal air (78 % N_2) for concentration measurements. A detailed description of the precision and accuracy of measurement is presented in Russow and Förstel (1993). Contamination of samples by N_2 in the laboratory atmosphere was prevented by flushing the injection system and the sample loop of the GC with helium before injection of the sample. The amount of N_2 derived from denitrification was calculated according to non-random distribution of the masses 28, 29 and 30 (Hauck et al. 1958; Siegel et al. 1982).

NO_3^- and NH_4^+ analyses

NO_3^- and NH_4^+ concentrations and isotopic composition ($^{15}N/^{14}N$, at. %) of in- and outflowing waters were determined three times for each NO_3^- concentration (4th, 5th and 6th incubation days). Both water and sediment samples were stored at -20 °C prior to analyses. Nitrate concentrations were measured with an ion chromatograph (Dionex DX-130, Sunnyvale, US, with an anion column A59-HC, 12 mM Na_2CO_3 as an eluent). Ammonium was determined photometrically according to the standard SFS 3032 (SFS standardization 1976). The isotopic compositions (at. %) of NO_3^- and NH_4^+ were determined with a R/CF-QMS (Reaction/Continuous Flow - Quadrupule Mass Spectrometer) (Russow 1999; Stange et al. 2007).

Oxygen and pH measurements

At the end of each incubation week, the oxygen (O_2) concentrations and pH of overlying water (1 cm above the sediment surface) were measured. Oxygen concentrations were measured with an oxygen electrode (dissolved oxygen meter Oxi 330 with dissolved oxygen probe CellOx 325, WTW, Germany) and pH was measured with a pH electrode (Microprocessor pH meter pH 320, WTW, Germany, with Hamilton pH electrode).

Data processing

Denitrification (Dtot) in the system can be divided into Dn - coupled nitrification denitrification, and Dw - denitrification from the added NO_3^- in the overlying water (and carried by diffusion into the sediment pore water) e.g. uncoupled denitrification. Total denitrification (Dtot) was calculated as the sum of measured N_2O and N_2. Dn was calculated from the difference between the ^{15}N label (at. %) of the output NO_3^- and the output N_2 (and N_2O). The isotopic dilution gives a value for the formation of N_2 (and N_2O) from sediment-derived nitrogen. Dw (with the substrate of denitrification being the NO_3^- in the overlying water) was calculated as the remaining part of the total denitrification (Dtot-Dn).

The response of total denitrification to NO_3^- addition was fitted to a Michaelis-Menten type function (the Lineweaver-Burk transformation). In this function the reciprocal of the reaction (denitrification) rate is plotted against the reciprocal of the substrate (NO_3^-) concentration. Extrapolating the linear regression line of the function to its intercept on the abscissa gives the negative reciprocal of the half-saturation concentration (K_m). This parameter gives an estimate of the NO_3^- concentration at which the denitrification rate is half maximal (Eq.4).

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \qquad (4)$$

where, v = Reaction rate, i.e the rate of total denitrification (µmol N m^{-2} d^{-1}) V_{max} = Maximal reaction rate, i.e. maximal rate of total denitrification (µmol N m^{-2} d^{-1}),[s] = concentration of substrate (NO_3^-) (µM), K_m = Substrate concentration, at which the reaction rate is half maximal (µM)

Statistical analyses

Statistical analyses were done using the SPSS statistical package (SPSS Inc. US). The normal distribution of the variables was tested with the Kolmogorov-Smirnov Test. As the responses of the parameters to NO_3^- addition were not linear, non-parametric Spearman correlation coefficients were applied to study the interactions between various parameters.

Results

Denitrification and N_2O effluxes

N_2 and N_2O effluxes and uncoupled denitrification (Dw) increased with increasing NO_3^- load (Table 1, Fig. 2). The greatest N_2 effluxes (1650 ± 210 µmol N_2 m^{-2} d^{-1}) were detected with the highest NO_3^- load, as were the greatest N_2O effluxes (68 ± 12 µmol N_2O m^{-2} d^{-1}). Both N_2 (0.587, $p < 0.01$) and N_2O (0.865, $p < 0.01$) effluxes calculated from the non-averaged data show a positive correlation with the NO_3^- load (Table 1). The treatment averages of N_2 and N_2O effluxes and Dw exhibited a logarithmic response to NO_3^- load (p of the regression < 0.01 for all three parameters)(Fig. 2) With lower concentrations (10-100 µmol NO_3^- l^{-1}) the effluxes increased linearly, but as the input NO_3^- increased to 300 µmol NO_3^- l^{-1}, the response levelled off (Fig. 2). Uncoupled denitrification (Dw) always accounted for a greater part of denitrification than denitrification coupled with nitrification (Dn). The proportion of Dw/Dtot showed a significant positive correlation with NO_3^- load (0.550, $p < 0.01$, Table 1). The response of total denitrification to NO_3^--N addition fitted to a Michaelis-Menten type curve ($R^2 = 0.90$) exhibited an apparent K_m value of 20 µM NO_3^-

(Fig. 3). The K_m value obtained is an apparent rather than actual K_m as it includes the limitation of diffusion of NO_3^- to the denitrifiers created by the undisturbed sediment cores.

The ratio of N_2O to N_2 was always low (< 0.04). The ratio increased with increase in NO_3^- load up to 100 µmol NO_3^- l^{-1} but decreased at 300 µM. The maximum proportion of N_2O in the nitrogenous gases was 3.9%.

The amount of NO_3^- reduced to nitrogenous gases increased with increasing NO_3^- load (Table 1), but at the same time the proportion of NO_3^- reduced in denitrification decreased as the NO_3^- load increased. With a 30 µM NO_3^- load, 7.6 % of the NO_3^- was denitrified, but at 300 µM NO_3^-, only 1.8 % was denitrified (Table 1). The sediments were always a sink for NO_3^- from overlying water (Table 1). The total amount of NO_3^- removed at the sediment-water interface increased with increasing NO_3^- load. The proportions of $^{15}NO_3^-$ removed from the overlying water were 9.3 ± 2.0, 13 ± 1.0, 17 ± 3.0 and 42 ± 11 % with treatments of 10, 30, 100 and 300 µM NO_3^-, respectively (Table 1).

Ammonium effluxes were scattered, and due to the high variation only a low, statistically insignificant positive correlation with NO_3^- concentration was observed (Table 1). However, the treatment averages show a significant logarithmic increase as a function of NO_3^- load ($R^2 = 0.99$, $p < 0.01$, Fig. 2). The O_2 concentrations in the input water were 8.0, 8.5, and 8.0 mg O_2 l^{-1} at the 30, 100 and 300 µM NO_3^-, respectively. The oxygen was consumed efficiently, especially at the highest NO_3^- concentration. 30 and 100 µmol NO_3^- treatments exhibited oxygen concentrations of 4.1 and 5.2 mg O_2 l^{-1} at the sediment surface. At the highest NO_3^- concentration, the O_2 concentration was 3.4 mg O_2 l^{-1}. Both DIC fluxes and O_2 consumption exhibited a high variation but increased with the highest NO_3^- treatment (Table 1, Fig. 2).

The pH increased slightly, from 6.1 to 6.3, with increasing NO_3^-, but the observed positive correlation was low (0.151) and statistically insignificant.

Discussion

Denitrification rates as affected by NO_3^- concentration

The denitrification rates measured in this study exhibited a positive correlation (0.617, $p < 0.01$, Table 1) with NO_3^- load. This result is consistent with many site-specific studies from different ecosystems which have demonstrated a positive relationship between denitrification rates and NO_3^- concentration in lake (Anderssen 1977), estuarine and marine sediments (Oren and Blackburn 1979; Oremland et al. 1984; Nielsen et al. 1995; Kana et al. 1998) and in sediments of rivers in temperate regions (Royer et al. 2004; Garcia-Ruiz et al. 1998). The N_2 fluxes, Dw and N_2O fluxes that represent denitrification scaled over the entire studied NO_3^- addition range exhibit a logarithmic response to NO_3^- (Fig. 2). At the lowest three concentrations the response is linear, but the response plateaus at the highest concentration. At the lowest NO_3^- concentrations, denitrification is probably limited by the availability of NO_3^- but as the concentration increases, denitrification reaches its maximum rate (K_m was 20 µM NO_3^-). A similar logarithmic response was found from intertidal mudflats of San Francisco Bay, where NO_3^- addition increased denitrification rates linearly only up to ~100 µM NO_3^- (Oremland et al. 1984).

Denitrification rates measured in this study were similar to or higher than the rates measured from open sea sediments of the Bothnian Bay (0-940 µmol N $m^{-2}d^{-1}$, Stockenberg and Johnstone 1997) and the Gulf of Finland (150-650 µmol N m^{-2} d^{-1}; Tuominen et al. 1998), and they are considerably higher than the rates found for estuary sediments of the Gulf of Finland (30-50 µmol N m^{-2} d^{-1}, Gran and Pitkänen 1999). Nitrate concentrations of near-bottom waters in both the Bothnian Bay and Gulf Finland (8-14 µM in the Neva Estuary, <14 µM in the central Gulf of Finland, <10 µM in the Bothnian Bay (Stockenberg and Johnstone 1997; Tuominen et al. 1998; Gran and Pitkänen 1999), were similar to our lowest

NO_3^- treatment (10 µM), which exhibited a denitrification rate of 440 µmol N $m^{-2}d^{-1}$

The denitrification rates measured in this study (440 - 1,718 µmol N m^{-2} d^{-1}, Table 1) were closest to denitrification rates found in marine sediments (up to 1,440 µmol N m^{-2} d^{-1}) (Piña-Ochoa and Álvares-Cobelas 2006 and references therein). River sediments generally exhibit higher denitrification rates (up to 79,000 µmol N m^{-2} d^{-1}) than either lake sediments (up to 7,500 µmol N m^{-2} d^{-1}) or estuary sediments (up to 14,200 µmol N m^{-2} d^{-1}). The higher denitrification rates measured in rivers and estuaries than in coastal areas and oceans could be due to higher anthropogenic loading. The rates measured here were an order of magnitude lower than the rates measured from very eutrophic rivers at lower latitudes (García-Ruiz et al. 1998a), presumably as the denitrifiers have adjusted to the lower NO_3^- availability. Piña-Ochoa and Álvares-Cobelas (2006) plotted a data set of denitrification rates from all the main aquatic environments around the world in a multiple regression model with the main factors controlling denitrification, and they found that only dissolved oxygen and NO_3^- concentrations significantly explained the denitrification rates, the latter being responsible for 70 % of the variation in the rates.

Denitrification rates obtained in this study were always primarily based on added NO_3^- (Dw/Dtot 52-69 %, Table 1). The proportion of Dw/Dtot showed a significant positive correlation with NO_3^- load. In contrast to our study, denitrification in open sea sediments of the Bothnian Bay and Gulf of Finland has been found to be mostly due to denitrification coupled with nitrification (Dn) (Stockenberg and Johnstone 1997), which can be explained by the lower external NO_3^- availability in open sea sediments than in the sediments in our laboratory experiments. Although the NO_3^- concentrations in those studies were similar to our lowest treatment, the continuous loading with NO_3^- in the river sediments increased the penetration of NO_3^- into sediments and consequently the availability of NO_3^- to denitrifiers (e.g. Law and Owens 1990; Kana et al 1998). Several studies from coastal and marine environments report high proportions of coupled denitrification, suggesting that the low availability of NO_3^- from the overlying water enhances the role of nitrification in sediment as the provider of the substrate for denitrification. For example, Rysgaard et al. (1993) showed that when NO_3^- concentrations in the water phase were low (~5µM), coupled denitrification accounted for a larger fraction of the total denitrification than when the NO_3^- concentration in the water column was higher. The sediments studied here under higher NO_3^- concentrations (10-300µM) always exhibited a predominance of uncoupled denitrification, and thus support the conclusions by Rysgaard et al. (1993) presented above.

The apparent half-saturation concentrations (K_m) measured in this study (20 µM NO_3^-) fall well within the lower end of the range measured in previous studies, indicating that the bacteria are well adjusted to living under low NO_3^- availability, i.e. they have a high affinity for NO_3^-. K_m values for marine sediments using the slurry technique generally range from 27 to 53 µM (Seitzinger 1988), with a value of 344 µM reported in one study. Results obtained from the Swale-Ouse river continuum in NE England varied between 13.1 and 90.4 µM NO_3^- (Garcia-Ruiz et al 1998b). Since the apparent K_m value in this study was measured with intact sediment samples, it reflects the actual conditions in these sediments and thus offers a tool for integrated denitrification models for similar sediments. However, spatial and temporal variation of denitrification is well known (McClain et al. 2003), and therefore caution must be practiced when the denitrification rates obtained in the laboratory are extrapolated to the river or landscape level.

In this experimental set-up, neither the possibility of an increase in the measured N_2 pool by annamox from non-labelled NH_4^+ and NO_2^- in the sediment nor the contamination of the N_2 pool by airborne nitrogen contamination can be eliminated. Mathematical approaches for separating anammox, denitrification and airborne nitrogen contamination have been presented recently (Thamdrup and Dalsgaard 2002; Risgaard-Petersen et al. 2003; Trimmer et al. 2006; Spott and Stange 2007). However, all of these approaches require an accurate time-dependent quantification of NO_2^- and are therefore not suitable for this data. Both of these problems decrease the proportion of Dw from Dtot in favor of Dn. In the case of airborne contamination, estimated NO_3^- removal via denitrification would be even lower.

N_2O effluxes as affected by increasing the NO_3^- load

In our study, N_2O effluxes showed an increasing trend with increasing NO_3^- concentrations (Table 1, Fig. 2), but the fraction of the N_2O from the end products was, at most, only 3.9 %. Thus, the contribution of N_2O production via denitrification is consistently a small fraction of the total denitrification and NO_3^- consumption rates throughout the studied range of NO_3^- concentrations in this study.

Several studies in terrestrial (Blackmer and Bremner 1978; Weier et al. 1993) and aquatic (Oren and Blackburn 1979; Oremland et al. 1984; Koch et al. 1992) ecosystems have shown that the presence of high NO_3^- concentrations limits the conversion of N_2O to N_2 and results in higher N_2O/N_2 ratios. Thus, it is possible that increased local N_2O emissions due to denitrification activity in boreal eutrophic rivers and estuaries have resulted from the increase in the N_2O/N_2 ratios (Seitzinger 1998; García-Ruiz et al. 1999). Seitzinger (1988) reported that in eutrophic water ecosystems, up to 5 % of the gases produced in denitrification were released as N_2O. Ratios as high as 80 % have been measured from very eutrophic rivers in NE-England (García-Ruiz et al. 1998b).

The N_2O production rates measured in this study (4-68 µmol N_2O-N $m^{-2}d^{-1}$) from boreal river sediments were lower than the rates reported for rivers in general (Elkins et al. 1978; García-Ruiz et al. 1999; de Bie et al. 2002; Laursen and Seitzinger 2004). N_2O production rates have not been measured in the rivers of the northern Baltic Sea before, but in shallow profundal sediments of a freshwater lake of the same latitude, the production rates in aerobic conditions were of the same magnitude (up to 17 µmol N_2O-N m^{-2} d^{-1}) (Liikanen et al. 2002b) as those measured from the rivers in this study.

Nitrate removal and sediment metabolism

Our main goal was to study the effect of increasing NO_3^- loads on denitrification and the N_2O/N_2 ratio. The experimental set-up was designed for studying those processes and therefore has a limited ability to detect or study other potential NO_3^- removing processes. However, the results of this study show that processes other than denitrification are important in the N cycling of the boreal river sediments studied. Although denitrification rates increased with increasing NO_3^- load, only a small fraction (<10%) of the added NO_3^- was removed by denitrification, an effect that was particularly strong at the highest NO_3^- load, where only 1.8% of the added NO_3^- was denitrified and 96% of the removed NO_3^- remains unaccounted for. There are two processes that could be responsible for the unaccounted-for removal: dissimilatory NO_3^- reduction to NH_4^+ (DNRA) and assimilation of NO_3^- to microbial biomass. In contrast to denitrification, the end product of DNRA (NH_4^+), is immediately available to primary producers, and can be assimilated into microbial biomass as is NO_3^-, being therefore only temporarily removed. Similarly, the assimilated N can be released to the water during degradation of biomass. In prior studies, denitrification has been recognized as the most important process in removing NO_3^-, but several studies have reported the importance of DNRA, especially in marine sediments (e.g. Bonin 1996; An and Gardner 2002). Brunet and Garcia-Gill (1996) reported as high as 30 % NO_3^- removal via DNRA in temperate river sediments. Only a few studies exist on assimilation into microbial biomass in estuaries (Jørgensen 1989) and riparian wetlands (Matheson et al. 2002), showing a great variation (< 5 - 22 %) in N removal. To our knowledge, no studies on N assimilation exist from boreal river sediments.

Microbial activity in the sediment seems to be generally enhanced as a function of increased NO_3^- loading, which can be seen as increased DIC effluxes and enhanced O_2 consumption. The DIC produced in denitrification contributes only a small fraction of the total DIC efflux (according to Eq. 2), indicating that in this sediment NO_3^- has a more profound function as a source of N than as an electron acceptor. Therefore, it seems that the sediment metabolism is generally limited by N availability, and a great part of the added NO_3^- was probably due to microbial growth (biomass production). There was evidence that the addition of NO_3^- to this system enhanced both assimilation and mineralization. The enhanced mineralization was seen as an increase in the DIC and NH_4^+ effluxes. As the output NH_4^+, although well correlated to NO_3^-, exhibited only a low level of ^{15}N labelling (< 4 excess at. %) at the highest NO_3^- treatments, direct reduction of NO_3^- to NH_4^+ (DNRA) does not explain the observed NO_3^- removal. DNRA would have produced NH_4^+ with levels of ^{15}N labelling more similar to those of the added NO_3^- (98 at. %). Therefore, the NH_4^+ efflux most likely increases as the mineralization of the top sediment layers is enhanced. The low ^{15}N enrichment in NH_4^+ probably originates from the labelled NO_3^- that was assimilated during earlier treatments and further remineralized.

Conclusions

This study provides the first information on denitrification and N_2O fluxes, and their regulation by NO_3^- load, in eutrophic high latitude rivers. Increased NO_3^- loading in boreal rivers enhances denitrification. However, denitrification has a limited capacity to remove the NO_3^- from rivers (1.2 -7.9 % of the added NO_3^-), especially at very high NO_3^- concentrations. An increased availability of NO_3^- also stimulates N_2O production, but the N_2O/N_2 ratio in riverine denitrification remais low even with very high NO_3^- concentrations. Therefore, NO_3^- removal during denitrification in rivers will not lead to large emissions of N_2O, an efficient greenhouse gas, to the atmosphere. Additionally, the results of this study show the potential of other processes, especially assimilation to microbial biomass, for removing NO_3^-, and the impact of increased N loads on overall metabolism in sediments.

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Table 1. Measured parameters of denitrification and NO_3^- removal under different NO_3^- treatments

NO_3-N LOADING TO CF-MICROCOSM			DENITRIFICATION				NO_3^- REMOVAL		BACKGROUND PARAMETERS		
Treatment (µM)	NO_3-N Load (mmol $Nm^{-2}d^{-1}$)	N	N_2 (µmol N $m^{-2}d^{-1}$)	N_2O (µmol N $m^{-2}d^{-1}$)	N_2O/N_2 (%)	Dw/Dtot (%)	Total (%)	Via denitrification (%)	O_2 (mg l^{-1})	DIC (mmol C $m^{-2}d^{-1}$)	NH_4^+ (µmol N $m^{-2}d^{-1}$)
10	3.3 (0.03)	5	440 (89)	4.1 (0.5)	1.0 (0.01)	52 (1.3)	9.3 (2.0)	5.3 (1.3)	ND	22 (3.3)	730 (180)
30	5.2 (0.01)	5	610 (97)	19 (3.0)	2.8 (0.01)	59 (2.0)	13 (1.0)	7.6 (1.0)	3.8 (0.1)	22 (5.2)	850 (240)
100	11 (0.9)	5	960 (200)	39 (5.2)	3.9 (0.01)	67 (0.2)	17 (3.0)	5.6 (1.2)	4.8 (1.4)	23 (6.3)	1120 (320)
300	49 (6.5)	5	1650 (210)	68 (12)	3.4 (0.01)	69 (0.01)	42 (11)	1.8 (0.5)	2.5 (0.8)	35 (2.5)	1590 (540)
			Spearman correlations of parameters with NO_3- treatments (µM)								
			0.587 **	0.865 **	0.736 **	0.884 **	0.633 **	-0.390 *	-0,327	0.71	0.146

Averages and standard deviations in parenthesis

$^{**}p<0.01$, $^{**}p<0.05$

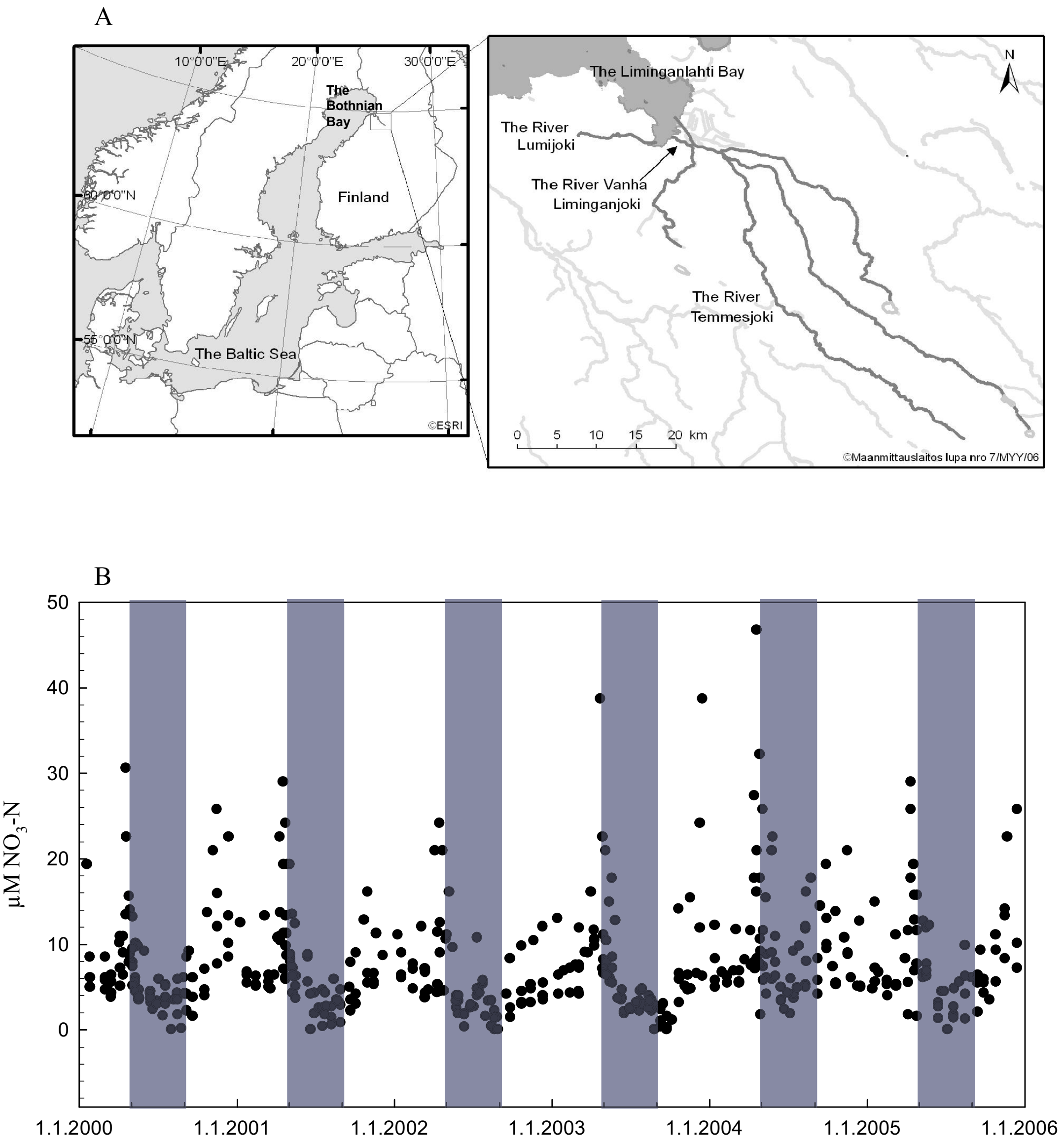

Fig. 1 a The study site. **b** Annual variation in the NO_3^- concentrations (µM) in surface waters (depth 0.5 m) of the Temmesjoki River in years 1999-2005 (Data from the Environmental Information System, HERTTA). Summers (May-August) are shaded grey.

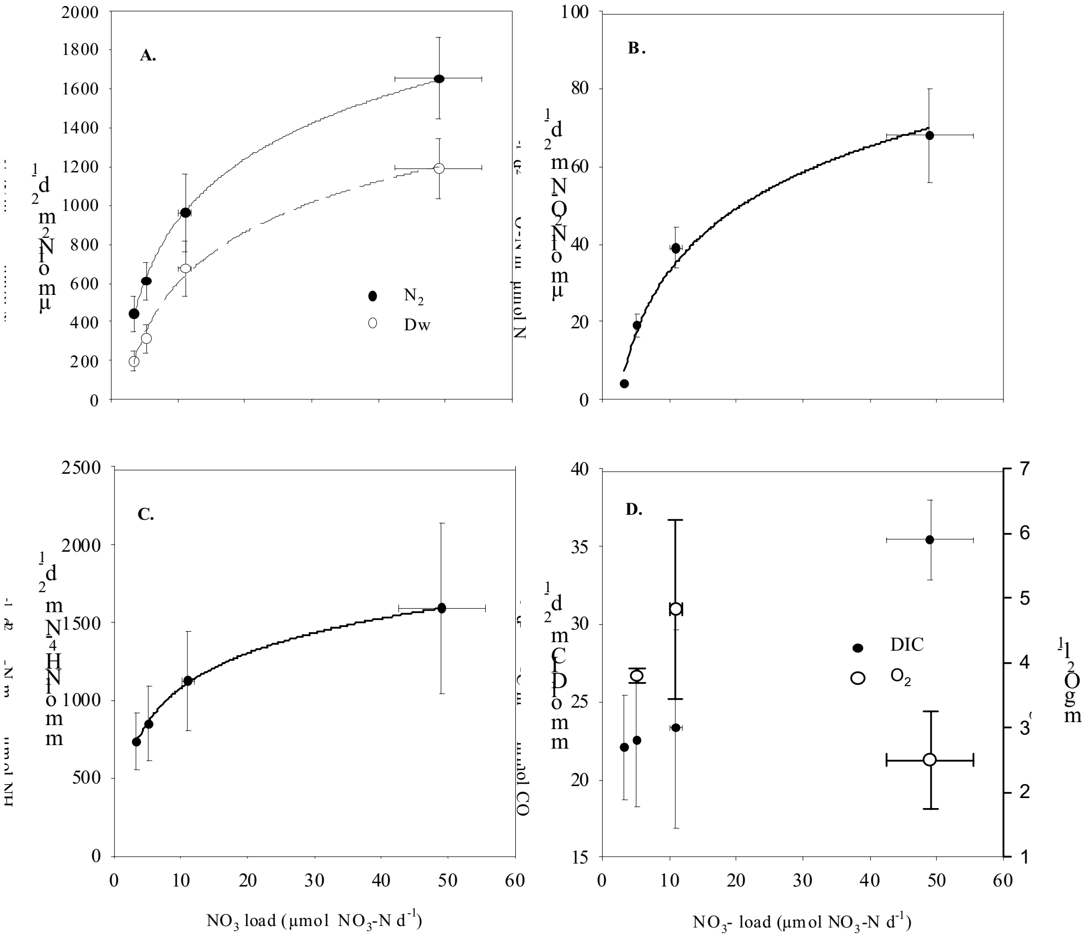

Fig. 2. A N_2 effluxes and Dw as a function of NO_3^- load. Logarithmic curve fit for N_2: $y = 435 \ln(x) - 119$, $R^2 = 0.99$, $p < 0.01$; and for Dw: $y = 377 \ln(x) - 268$, $R^2 = 0.99$, $p < 0.01$. **B)** N_2O effluxes as a function of NO_3^- load. Logarithmic curve fit $y = 23 \ln(x) - 20$, $R^2 = 0.98$, $p < 0.01$ **C)** NH_4^+ fluxes as function of NO_3^- load. Logarithmic curve fit $y = 322 \ln(x) + 336$, $R^2 = 0.99$, $p < 0.01$. **D)** DIC effluxes and O_2 consumption as a function of NO_3^- load. In all figures NO_3^- load is the NO_3^- input (µmol/day) into the sediment.

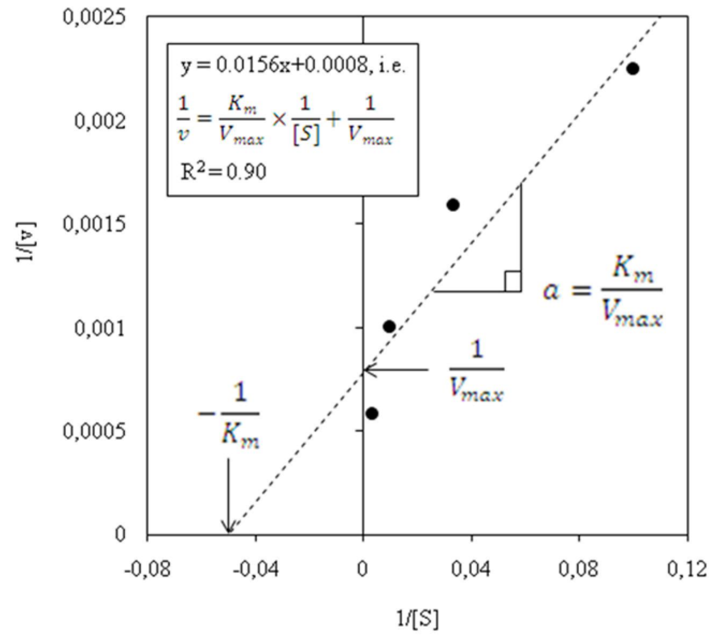

Fig 3. Lineweaver-Burk transformation of the Michaelis-Menten type curve for total denitrification. V is the measured total denitrification rate (mmol N_2 m^{-2} d^{-1}) and [S] is the NO_3^-concentration (µM NO_3^-). The intercept of the linear regression estimate on the abscissa gives a value of -0.05 µM NO_3^-, which is a negative reciprocal of the K_m-value (20 µM NO_3^-)

CHAPTER IV:

DENITRIFICATION AND N_2O EFFLUXES IN THE BOTHNIAN BAY (NORTHERN BALTIC SEA) RIVER SEDIMENTS AS AFFECTED BY TEMPERATURE UNDER DIFFERENT OXYGEN CONCENTRATIONS.

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ORIGINAL PAPER

Denitrification and N_2O effluxes in the Bothnian Bay (northern Baltic Sea) river sediments as affected by temperature under different oxygen concentrations

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Abstract Denitrification rates and nitrous oxide (N_2O) effluxes were measured at different temperatures and for different oxygen concentrations in the sediments of a eutrophied river entering the Bothnian Bay. The experiments were made in a laboratory microcosm with intact sediment samples. ^{15}N-labelling was used to measure denitrification rates (Dw). The rates were measured at four temperatures (5, 10, 15 and 20°C) and with three oxygen inputs (<0.2, 5, and 10 mg O_2 l^{-1}). The temperature response was highly affected by oxygen concentration. At higher O_2 concentrations (5 and 10 mg O_2 l^{-1}) a saturation over 10°C was observed, whereas the anoxic treatment (<0.2 mg O_2 l^{-1}) showed an exponential increase in the temperature interval with a Q_{10} value of 3.1. The result is described with a combined statistical model. In contrast with overall denitrification, the N_2O effluxes from sediments decreased with increasing temperature. The N_2O effluxes had a lower response to oxygen than denitrification rates. The N_2O/N_2 ratio was always below 0.02. Increased temperatures in the future could enhance denitrification rates in boreal river sediments but would not increase the amount of N_2O produced.

Keywords Baltic Sea · Denitrification and nitrous oxide effluxes · ^{15}N · Oxygen concentration · River sediments · Temperature

Introduction

Baltic Sea is suffering from severe eutrophication, mainly caused by human activity, for example agriculture, forestry, and peat mining in the surrounding catchments (HELCOM 2003). Lakes, peatlands, rivers, and estuaries within the catchments provide natural filters for the nitrogen load heading to the Baltic Sea (Lepistö et al. 2006). The northernmost part of the Baltic Sea, the Bothnian Bay, is the most intact part of the Baltic Sea; deep sea areas are in a near-pristine state and primary production is, in contrast with the southern areas (e.g. Gulf of Finland), phosphorus, not nitrogen, limited. The shoreline on the Finnish side, however, shows increased nitrate (NO_3^-) fluxes from several eutrophied rivers. The rivers entering the Bothnian Bay, in contrast to most of the catchments entering to the Gulf of Finland, are profitable for studying the importance of the river channel in nitrogen removal, as their catchments have low lake coverage.

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Therefore, the changes in the NO_3^- removal capacity of the river sediments directly reflect the NO_3^- loading entering Bothnian Bay.

Microbial processes have key importance in diminishing anthropogenic nutrient inputs to aquatic ecosystems (Ogilvie et al. 1997; Seitzinger 1988). Denitrification (reduction of NO_3^- to gaseous N compounds) has been estimated to remove up to 40–50% of inorganic nitrogen in estuaries (Seitzinger 1988), 30% of the annual N loading in the Gulf of Finland, and 23% in the Bothnian Bay (Stockenberg and Johnstone 1997). However, there are only few studies on the nitrogen cycle, including denitrification, in boreal estuaries and rivers (Nielsen et al. 1995; Silvennoinen et al. 2007; Stepanauskas et al. 2002) and those studies have reported only minor removal of inorganic nitrogen by denitrification.

Denitrification in aquatic ecosystems is controlled by several environmental factors including NO_3^- concentration, oxygen and carbon availability, temperature, and retention time. A collective study from several aquatic ecosystems showed that NO_3^- and oxygen availability generally limit denitrification rates (Piña-Ochoa and Álvares-Cobelas 2006). The importance of temperature in controlling denitrification was found to be minor in the global state (Piña-Ochoa and Álvares-Cobelas 2006). However, there are few published data on the effect of temperature on denitrification and, as far as we are aware, none on boreal rivers. Most studies show an increase in denitrification rate with increasing temperature (Nowicki 1994; Cavari and Phelps 1977; Sørensen et al. 1979; van Luijn et al. 1996; Seitzinger 1988 and references therein). In boreal regions, seasonal variation in water temperature is great and should determine microbial activity, including denitrification.

Oxygen availability has a dual effect on denitrification in sediments: Nitrification requiring oxygen provides NO_3^- and nitrite for denitrification, especially in NO_3^- depleted marine ecosystems (Knowles 1981; Jensen et al. 1993). Anaerobic conditions in marine sediments limit nitrification. In contrast, denitrification requires low oxygen concentrations ($\sim$0.2 mg l^{-1} or less) (Seitzinger 1988). These processes occur simultaneously in sediments resulting from their different distribution in the sediment profile, nitrification being in the uppermost aerobic sediment layers (Vanderborght et al. 1977; Billen 1978).

The effect of oxygen availability on denitrification in river sediments has not been investigated, presumably because river waters are flowing and turbulent and therefore usually well aerated. This is true also for boreal rivers during spring and autumn when flow rate is high. However, during summer the flow rate is reduced and near bottom oxygen concentrations are diminished. The flow rate is low also during winter time when the rivers have an ice-cover for approximately 5 months. The ice cover limits the gas exchange between air and water. The effects of both temperature and oxygen availability on denitrification need to be investigated in boreal rivers. These results can be used in models to make regional estimates of annual denitrification.

Nitrous oxide (N_2O), an effective greenhouse gas, is produced in denitrification and in nitrification. If NO_3^-, causing eutrophication, is reduced to molecular nitrogen (N_2) by denitrification there is no atmospheric greenhouse effect. In aquatic ecosystems, the amount of N_2O produced in denitrification is generally minor compared with the amount of N_2. However, the enzyme (nitrous oxide reductase) catalysing the last reduction step of denitrification is sensitive to environmental factors. Increased NO_3^- concentrations and low temperature have been reported to favour the production of N_2O (Knowles 1982).

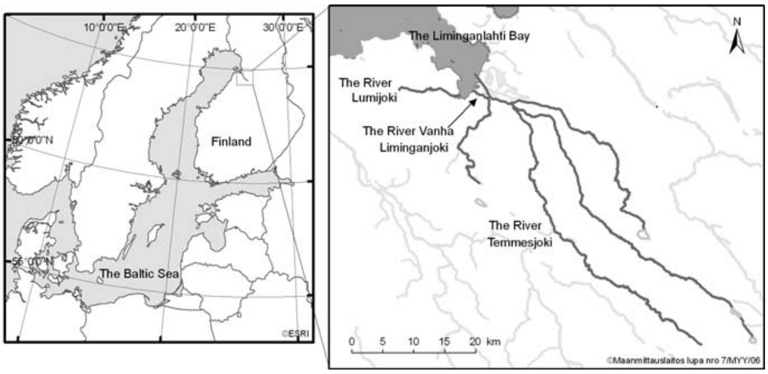
This study provides the first information on the effects of oxygen availability and temperature on denitrification and N_2O efflux rates in boreal river sediments.

Material and methods

Site description

Sediments were collected in June 2003 from the river mouth of the River Temmesjoki (64°84′ N, 25°37′ E) (Fig. 1). The River Temmesjoki is characterised as a eupolytrophic river for total N concentration. The drainage basin of the Temmesjoki consists of different catchments including forests, wetlands, and agricultural areas. The Temmesjoki has a drainage basin of 1190 km^2 with high coverage of agricultural land (15%). The river has a small annual mean flow (11 m^3 s^{-1}). Thus, the annual N load to the Bothnian Bay is not higher than 520 tons despite the high NO_3^- concentrations in the river water.

Fig. 1 The study site



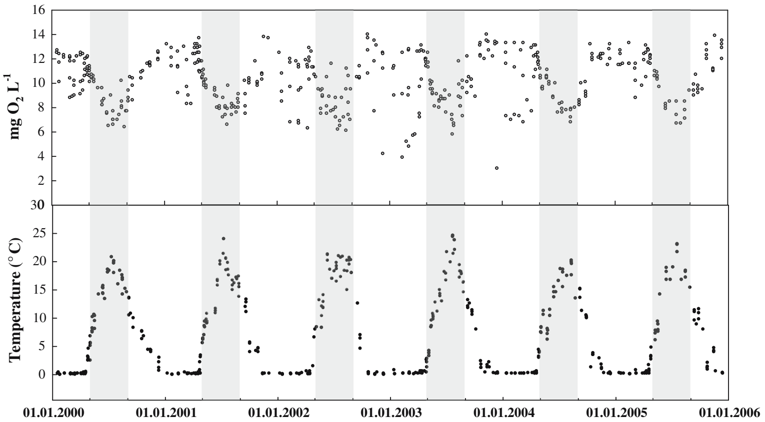
Annual variation in temperatures and oxygen concentrations of the river Temmesjoki

Annual variation in temperatures and oxygen concentrations (as dissolved oxygen, DO, mg O_2 l^{-1}) in the river Temmesjoki is shown in Fig. 2 (data from Environmental Information System, HERTTA). These values were measured from the surface water at the depth of 0.2–1.0 m and, therefore, the actual temperature close to the sediments (depth 5–6 m) is likely to be lower in summer and higher in winter. Furthermore, the oxygen saturation is probably lower close to the sediments all year around, especially during summer and winter when the flow rate is low and the vertical turbidity is negligible. Temperatures in the presented dataset of surface waters varied from 0.1 to 24.6°C, and oxygen concentrations from 3 to 14 mg O_2 l^{-1}.

Sampling and experimental set-up

Sediments (12 replicate cores) were collected directly in transparent acrylic tubes (ø 94 mm, height 650 mm). The height of the collected intact sediment profile was ≤200 mm. The sediments were placed in a laboratory microcosm equipped with continuous water flow (Liikanen et al. 2002b). The microcosm was situated in a dark, temperature-controlled room. Water was pumped from an 80-l water reservoir over the cores by a peristaltic pump (IPC-24, Ismatec, Glattbrugg-Zürich, Switzerland) at a rate of 50 ml h^{-1}. Overlying water in the cores was gently stirred with a rotating magnet to prevent stratification (Liikanen et al. 2002b). A vacuum was applied to the water reservoir three times, to remove N_2, and the water was flushed continuously with a gas mixture consisting of Ar/O_2 (AGA, Finland). N_2 was replaced

Fig. 2 Seasonal variation of temperatures (°C) and oxygen concentrations (mg l^{-1}) in the River Temmesjoki during the years 2000–2005. Data from Environmental Information System (HERTTA). Summers (May–August) are shaded *gray*



with argon to improve analysis of N_2 derived from denitrification. The anoxic treatment was fed with exclusively argon-treated water, whereas water treated with 90/10% Ar/O_2 and 80/20% Ar/O_2 mixtures was fed to 5 and 10 mg l^{-1} treatments, respectively.

The sediments were supplied with distilled water amended with 30 μM $K^{15}NO_3^-$ (60 at%) and in-situ concentrations of sulfate (0.2 mM) and chloride (0.5 mM) (added as $MgSO_4$ and $CaCl_2$) and incubated with 0, 5 and 10 mg O_2 l^{-1} oxygen inputs (three replicate sediment cores per oxygen level) for four weeks. During the four weeks temperature was decreased gradually from 20 to 5°C, allowing the sediments to be adapted to each temperature for four days. The samples at various temperatures were taken during the last three days of the experiment week. During the first week (beginning of the experiment) the sediments were incubated at 20°C (close to the water temperature (17°C) at sampling); during the second, third, and fourth weeks temperatures of 15, 10 and 5°C were applied.

The concentrations of N_2, N_2O, and dissolved inorganic carbon (DIC) in water were measured and the effluxes were calculated from the difference between the concentrations in the in-flowing and outflowing waters and by taking into account the water flow rates and sediment surface area (69 cm^2).

Analyses of N_2, N_2O and DIC

For determining N_2, N_2O, and DIC effluxes, water samples (~30 ml) were taken in syringes and preserved with sulfuric acid (1 ml H_2SO_4 20% *v*/*v*) and balanced for 1 day with Ar headspace. The headspace was then measured for the gas concentrations and isotopic composition of N_2. The gas concentrations in the original sample were calculated according to Henry's law (modified by McAuliffe 1971). Due to sulfuric acid preservation all the inorganic carbon was liberated to the headspace as CO_2 and, therefore, the values presented here are DIC (dissolved inorganic carbon) effluxes. Nitrous oxide concentrations and CO_2 were analysed by GC (Hewlett–Packard, Palo Alto, USA: Series II) equipped with two 2-m long packed columns (Hayesep Q (80/100 mesh), an electron-capture detector for N_2O analyses, and a thermal conductivity detector for CO_2 analyses (Nykänen et al. 1995). N_2 concentrations and isotopic compositions were measured by gas chromatography coupled with a quadrupole mass spectrometer (GC–QMS) (QP 2000; Shimadzu, Japan) (Russow et al. 1996). The masses 28, 29, and 30 were measured and the peaks were calibrated against normal air (78% N_2) for concentration measurements. Contamination of samples by N_2 in the laboratory atmosphere was prevented by flushing the injection system and the sample loop of the GC with helium before injection of the sample. The amount of N_2 derived from denitrification was calculated according to non-random distribution of the masses 28, 29, and 30 (Hauck et al. 1958; Siegel et al. 1982; Russow et al. 1996). The isotopic composition in N_2O was assumed to be similar to that of N_2.

Oxygen, redox, and pH measurements

At the end of each incubation week oxygen concentrations in the overlying water (1 cm above the sediment surface) were measured with an oxygen electrode (Oxi 330 dissolved oxygen meter with CellOx 325 dissolved oxygen probe; WTW, Germany). pH and redox were measured with a pH320 microprocessor-controlled pH meter (WTW) with Hamilton pH electrode and InLab 501 redox electrode.

Model for combining O_2 concentration and temperature responses of denitrification

Observed temperature response in the interval investigated shows an exponential increase in the denitrification rate (Fig. 3a–c). It can be described by a simple exponential function or by the more process-based Arrhenius equation. The influence of O_2 concentration was described with a simple linear relationship. In most models the response functions are combined by multiplication, based on the assumption that the factors are independent. The results of this study pointed out that this is not true for the relationships of temperature and O_2 concentrations with denitrification in sediments. Therefore the approach presented by Stange (2007) was used in the generalised form:

$$\mathrm{g(temp,\ O_2)} = \mathrm{den_{max}}\ \left[(\mathrm{f(temp)})^{-m} + (\mathrm{f(O_2)})^{-m}\right]^{-\frac{1}{m}} \tag{1}$$

where, g(temp, O_2) is the combined response function, $\mathrm{den_{max}}$ is the maximum denitrification rate

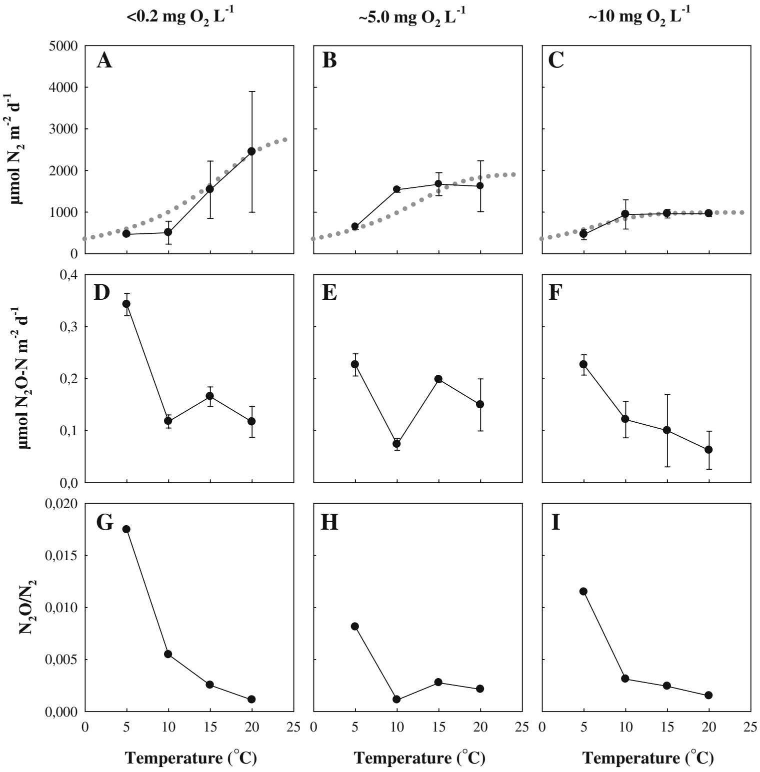


Fig. 3 Denitrification rates (**a–c**), N_2O effluxes (**d–f**) and N_2O/N_2 ratios (**g–i**) for different temperatures and oxygen concentrations. N for each point is 3. The *error bars* represent the standard deviations. The *dotted dark grey lines* in **a–c** represent the results of the combined model

(μmol N $m^{-2}d^{-1}$), f(temp) is the temperature response function (0.1), f(O_2) is the oxygen response function (0.1), and *m* is a parameter (fixed at 4).

The results demonstrated that temperature and O_2 concentration interact and parameter *m* represents the strength of the interaction between these two factors. If $m = 1$, the original approach from Stange (2007) was used. If *m* tended to infinity the minima approach was used. This parameter is highly sensitive in the interval from 0 to 3 but insensitive >4. Because insensitivity results in a high uncertainty of parameter estimation the parameter is fixed at a value of 4.

The exponential function for the temperature response was standardised in the interval from 0 to 1 for the investigated temperature interval ≤20°C:

$$\text{f(temp)} = \exp\,(\text{P1} * (\text{temp} - 20^{\circ}\text{C})) \tag{2}$$

where, temp is temperature (°C), P1 is the temperature influence parameter (°C^{-1}) and f(temp) is the temperature response function (0.1).

Also the oxygen response function was standardised for observed oxygen concentrations >0 mg O_2 l^{-1}:

$$\text{f}(O_2) = 1 - \text{P2} * O_2 \tag{3}$$

where P2 is the slope of the linear relationship (mg^{-1} O_2 l).

Parameter estimations for denitrification rate were carried out using individual measurements ($n = 36$) and also with aggregated mean values ($n = 12$) with the non-linear parameter estimation procedure in Statistica 7.1 software.

Results

Denitrification rates

Denitrification rates increased with increasing temperature in all oxygen treatments, exhibiting values of 460–2450, 650–1630, and 460–960 μmol N m^{-2} d^{-1} for 0, 5, and 10 mg l^{-1} oxygen treatments, respectively (Fig. 3a–c). For the lowest oxygen treatment the increase in denitrification rate with increasing temperature was exponential, exhibiting a Q_{10} value of 3.06, and the highest denitrification rate measured during the experiment was at 20°C (Fig. 3a). For 5 and 10 mg $O_2\ l^{-1}$ treatments denitrification rates increased from 5 to 10°C but did not increase further at higher temperatures (Fig. 3b–c). At the highest temperatures, the anoxic treatment exhibited nearly twofold denitrification rates in comparison with those in the 5 mg l^{-1} treatments and nearly threefold rates in comparison with those in the 10 mg l^{-1} treatments (Fig. 3a–c). The presented model, which considers the relationship between temperature, oxygen concentration, and denitrification rates, explains 55% of the variation in the data (Fig. 3a–c). All three fitted parameters differ significantly from zero ($p < 0.001$) and are given in Table 1. It must be noted that the unexplained variation is caused by the high variation in the treatment replicates rather than the model approach. Using mean value ($n = 12$) instead of all measurements ($n = 36$) the fitted values for the parameter den_{max}, P1, and P2 are exactly the same, but the R^2 value increased to 0.838. This is considerably better than a multiple linear regression model ($R^2 = 0.607$) or a multiplicative model with exponential response function ($R^2 = 0.665$) using the mean values by calculation. Using the unaggregated data the explainable variation is only 40% and 44% for the multiple linear regression model and the multiplicative model, respectively.

N_2O effluxes and N_2O/N_2 ratio

In contrast with denitrification rates ($N_2 + N_2O$ efflux) the N_2O effluxes generally decreased with increasing temperature. for all O_2 treatments the lowest temperature (5°C) exhibited the highest N_2O effluxes—12, 8, and 8 μmol N_2O–N $m^{-2}\ d^{-1}$ with increasing O_2 input (Fig. 3d–f). The decreasing trend was most obvious for 0 and 10 mg $O_2\ l^{-1}$ treatments; in the linear regression model the increase in temperature in those treatments explained 58 and 88% of the variation in the N_2O efflux, respectively. However, in the 5 mg l^{-1} treatment it explained only 4% of the decrease in the N_2O efflux. The N_2O/N_2 ratio is generally below 0.01 but for the lowest temperature and lowest O_2 input it increased and exhibited a value of 0.17 (Fig. 3g–i).

DIC effluxes, oxygen consumption, redox potential, and pH

DIC effluxes increased with increasing temperature in all O_2 treatments (Table 2), which is an indication of increasing microbial activity in response to rising temperature. The oxygen consumption (the oxygen efflux from water to the sediment) could not be determined for the anoxic treatment, since the output water had no oxygen (Table 2). The molar ratio of DIC effluxes to oxygen consumption increased with increasing temperature. At high temperatures (15–20°C) the ratio was higher in the 5 mg l^{-1} treatment than in the 10 mg l^{-1} treatment (Table 2). The redox potential was lowest in the anoxic treatment but was also on the negative side in the 5 and 10 mg $O_2\ l^{-1}$ treatments (Table 2). The pH was generally lowest in anoxic treatment but there were no major differences between temperatures (Table 2).

Discussion

Denitrification rates as affected by temperature

Denitrification rates measured in the sediments of the rivers entering the Bothnian Bay with same NO_3^- input as here exhibited values from 330 to 910 μmol N $m^{-2}\ d^{-1}$ at 15°C and with ~10 mg $O_2\ l^{-1}$ (Silvennoinen et al. 2007). The rates measured here with similar temperature and oxygen concentration fall into this range (960 ± 108). The

Table 1 Results from parameter estimation using the presented approach

The optimised values, standard errors (SE), significance levels of the parameters, and confidence intervals are given

	Estimation	SE	Significance value *p*	Lower confidence limit	Upper confidence limit
Den_{max}	2838.2	273.9	0.000000	2280.9	3395.49
P1	0.106	0.0171	0.000001	0.071	0.140
P2	0.066	0.0085	0.000000	0.048	0.083

Table 2 O_2 consumption, redox potentials, pH values, DIC (dissolved inorganic carbon) effluxes, and respiratory quotients for various temperatures and oxygen concentrations

Temperature (°C)	<0.2 mg O_2 l^{-1}	5 mg O_2 l^{-1}	10 mg O_2 l^{-1}
O_2 consumption (mg O_2 m^{-2} d^{-1})			
5	ND*	550 (17)	887 (60)
10	ND*	1100 (280)	950 (130)
15	ND*	375 (160)	1200 (160)
20	ND*	542 (70)	1100 (114)
Redox potential (mV)			
5	−121 (16)	−54 (17)	−11 (13)
10	−161 (4)	−100 (13)	−12 (5.2)
15	−144 (7)	−110 (27)	−70 (10)
20	−126 (10)	−79 (14)	−75 (13)
pH			
5	6.5 (0.1)	6.2 (0.1)	6.6 (0.1)
10	6.4 (0.1)	6.2 (0.1)	6.6 (0.0)
15	6.4 (0.1)	6.2 (0.1)	6.2 (0.1)
20	6.5 (0.1)	6.6 (0.1)	6.2 (0.1)
DIC (mmol C $m^{-2}d^{-1}$)			
5	19 (0.2)	15 (2.4)	20 (1.0)
10	17 (2.0)	24 (2.6)	24 (2.0)
15	36 (3.0)	42 (0.8)	44 (3.0)
20	88 (9.0)	86 (16)	78 (4.0)
Respiratory quotient[a]			
5	ND*	0.8	0.7
10	ND*	0.7	0.8
15	ND*	3.6	1.7
20	ND*	5.1	2.3

ND* = Not determined. The oxygen effluxes could only be measured when O_2 concentration in the outflowing water was measurable

[a] Molar ratio of DIC production to O_2 consumption (mmol C m^{-2} d^{-1}/mmol O_2 m^{-2} d^{-1})

results presented here are the "Dw" values i.e. the denitrification originates from the overlying NO_3^-. These rates are considerably lower those measured in rivers in temperate regions, e.g. García-Ruiz et al. (1998a) measured rates up to 13,800 μmol N m^{-2} d^{-1} in a river in NE England. The higher rates obtained in lower latitude rivers are probably due to both higher nutrient input and higher temperature with more abridged annual variation.

There are several studies in which seasonal changes in denitrification rates and N_2O effluxes in different water ecosystems are discussed, and often the seasonality of denitrification rates has been found to reflect the changes in nutrient inputs and the availability of carbon and oxygen (Jørgensen 1989; Piña-Ochoa and Álvares-Cobelas 2006 and references therein). The processes supporting denitrification, e.g. carbon and nitrogen mineralization, the discharge supplying the river with alloctonous carbon and nutrients, and the turbidity supporting aeration, vary with seasons and most of them are also temperature related. Thus, there are difficulties distinguishing between the forces driving denitrification.

The positive effect of temperature on denitrification is well known for various aquatic ecosystems (Nowicki 1994; Cavari and Phelps 1977; Pattinson et al. 1998; Sørensen et al. 1979; van Luijn et al. 1996; Seitzinger 1988 and references therein); there are only few studies reporting a negative response (Sørensen et al. 1979). The results obtained in this study support the findings that denitrification rate increases with temperature, although the correlation was weak. The high variability in denitrification rates, also in aquatic ecosystems, is well known (e.g. Middelburg et al. 1995), and is clearly seen in these results also (Fig. 3a–c).

Long-term incubation experiments create a risk of heterotrophic processes becoming limited by the availability of labile organic carbon. We studied that possibility by supplying sediments from the same study site in a four-week incubation experiment at 15°C with extra carbon source. The in-situ concentrations of acetate, formate, oxalate, and lactate in inflowing water did not increase the denitrification or respiration rates (data not shown). The result shows that the sediment supplies heterotrophic processes with labile organic matter throughout the four-week incubation experiments without an extra carbon source.

Anaerobic processes become more important in carbon degradation as the temperature increases. The molar ratio of DIC production to O_2 consumption (respiratory quotient, RQ) increases as temperature increases (Table 2). At lower temperatures RQ is <1, indicating that carbon is mainly degraded in aerobic respiration. As the temperature increases, microbial activity accelerates using all of the available oxygen, which leads to use of other electron acceptors in degradation of organic matter (Rich 1975). This can be seen as higher RQs. It can be assumed, as found here, that moderate oxygen concentrations exhibit higher RQs than the high oxygen concentrations.

The possibility, that anammox (anaerobic ammonium oxidation) causes inaccuracies in determination of N_2 flux during denitrification cannot be neglected. If the input $^{15}NO_3^-$ was assimilated to microbial biomass and remineralized as NO_2^- or NH_4^+ or reduced to NO_4^+ in DNRA, the measured N_2 pool could have received labelled N_2 via anammox (Kartal et al. 2007). However, there is, so far, no evidence on anammox existing in boreal river ecosystems.

Temperature effect on N_2O effluxes—inhibition of the N_2O reductase at low temperatures

The denitrification rates and N_2O effluxes presented in this study represent the ^{15}N-labelled N-pool, i.e. the total denitrification is the denitrification originating from the added $^{15}NO_3^-$ and the same is true for the N_2O. Nevertheless, it is well known that N_2O is produced not only in denitrification but also in nitrification. During the experiment the added $^{15}NO_3^-$ could have been assimilated to microbial biomass and further liberated to overlying water as $^{15}NH_4^+$. Additionally, the added $^{15}NO_3^-$ could have been reduced to $^{15}NH_4^+$ in dissimilatory nitrate reduction to ammonium (DNRA). Therefore, additional ^{15}N measurements would be required to identify whether the measured N_2O is produced in nitrification or in denitrification. However, the greatest N_2O effluxes were measured in the anoxic treatment (<0.2 mg O_2 l^{-1}), where the nitrification activity is expected to be suppressed in oxygen deficiency, indicating that most of the N_2O effluxes in this study were produced by denitrification.

The most distinguishable result of this study was the decrease in N_2O effluxes with increase in temperature, a response opposite to that for overall denitrification. It has also been found by García-Ruiz et al. (1998b), that N_2O production occasionally exhibits greater variability than denitrification in an NE England river. They summarized that the relative effect of the environmental factors influencing N_2O removal may differ from the initial steps in denitrification. In their data, N_2O production was detected at low temperatures (2°C), and at low NO_3^- concentrations, when denitrification was already reduced. It is well known that the increase in NO_3^- concentration leads to partial inhibition of N_2O reductase in soils (Blackmer and Bremmer 1976; Letey et al. 1981) and in sediments (Terry and Tate 1980). Nevertheless, several studies from soil ecosystems have also shown that the increase in temperature reduces the N_2O/N_2 ratio in denitrification by suppressing N_2O reductase (Knowles 1982; Maag and Vinther 1996). It has been suggested that the changes in N_2O/N_2 ratio may not be due to higher activation energies for N_2O reduction in comparison to N_2O production but due to anomalies at critically low temperatures (Holtan-Hartwig et al. 2002; Öquist et al. 2004).

Global N_2O emissions from rivers, estuaries, and continental shelves are calculated to be 4.9 (1.3—13.0) Tg N in 2050, of which two-thirds are from rivers (Kroeze and Seitzinger 1998). The N_2O effluxes measured in this study (8–12 µmol N_2O-N m^{-2} d^{-1}) are slightly lower, especially at the higher temperatures, than fluxes from the Bothnian Bay river sediments measured earlier (19–27 µmol N_2O-N m^{-2} d^{-1}) (Silvennoinen et al. 2007). N_2O production rates in boreal river sediments measured in this study are a factor of ten lower than rates measured in temperate rivers (García-Ruiz et al. 1998b), but they are in the range measured from freshwater lakes of the same latitude (Liikanen et al. 2002a). Very high N_2O/N_2 ratios have been measured from eutrophied rivers (García-Ruiz et al. 1999), and Seitzinger (1988) reported that in eutrophied water ecosystems the N_2O/N_2 ratio would be 0.05. The highest ratio measured here, even at cold temperatures with enhanced N_2O production, was 0.017. According to our results, the amount of N_2O produced in denitrification is lower in boreal rivers than in other river ecosystems. Increasing anthropogenic nutrient loading may, however, further increase the N_2O/N_2 ratio and, consequently, N_2O production in these rivers.

Impact of oxygen availability on denitrification rates and N_2O effluxes

Denitrification rates measured at the highest temperature were two and threefold higher in the anoxic treatment than in the 5 and 10 mg O_2 l^{-1} treatments, respectively. The anoxic treatment also exhibited the highest N_2O effluxes, but the response of N_2O to O_2 input was more indistinguishable than that of denitrification rates. To the best of our knowledge, impact of oxygen concentration on denitrification has not been studied in boreal rivers, and studies in all water ecosystems are few, but the results obtained here support the earlier observations from other water ecosystems (Piña-Ochoa and Álvares-Cobelas 2006; Rysgaard et al.1994). Furthermore, oxygen availability together with NO_3^- availability was among the most important factors affecting denitrification in a cross-ecosystem analysis (Piña-Ochoa and Álvares-Cobelas 2006).

Restriction of the denitrification potential (assuming it is close to the denitrification rate in anoxic treatment) by O_2 was observed at high temperatures. If the boundary value of 0.2 mg O_2 l^{-1} (Seitzinger 1988) is valid for our experiment, it can be assumed that denitrification is not limited by increased oxygen concentrations throughout the sediment core, but in the water and the sediment surface only. Consequently, denitrification could proceed in the deeper sediment layers and might be limited by the diffusion of NO_3^- into the deeper sediment layers. Therefore, in oxic treatments denitrification is limited by the vertical diffusion of NO_3^- in addition to temperature and high oxygen concentrations in the overlying water.

Conclusions

River waters are well aerated most of the year, so the results obtained for 10 mg O_2 l^{-1} best reflect the O_2 conditions in rivers. However, the O_2 concentrations in surface waters of the River Temmesjoki vary from 3 to 14 mg l^{-1} at the surface and almost anoxic conditions could develop in the river sediment during summer, when the flow rate and turbidity are low and during winter when the rivers are ice-covered, limiting gas-exchange.

Future scenarios have predicted the climate to warm in boreal regions. These results indicate that for prolonged warm periods or increases in temperature the yearly amount of NO_3^- removed by denitrification would increase and, in addition, at higher temperatures the amount of N_2O produced would decrease. However, if winter temperatures in river waters increased from the current value, which is close to zero, even a few degrees rise would increase the overall denitrification rate.

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CHAPTER V:

GREENHOUSE GAS FLUXES FROM THE EUTROPHIC TEMMESJOKI RIVER AND ITS ESTUARY IN THE LIMINGANLAHTI BAY

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Greenhouse gas fluxes from the eutrophic Temmesjoki River and its Estuary in the Liminganlahti Bay (the Baltic Sea)

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Abstract We studied concentrations of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) in the eutrophic Temmesjoki River and Estuary in the Liminganlahti Bay in 2003–2004 and evaluated the atmospheric fluxes of the gases based on measured concentrations, wind speeds and water current velocities. The Temmesjoki River was a source of CO_2, CH_4 and N_2O to the atmosphere, whereas the Liminganlahti Bay was a minor source of CH_4 and a minor source or a sink of CO_2 and N_2O. The results show that the fluxes of greenhouse gases in river ecosystems are highly related to the land use in its catchment areas. The most upstream river site, surrounded by forests and drained peatlands, released significant amounts of CO_2 and CH_4, with average fluxes of 5,400 mg CO_2–C m^{-2} d^{-1} and 66 mg CH_4–C m^{-2} d^{-1}, and concentrations of 210 μM and 345 nM, respectively, but N_2O concentrations, at an average of 17 nM, were close to the atmospheric equilibrium concentration. The downstream river sites surrounded by agricultural soils released significant amounts of N_2O (with an average emission of 650 μg N_2O–N m^{-2} d^{-1} and concentration of 22 nM), whereas the CO_2 and CH_4 concentrations were low compared to the upstream site (55 μM and 350 nM). In boreal regions, rivers are partly ice-covered in wintertime (approximately 5 months). A large part of the gases, i.e. 58% of CO_2, 55% of CH_4 and 36% of N_2O emissions, were found to be released during wintertime from unfrozen parts of the river.

Keywords Carbon dioxide · Estuary · Greenhouse gas · Methane · Nitrous oxide · River

Abbreviations

DIC	Dissolved inorganic carbon
ECD	Electron capture detector
FID	Flame ionization detector
GC	Gas chromatograph
GWP	Global warming potential
k_{600}	Gas transfer velocity normalized to a Schmidt number of 600
pCO_2	Partial pressure of CO_2 in water
TIC	Total inorganic carbon
TOC	Total organic carbon
tot-N	Total nitrogen
tot-P	Total phosphorus

Introduction

Traditionally, rivers have been seen as links between terrestrial and marine ecosystems, as they receive, transport and process nutrients and organic matter

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(Raymond and Bauer 2001; Wetzel 2001). They also act as important conduits for gas exchange between terrestrial ecosystems and the atmosphere (Kling et al. 1991; Cole and Caraco 2001a; Hope et al. 2001; Richey et al. 2002; Mayorga et al. 2005; Cole et al. 2007). Surface runoff and groundwater that rivers receive contain high amounts of greenhouse gases, namely CO_2, CH_4 and N_2O, originally produced in terrestrial ecosystems (Jones and Mulholland 1998a, b; Hasegawa et al. 2000; Hope et al. 2001). Gases emitted from rivers can also be produced in rivers in situ when the nutrients and organic matter that rivers receive are transformed by microbial processes to gaseous products (Raymond and Bauer 2001). Hence, most of the gases emitted from rivers are of terrestrial origin, being derived from either gases leached from terrestrial ecosystems or gases produced in rivers from the organic matter derived from catchments (de Angelis and Lilley 1987; Jones and Mulholland 1998a; Reay et al. 2003; Mayorga et al. 2005). By processing the nutrients and organic matter to gases, river ecosystems can remove part of the C and N load of marine ecosystems, but at the same time they increase the atmospheric load of greenhouse gases. However, a part of the nutrients, organic matter and greenhouse gases that rivers receive ends up in estuaries, which are considered to be important sources of greenhouse gases in marine ecosystems (Bange et al. 1994, 1996; Frankignoulle et al. 1998; Seitzinger and Kroeze 1998; Borges 2005).

Estuaries and near-shore coastal areas are known to be significant sources of marine CO_2 (Borges 2005; Borges et al. 2006), CH_4 (Bange et al. 1994; Upstill-Goddard et al. 2000) and N_2O (Seitzinger and Kroeze 1998; Seitzinger et al. 2000). Estuaries and coastal areas are estimated to contribute up to 35–60% of total marine N_2O emissions (Bange et al. 1996; Seitzinger et al. 2000), and up to 75% of total marine CH_4 emissions (Bange et al. 1994). Estuaries are generally sources of CO_2, while marginal seas are net sinks for atmospheric CO_2 (Borges 2005). Recent reviews concerning CH_4 and CO_2 emissions from estuaries have pointed out that minimal data are available for high latitudes (Upstill-Goddard et al. 2000; Borges 2005). Global estimations of N_2O emissions between rivers and estuaries are based on nitrogen input from watersheds to aquatic ecosystems (Seitzinger and Kroeze 1998; Seitzinger et al. 2000) but few field measurements have been made.

Greenhouse gas content in and emissions from rivers reflect the properties of the surrounding catchments, such as topography, soil type and texture, and land use and other anthropogenic activities (Jones and Mulholland 1998a; Reay et al. 2003). High amounts and fluxes of CO_2 and CH_4 have been found in small streams and rivers surrounded by peatlands and forests (Lilley et al. 1996; Jones and Mulholland 1998a; Neal et al. 1998; Hope et al. 2001; Dawson et al. 2002), whereas high fluxes of N_2O have been measured from rivers receiving N containing wastewaters or surrounded by N fertilized agricultural soils (McMahon and Dennehy 1999). The CO_2 concentrations and fluxes in terrestrial and aquatic ecosystems depend on photosynthesis and degradation of organic matter. Methane is produced during anaerobic carbon degradation, and high CH_4 concentrations and fluxes have been found in aquatic ecosystems and water saturated terrestrial ecosystems with anaerobic conditions (Cicerone and Oremland 1988). In aerobic conditions a fraction of the CH_4 is consumed by aerobic methane oxidation (Hanson and Hanson 1996). Nitrous oxide is produced as an intermediate of denitrification (Knowles 1981), a by-product of nitrification (Firestone and Davidson 1989) and by dissimilatory reduction of NO_3^- to NH_4^+ (Smith and Zimmerman 1981). The presence of N_2O in terrestrial and aquatic ecosystems is therefore related to the availability of inorganic nitrogen.

We studied both temporal and spatial variations in greenhouse gas dynamics in the Temmesjoki River and Estuary in the Liminganlahti Bay, in the Bothnian Bay of the northern Baltic Sea. We show how gas dynamics vary along the river as land use in the catchments changes. Additionally, our data reveal a seasonal pattern of gas dynamics along the river continuum and the impact of wintertime on aquatic gas concentrations and fluxes in boreal regions.

Materials and methods

Study sites

The dynamics of CO_2, CH_4 and N_2O emissions were measured along the course of the Temmesjoki River and Estuary and in the Liminganlahti Bay (64°52′N, 25°21′E, Finland), which is located in the north of the Gulf of Bothnia, Finland (Fig. 1). The Temmesjoki

River is a eutrophic river having a catchment mostly occupied by forests (52%), peatlands (20%) and agricultural soils (17%). Before entering the bay, the Temmesjoki River receives waters from the Tyrnävänjoki River and the Ängeslevänjoki River. The Temmesjoki Estuary is a freshwater estuary, since the Liminganlahti Bay has a salinity of <1‰, and the salinity of the northern parts of the Bothnian Bay is <2‰ (Kronholm et al. 2005). On average, 40% of the water in the Liminganlahti Bay has a riverine origin and most of it comes from the Temmesjoki River. The surface area of the Liminganlahti Bay is 109 km^2 and its mean depth is 2.6 m. The sea level and water movements in the bay vary irregularly depending mostly on wind and atmospheric pressure, tidal variations being negligible. With south and west winds, water flows from the sea up the Temmesjoki River. During the measurement period, the prevailing wind direction in the Liminganlahti Bay was 115° (in 2003 May–August) and 150° (in 2004 May–October) (Finnish Meteorological Institute, Oulu Airport).

Characteristics of the study sites and sampling strategy

There were five intensive study sites (sites 1–5, from upriver to downriver) in the Temmesjoki River (Fig. 1). For sites 1 and 2, forests and peatlands affected by forestry and peatland ditching dominated in the catchments (Table 1). Agricultural activity was substantial after site 2, and coverage of agricultural soils increased towards the estuary (site 5) (Table 1). In surface waters of the Temmesjoki River and Estuary in 2003–2004, at a depth of 10 cm, we measured the concentrations of gases, nutrients (NH_4^+–N, $NO_2^- + NO_3^- - N$, total N i.e. tot-N, total organic C. i.e., TOC, total inorganic C, i.e., TIC, tot-P, SO_4^{2-}) and O_2 as well as pH and temperature. At sites 1–5, measurements were done four times in 2003 (once in May, July, August and October) and eight times between April and August 2004 (1–3 times a month) (Table 2). Water currents and depths were measured (sites 1–4) five times during summer 2004.

Three sites were studied in Liminganlahti Bay (sites 6–8). Site 6 was located near the mouth of the Vanha Liminganjoki River, site 7 near the mouth of the Lumijoki River, and site 8 in the middle of the bay (Fig. 1). In the Liminganlahti Bay, measurements were done four times in 2004, twice in June and twice in July (Table 2).

In addition to sites 1–8, there was one site in the Temmesjoki River (site A), which was located about 1 Km upstream from site 5 (Fig. 1, black square). At site A, similar measurements to those for sites 1–5 were done during all seasons (once or twice a month

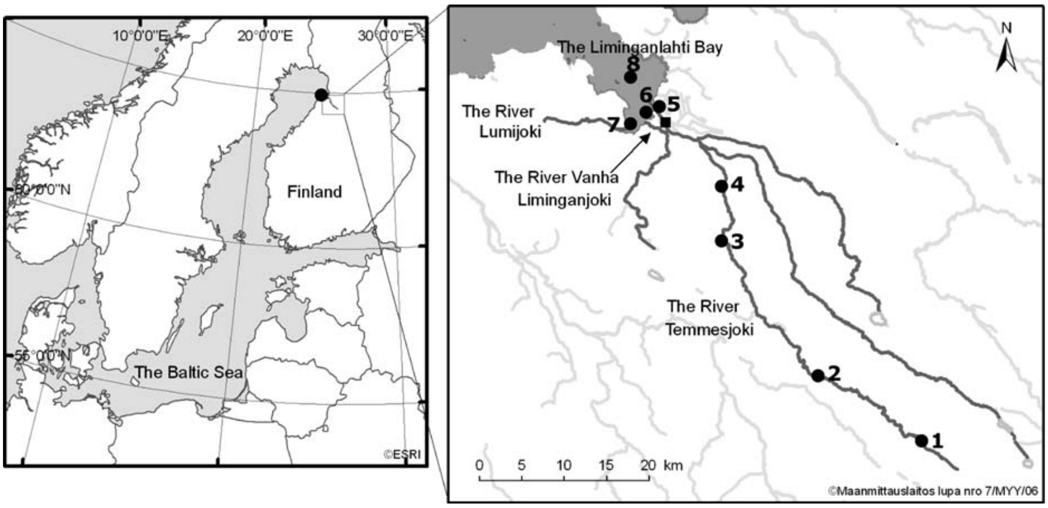

Fig. 1 The study sites in the River Temmesjoki (1–5) and in the Liminganlahti Bay (6–8). Black circles represent the study sites 1–8. Black square represents the study site A

Table 1 Characteristics of catchment area and river channel

Site	Catchment					River channel		
	Area (km^2)	Land use (%)				Width (m)	Length (m)[a]	Flow (m^3 s^{-1})[b]
		Wetland	Forest	Agriculture	Other			
1	43	52	46	0	2	3	6,256	0.4
2	105	41	53	0	6	7.5	30,554	0.99
3	261	24	61	6	9	8	48,410	2.5
4	311	21	62	7	10	6	56,528	2.9
5	1,180	20	52	17	11	41	71,500	11

[a] Length of river channel from the starting point of the river to the study site

[b] Averages of the modeled flow in 2003–2004

Table 2 Sampling strategy at the various sites

	River (site A)	River (sites 1–5)	Bay (site 6–8)
Fluxes with floating chamber	–	–	4 (2004, summer)
Concentrations of gases	21 (2003–2004)[b]	12 (2003–2004)[c]	4 (2004, summer)
Water quality[a]	21 (2003–2004)[b]	12 (2003–2004)[c]	4 (2004, summer)
Water current and depth	–	5 (2004, summer)	–

Number of measurements and time of measurements in parenthesis

[a] Water quality: NH_4–N, NO_2–N, tot-N, TOC, TIC, tot-P, SO_4^{2-}, O_2 conc., pH, temperature

[b] All seasons: once or twice a month from May 2003 to November 2004

[c] In May, July, August and October of 2003, eight times in 2004 between April and August

between May 2003 and November 2004) (Table 2). Water currents and depths were not determined at site A.

Measurements

The concentrations of CH_4 and N_2O, and dissolved inorganic carbon (DIC, the sum of CO_2, HCO_3^-, and CO_3^-), in water were determined by the headspace equilibration technique (McAuliffe 1971). In 2003, water samples from sites 1–5 were taken and stored in glass bottles (V, 120 ml), which were closed with rubber septa without headspace. Samples were preserved with sulfuric acid (2 ml 20% H_2SO_4). Acid was injected into the bottles through the septa using two needles. The first needle was used to inject acid and the second one allowed outflow of excess fluid. Within 4 days, water samples of 30 ml were drawn into polypropylene syringes (Terumo Europe, Leuven, Belgium) from the storage bottles for gas analysis. In 2004, water samples (30 ml) from sites 1–8 were drawn directly into the syringes with the help of a 10–15 cm long piece of PVC-tube in water. From study site A, water samples were drawn into syringes from a Limnos water sampler (Limnos Oy, Turku, Finland). The 30 ml water samples in the syringes were preserved with sulfuric acid (1 ml 20% H_2SO_4) in the field and were analyzed within 2 days. In the laboratory, the acidified water in the syringe was equilibrated with added N_2 gas (30 ml) and the headspace gas concentrations were analyzed with GC.

The concentrations of dissolved CH_4, DIC, and N_2O in the water samples were calculated from the headspace gas concentrations according to Henry's law using the values from Lide and Fredrikse (1995). Preservation of the water samples with sulfuric acid dropped the water pH to <4. At a pH below four, all inorganic carbon in water (CO_3^{2-}, HCO_3^- and CO_2) is present as CO_2, thus the measured CO_2 concentration from an acidified sample represents the DIC of the water. The proportion of CO_2 in the water samples was calculated from the DIC using the pH and temperature of the water determined in the field and the equations and constants published by Buttler (1982).

At the bay sites (sites 6–8), the fluxes of gases (CH_4, CO_2 and N_2O) across the water–air interface were measured with floating static chambers (Huttunen et al. 2002). Dark chambers (area 60 × 60 cm^2, height 25 cm) made from aluminum were equipped with pontoons. The floating depth of the chambers was adjusted to 3–4 cm by placing weights as necessary on the tops of the chambers. Measurements were done from the boat and the chambers were allowed to float freely. Pressures in the chambers were allowed to equilibrate with atmospheric pressure by means of a 2 m long PVC-tube with an inner diameter of 2 mm. Headspace gas samples of 50 ml were taken into polypropylene syringes (Terumo Europe, Leuven, Belgium) equipped with three way stopcocks (Codan Steritex, Hoejvangen, Denmark) five times during the 30 min incubation period. Gas samples were analyzed with gas chromatography (GC). The samples were analyzed either directly from the syringes within 24 h or were injected into evacuated vials (12 ml, Labco Exetainer®, Labco Co., UK) to be analyzed within 2 weeks. The gas fluxes (mg CO_2–C m^{-2} d^{-1}, mg CH_4–C m^{-2} d^{-1}, and μg N_2O–N m^{-2} d^{-1}) were calculated by taking into account the change in gas concentration with time in the chamber and the surface area of the chamber. Fluxes were accepted when the coefficient of determination of the linear regression of gas partial pressure versus time was higher than 0.9. However, if gas fluxes were negligible, non-linear ($r^2 < 0.9$) measurements were also taken into account and the criteria that coefficients of determination have to be >0.9 was not used. In these cases, the fluxes were obtained from the slope of the linear regression plotting gas concentration against time, regardless of the value of the coefficient of determination.

Gas analyses were performed with two different gas chromatograph systems. The air samples and the water headspace samples stored in syringes were analyzed with GC (Hewlett Packard 5890 Series II, Hewlett Packard, Palo Alto, California) equipped with a flame ionization detector (FID) for CH_4, a thermal conductivity detector for CO_2 and CH_4 (>1,000 ppm CH_4), and an electron capture detector (ECD) for N_2O (see Nykänen et al. 1995 for details). The gas samples stored in glass vials were analyzed with a GC (Agilent 6890N, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) equipped with a peristaltic pump (Minipuls 3, Gilson Inc., Middleton, USA) and an autosampler (Gilson autosampler 222XL, Gilson Inc., Middleton, USA). The Agilent GC had two columns (HaysepQ 80/100″ × 0.9 m and HaysepQ 80/100″ × 2.7 m), FID and ECD. The temperature of the oven was 60°C and the carrier gas was N_2 (flow = 35 ml min^{-1}). The sensitivity of the ECD was improved by flushing it with a 5% CH_4/95% Ar gas mixture (flow = 0.4 ml min^{-1}). The calibration of the GCs was done with the following standards: 1.98 ppm CH_4, 396 ppm CO_2, and 389 ppb N_2O (Linde Gas, Germany).

At sites 1–8, water samples for nutrient analysis were taken directly into plastic bottles, whereas from site A water samples were taken into bottles with the Limnos water sampler (Limnos Oy, Turku, Finland). Water was allowed to overflow from the bottles prior to sample collection. At sites 1–8, pH and O_2 concentrations were measured with electrodes (Microprocessor pH meter pH 320, WTW, Germany with Hamilton pH electrode, Switzerland; Dissolved Oxygen Meter Oxi 330 with Dissolved Oxygen Probe CellOx 325, WTW, Germany) and temperature was measured with the thermometer built into the O_2 meter or with a separate thermometer (Fluke 51 K/J Thermometer, Fluke Corporation, WA, USA). The pH electrode was calibrated with pH 7.00 (model STP 7) and pH 4.01 (model STP 4) buffers (WTW, Weilheim, Germany). At site A, water temperature was measured from a mercury thermometer installed in the water sampler, and water O_2 concentrations and pH were determined for the water samples in the laboratory (within a few hours of sampling) according to SFS-EN 25813 (SFS Standardization 1993) and SFS-3021 (SFS Standardization 1979) standards, respectively.

Water samples for nutrient analysis were frozen within 24 h of sampling and stored at −20°C prior to analysis. Ammonium was analyzed photometrically according to Fawcett and Scott (1960) (sites 1–8) or following the SFS 3032 standard (SFS Standardization 1976; site A). Combined NO_2^- + NO_3^- was analyzed with an ion chromatograph (DIONEX 2010i equipped with DIONEX Ion Pac® AS4A-SC 4 mm column, Dionex Corp., USA; sites 1–8) or according to the SFS-EN ISO 13395 standard (SFS Standardization 1997b; site A). The concentration of SO_4^{2-} was analyzed with an ion chromatograph (see above; sites 1–8) or according to the SFS-EN ISO 10304 standard (SFS Standardization 1995; site A). Total organic and inorganic C (TOC and TIC) were analyzed according to the SFS-EN 1484 standard (SFS Standardization 1997a), total P was analyzed according to the SFS 3026 standard (SFS Standardization 1986) and total N according to the SFS-EN ISO 11905-1 standard (SFS Standardization 1998).

Data processing

The degree of saturation of gases in water was calculated by comparing the measured gas concentrations in the water samples to that in equilibrium with the atmosphere. For atmospheric gas concentrations we used an annual average of measured ambient air gas concentrations for the sites. Ambient air concentrations were measured at the intensive sites (1–5) during sampling. For the bay sites and site A annual averages from site 5 were used.

Gas transfer velocities (k_{600} cm h^{-1}) for the river sites were calculated according to Borges et al. (2004), assuming that the effects of water current velocity and wind speed are additive.

$$k_{600} = k_{600current} + k_{600wind} \tag{1}$$

The effect of water current velocity was calculated with the following parameterization, originally presented by O'Connor and Dobbins (1958), and normalized to a Schmidt number of 600 by Borges et al. (2004).

$$k_{600current} = 1.719w \times 0.5h - 0.5 \tag{2}$$

where w is the water flow (cm s^{-1}) and h is the water depth (m). For the dates when the flow and depth were not measured, they were approximated from the closest measurements. The effect of wind speed was calculated according to Borges et al. (2004) (Eq. 3) and according to Kremer et al. (2003a, b) (Eq. 4)

$$k_{600wind} = 1.98 + 0.18 \times u_{10} \tag{3}$$

$$k_{600wind} = 1 + 2.58 \times u_{10} \tag{4}$$

where u_{10} is the wind speed (m s^{-1}) at 10 m height. The average of the two parameterizations was used for $k_{600\mathrm{wind}}$ in Eq. 1. Gas transfer velocities for different gases (k_{gas}, cm h^{-1}) at each site were calculated with the following equation:

$$k_{gas} = k_{600}\left(\frac{Sc_{gas}}{600}\right)^{x} \tag{5}$$

where $\mathrm{Sc_{gas}}$ is the ratio of the kinematic viscosity of water and the diffusion coefficient. Schmidt numbers for the gases were calculated according to the values published by Wanninkhof (1992). The value used for exponent x was chosen to be −0.5, which has been used for rivers and estuaries having turbulent conditions but moderate wind speeds (Jähne et al. 1987). Fluxes were calculated using the calculated k_{gas} values and excess gas concentrations (calculated from measured concentrations) with the equation:

$$F = k_{gas} \times (C_w - C_a) \tag{6}$$

Wind speeds and directions were provided by the Finnish Meteorological Institute measured from the Oulu Airport (every 10 min), which is located next to the Liminganlahti Bay, 8 km north of the Temmesjoki Estuary.

For the bay sites, gas k_{600} values were also calculated from the gas concentrations measured in the water and the fluxes measured in the floating chambers. When the fluxes and concentrations of gases and Schmidt numbers are known, k_{gas} can be determined according to Eq. 6, and k_{600} according to Eq. 5.

Statistical analyses were made using the SPSS statistical package (SPSS Inc., USA). The significance of the linear correlations was analyzed by two-tailed Pearson tests. Correlation analysis was made (Pearson correlation coefficients, two-tailed significances) for gas fluxes, saturations and water quality parameters. Variation in gas fluxes and saturations between the sites was tested with One-Way ANOVA using Tukey's-b as a post hoc test.

Results and discussion

Gas transfer velocities at the Temmesjoki river and Liminganlahti bay

Gas transfer velocities (k_{600} cm h^{-1}) calculated for the three gases at various sites are presented in Table 3. At the river sites the gas transfer velocities varied from 12.5 to 15.8 cm h^{-1}. At the narrow and shallow upstream sites (1–2) the water current velocity had a greater impact on k_{600} than the wind

Table 3 K_{600} values calculated with wind speed (sites 1–8) and water current (sites 1–4), averages and standard errors of mean presented in parenthesis, and K_{600} values calculated from fluxes measured with floating chambers for CO_2, CH_4 and N_2O and the average K_{600} of the three gases (sites 6–8)

Site	K_{600} (cm h^{-1})		
	$K_{600\text{current}}$	$K_{600\text{wind}}$	$K_{600\text{current + wind}}$
Calculated with water current and wind speed			
River			
1	10.0 (0.2)	5.8 (0.6)	15.8 (0.7)
2	11.5 (1.4)	4.0 (1.0)	15.5 (1.2)
3	7.1 (0.4)	7.5 (0.3)	14.6 (0.5)
4	5.6 (0.3)	7.7 (0.6)	12.5 (1.4)
5	ND	ND	ND
Bay			
6	–	7.7 (0.9)	7.7 (0.9)
7	–	7.6 (0.9)	7.6 (0.9)
8	–	7.2 (0.3)	7.2 (0.3)

	CO_2	CH_4	N_2O	Average
Calculated from the fluxes measured with floating chambers				
6	3.8	15.1 (2.9)	3.3 (2.0)	8.6 (2.5)
7	20.4 (5.1)	50.6 (28.6)	11.1 (5.1)	30.7 (13.4)
8	–	27.1 (9.3)	4.6	22.6 (8.5)

speed. The effect of wind speed increased at the downstream sites (3–4). This result is in agreement with prior studies that showed higher k_{600} values in shallow, fast running, turbulent streams than in slowly flowing streams and estuaries (O'Connor and Dobbins 1958; Raymond and Cole 2001).

At the bay, k_{600} values were also determined from the measured gas concentrations and fluxes measured with floating chambers. k_{600} values determined from chamber measurements were significantly higher than those calculated according to wind speeds and gas concentrations (Table 3). They were also higher than the values measured from similar ecosystems with low or moderate wind speeds (Raymond and Cole 2001; Borges et al. 2004; Abril and Borges 2005). It is well known from recent literature that chamber measurements could lead to artificially high k-values and thereby to overestimation of fluxes (Borges et al. 2004; Matthews et al. (2003). The wind speed was always below 6 m s^{-1} during measurements. At such low wind speeds it is likely that the currents and bottom stress of the shallow bay have an effect on gas transfer velocity. Turbulence and water flow in the Liminganlahti Bay can be heterogeneous and may vary from day to day. The bay is shallow and, depending on the direction of the wind, water flows from the bay towards the river or vice versa. Therefore, the gas transfer velocities calculated using wind speeds might be underestimations, especially as they fall into the lower end of values measured from other estuaries (Raymond and Cole 2001; Borges et al. 2004; Abril and Borges 2005).

Temporal variation in gas dynamics in Temmesjoki River—the importance of winter

The results from site A showed that the supersaturations of CO_2 and CH_4 in river water were highest in winter (Fig. 2; Table 4). There are three possible explanations for high carbon gas supersaturations during winter. (i) There is no surface runoff in wintertime in boreal regions since the soil is frozen. Thus, the water entering the Temmesjoki River in winter is mainly groundwater filtrated through anoxic soil layers, causing river water to be rich in carbon gases produced in terrestrial ecosystems. (ii) Parts of the rivers are frozen during winter. Prior studies from lake ecosystems have shown that as ice forms, a physical barrier to gas transfer between the water and atmosphere forms, and then high concentrations of CO_2 and CH_4 can accumulate in the water beneath the ice (Striegel and Michmerhuizen 1998; Semiletov 1999; Kortelainen et al. 2000). (iii) In winter, photosynthesis is suppressed in the cold and dark water, and therefore CO_2 is not being consumed (Semiletov 1999; Dawson et al. 2001). Snow cover above the ice also reduces penetration of light, further diminishing photosynthesis. Those parts of rivers remaining unfrozen in winter due to high turbulence can easily exhaust dissolved gases accumulated in the river water in ice-covered areas. Previous studies have shown various seasonal patterns in river water CO_2 and CH_4 concentrations. The Hudson River, which has only a short ice-cover period, shows higher CO_2 concentrations in summer (Raymond et al. 1997). Results from rivers without any ice-cover periods are heterogeneous; some rivers exhibit the highest concentrations of CO_2 in autumn and winter when photosynthesis is reduced (Dawson et al. 2001), some show the highest CO_2 concentrations in summer when discharge is low and DOC concentrations are high (Raymond et al. 2000) and some lack a clear

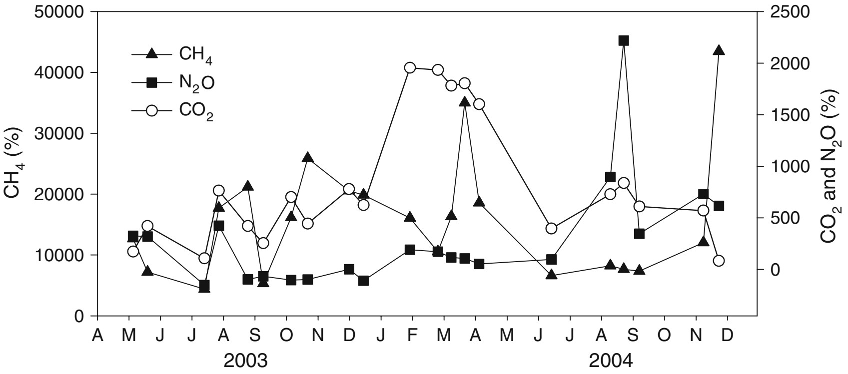

Fig. 2 Saturations of CO_2, CH_4, and N_2O in the river water at the site A in 2003 and 2004

seasonal pattern for CO_2 or CH_4 concentrations (Dawson et al. 2004). CH_4 concentrations in various Pacific Northwest rivers did not show any consistent seasonal trends (Lilley et al. 1996). The accumulation of CO_2 and CH_4 has been well documented in ice-covered lakes (Striegel and Michmerhuizen 1998; Semiletov 1999; Kortelainen et al. 2000), but to the best of our knowledge, this is the first time this phenomenon has been described for rivers.

In contrast to CH_4 and CO_2, the supersaturations of N_2O did not peak in the winter but were highest in spring and late summer (Table 4). N_2O can accumulate in waters beneath ice-cover, but not of such quantities as CH_4 and CO_2 (Kortelainen et al. 2000). The N_2O concentration seems to be more related to the amount of flooding and leaching of N and N_2O from the catchments. N_2O supersaturations measured from site A correlated positively with $NO_2^- + NO_3^- - N$ ($r = 0.845$, $P < 0.001$), tot-N ($r = 0.784$, $P < 0.001$), and TOC ($r = 0.708$, $P < 0.001$). Rivers in boreal zones receive high amounts of water and nutrients as snow in their catchments melts. Another peak in runoff occurs in late summer and autumn after rainstorms (Hyvärinen 1994; Kortelainen et al. 1997). Especially if catchments contain agricultural areas, runoff water in spring can contain high concentrations of nitrogen, phosphorus and organic matter as floodwater flushes bare agricultural soils. In addition to nutrients, a river may receive some N_2O dissolved in runoff waters from agricultural soils. Agricultural soils are known to have high concentrations of N_2O in spring during thawing periods (Syväsalo et al. 2004). Other studies have also shown the highest N_2O concentrations in river water in spring and late summer/autumn (Robinson et al. 1998; Cole and Caraco 2001b).

We evaluated gas fluxes from site A, according to the gas transfer velocities (*k*-values) determined for site 4. The calculated average annual gas fluxes from site A were 22,800 mg CO_2–C m^{-2} d^{-1}, 19 mg CH_4–C m^{-2} d^{-1}, and 2 mg N_2O–N m^{-2} d^{-1} (Table 4). Annual emissions from the site would then be 8,300 g CO_2–C m^{-2}, 7.0 g CH_4–C m^{-2}, and 0.8 g N_2O–N m^{-2}. We also calculated the average fluxes for different seasons (Table 4). If winter is assumed to last from the beginning of November to the end of March, winter emissions from the site would be 4,800 g CO_2–C m^{-2}, 3.8 g CH_4–C m^{-2}, and 0.3 g N_2O–N m^{-2}, representing 58, 55 and 36% of the annual emissions, respectively.

Due to high wintertime supersaturations, average supersaturations and evaluated fluxes for the river sites 1–5 do not represent yearly averages (see next chapter). Those sites were only measured during a period from early spring to autumn. The results from site A showed that the average gas concentrations measured year round (including winter) were 30% higher for CO_2, 59% higher for CH_4 and 11% higher for N_2O when compared to the averages calculated for the measurement period of sites 1–5 (Table 6).

The Temmesjoki River—land use in the catchment determines river water quality and gas dynamics

All the river sites were sources of CO_2 (70–14,300 mg CO_2–C m^{-2} d^{-1}) (Figs. 3a, 4a) and CH_4

Table 4 Gas saturations in surface water and calculated average gas fluxes at the site A in winter ($n = 22$), spring ($N = 6$), summer ($N = 15$) and autumn ($N = 8$), averages and standard errors of mean in parenthesis

	Average saturation (%)					Calculated average flux[a]				
	All seasons	Winter[b]	Spring[b]	Summer[b]	Autumn[b]	All seasons	Winter[b]	Spring[b]	Summer[b]	Autumn[b]
CO_2	890 (89)	1,220 (160)	730 (310)	560 (69)	520 (70)	22,800 (2,200)	31,900 (3,580)	21,900 (7,340)	15,600 (2,540)	11,900 (2,450)
CH_4	15,440 (1,420)	21,160 (2,450)	12,810 (2,460)	10,270 (1,620)	11,360 (3,040)	19 (1.7)	25 (0.3)	15 (0.3)	14 (2.0)	14 (3.4)
N_2O	264 (27)	234 (21)	231 (24)	366 (84)	178 (29)	2.2 (0.4)	1.8 (0.3)	1.7 (0.3)	3.5 (1.2)	1.0 (0.4)

[a] mg C m^{-2} d^{-1} for CO_2 and CH_4, mg N m^{-2} d^{-1} for N_2O

[b] Winter is from November to March, spring is from April to May, summer is from June to August and autumn is from September to October

(3.2–120 mg CH_4–C m^{-2} d^{-1}) (Figs. 3b, 4b) to the atmosphere throughout the measurement period. At sites 1–4, water was generally supersaturated with N_2O, whereas the estuary site (5) showed both under and supersaturations (Fig. 3c; Table 6). N_2O fluxes varied from −460 to 2,900 µg N_2O m^{-2} d^{-1} (Fig. 3c). CH_4 and CO_2 fluxes were similar to those found earlier for temperate and boreal rivers, 260–24,900 mg CO_2–C m^{-2} d^{-1} and 0.3–340 mg CH_4 m^{-2} d^{-1} (de Angelis and Lilley 1987; Lilley et al. 1996; Jones and Mulholland 1998a, b; Neal et al. 1998; Hope et al. 2001). Rivers, even in a pristine state, are generally known to be sources of CO_2 and CH_4 to the atmosphere (de Angelis and Lilley 1987). However, draining of peatlands, as in the catchment around site 1, likely elevates the concentrations of CO_2 and CH_4 in river waters. Supersaturations of N_2O in the Temmesjoki River were generally within the range of previously published values for rivers (McMahon and Dennehy 1999; Reay et al. 2003; Cole and Caraco 2001b), but lower than those measured from highly N loaded rivers (McMahon and Dennehy 1999; Reay et al. 2003). Low N_2O concentrations have been measured from rivers in pristine regions (Reay et al. 2003).

The river water gas concentrations strongly reflected the land use in the catchments. The carbon gas fluxes and supersaturations were highest from the sites surrounded by drained peatlands and forests, whereas the N_2O fluxes were related to the presence of agriculture in the catchment. The highest fluxes and supersaturations of both CO_2 (average of 5,400 mg CO_2–C m^{-2} d^{-1}, 1,036%; $P < 0.001$) (Fig. 4a; Table 6) and CH_4 (65 mg CH_4–C m^{-2} d^{-1}, 94,980%; $P \leq 0.001$) (Fig. 4b; Table 6) occurred at the most upstream site (site 1). There the Temmesjoki River received most of the water from the drained peatlands, resulting in low pH, low concentrations of O_2 and $NO_2^- + NO_3^- - N$ and a high concentration of inorganic carbon (TIC) (Table 5), and the river water probably received a lot of dissolved CO_2 and CH_4 from peatland-derived ground waters. The content of organic C, which remained at the same level at various river sites, was not the determining factor for C gas emissions. Other studies have stated that in small streams and rivers, most of the CO_2 and CH_4 emitted originate from terrestrial ecosystems (de Angelis and Lilley 1987; Jones and Mulholland 1998a; Mayorga et al. 2005).

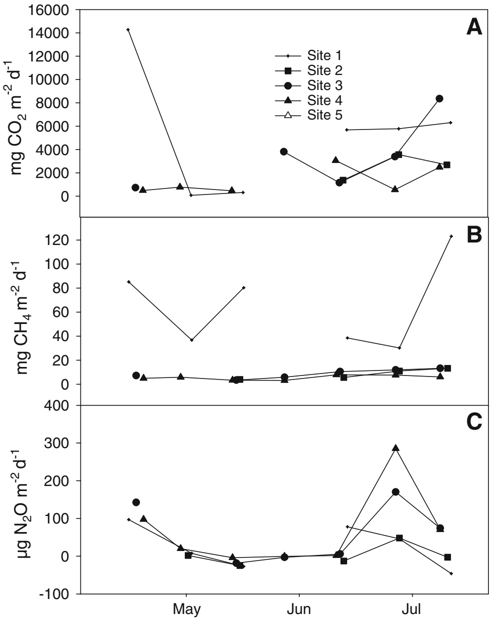

Fig. 3 (**a**) CO_2, (**b**) CH_4, and (**c**) N_2O fluxes calculated from measured gas concentration data and from gas transfer velocities in the Temmesjoki River (sites 1–5) in 2004, daily averages and standard errors of the means

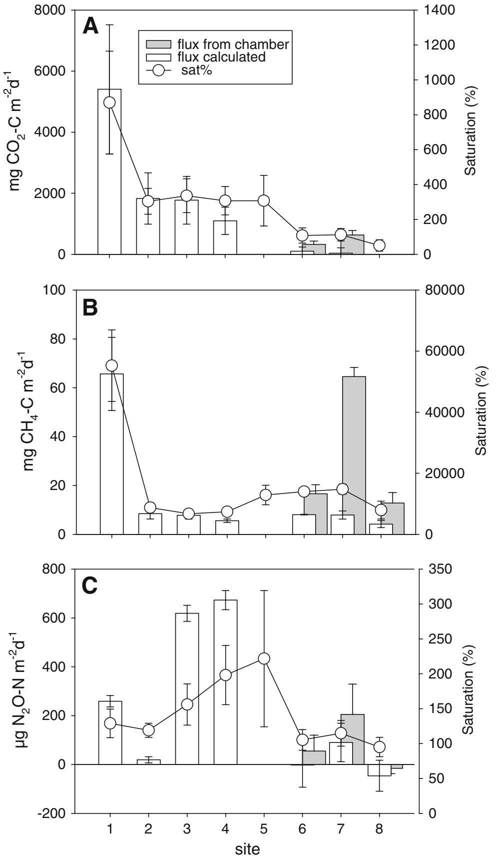

Fig. 4 (**a**) CO_2, (**b**) CH_4, and (**c**) N_2O saturations and fluxes at the river sites (1–5) and the bay sites (6–8). White bars represent the calculated fluxes (in the river and the bay) and gray bars represent the fluxes measured with floating chambers (in the bay). Averages and standard errors of the means are presented for the measurement periods in 2003 (saturations at the river sites) and 2004 (all the parameters)

Our results are consistent with the results of Hope et al. (2004), who found significant CO_2 and CH_4 fluxes from the upstream site of an upland Britain catchment containing drained peatlands. However, the highest concentrations of CO_2 and CH_4 at site 1 (up to 530 µM CO_2 and 15,360 nM CH_4) (Table 6) exceed maximum concentrations found previously (300 µM CO_2 and 2,000 nM CH_4) for temperate and boreal rivers (de Angelis and Lilley 1987; Lilley et al. 1996; Jones and Mulholland 1998a, b; Sansone et al. 1998, 1999; Upstill-Goddard et al. 2000; Hope et al. 2001; Dawson et al. 2002). In contrast to the carbon gases, the saturations and fluxes of N_2O were low at the upstream sites, and started to increase from site 3 (Fig. 4c), where agricultural soils appeared in the catchments and $NO_2^- + NO_3^- - N$ concentrations were orders of magnitude higher than at sites 1–2 (Table 5). In the downstream river sites, the average supersaturation (sites 3–5) was 175% and flux (sites 3–4) was 64 µg N_2O–N m^{-2} d^{-1} (Fig. 4c). The N_2O supersaturations had some positive correlations with the concentrations of $NO_3^- + NO_2^- -$ N ($r = 0.413$, $P = 0.004$) and total N ($r = 0.384$, $P = 0.014$) in river water. Correlations between the N species can be expected because they mostly originate from agricultural soils. Other studies have also found positive, but weak, correlations between N_2O and N species in river water (McMahon and Dennehy 1999; Harrison and Matson 2003; Reay et al. 2003). Reay et al. (2003) reported that N_2O is quickly exhausted from river water, whereas the levels of NO_3^- in river water remained high long after the discharge of agricultural drainage waters.

Table 5 Characteristics of water quality in 2003–2004, averages and standard error of the mean in parentheses

Sites	Carbon (mg C l^{-1})			Nitrogen (µg N l^{-1})			Other variables				
	N	TIC[a]	TOC[b]	NH_4^+	$NO_2^- + NO_3^-$	N-tot	P-tot (µg P l^{-1})	SO_4^{2-} (mg SO_4^{2-} l^{-1})	O_2 (mg O_2 l^{-1})	pH	Water *T* (°C)
1–8	53	2.6 (0.3)	19 (1.2)	61 (5.1)	310 (100)	810 (47)	49 (4)	29 (4.0)	9.7 (0.3)	6.8 (0.1)	13 (0.7)
River	42	2.7 (0.3)	20 (1.5)	58 (5.9)	370 (130)	790 (54)	49 (5.5)	20 (3.2)	9.5 (0.3)	6.5 (0.1)	12 (0.7
1	9	4.1 (1.0)	20 (4.6)	34 (11)	18 (12)	600 (110)	18 (3.0)	10 (9.1)	7.8 (0.7)	6.0 (0.1)	12 (1.4)
2	9	1.6 (0.3)	21 (3.8)	22 (6.6)	67 (30)	610 (100)	29 (4.0)	8 (4.0)	10.6 (0.7)	6.5 (0.2)	11 (1.6)
3	9	2.2 (0.5)	22 (2.7)	52 (6.5)	300 (100)	830 (85)	64 (13)	12 (1.5)	9.5 (0.5)	6.7 (0.2)	12 (1.7)
4	8	2.1 (0.6)	22 (2.5)	71 (7.9)	400 (140)	940 (140)	72 (16)	18 (1.4)	9.4 (0.7)	6.6 (0.1)	11(1.9)
5	7	4.2 (1.2)	16 (1.4)	122 (11)	1,180 (670)	1,050 (110)	69 (7.8)	56 (6.5)	10.3 (0.7)	7.1 (0.3)	14 (2.0)
Bay	12	2.0 (0.1)	15 (1.2)	72 (8.7)	73 (27)	866	51 (5.7)	68 (11)	10.7 (0.6)	7.7 (0.2)	17 (0.8)
6	4	2.0 (0.4)	17 (1.5)	69 (12)	44 (19)	824	46 (4.9)	54 (5.3)	9.6 (1.3)	7.4 (0.2)	17 (1.0)
7	4	2.0 (0.1)	18 (2.0)	94 (19)	170 (49)	1,014	70 (7.9)	52 (9.1)	12.2 (0.5)	7.4 (0.3)	16 (1.2)
8	4	2.0 (0.2)	12 (1.8)	55 (8.6)	1 (1)	761	36 (8.8)	100 (28)	10.1 (0.6)	8.1 (0.3)	17 (1.0)
A	21	8.9 (1.5)	14 (1.5)	280 (39)	300 (40)	1,130 (80)	120 (11)	37 (5.8)	9.0 (0.5)	6.8 (0.1)	11 (1.2)

[a] Total inorganic carbon

[b] Total organic carbon

Thus rapid degassing of the N_2O to the atmosphere is probably the reason for a poor correlation between the dissolved N species and the fluxes or supersaturations of N_2O. It has to be noted that we cannot evaluate here whether the N_2O emitted from river water was leached from catchments or was produced by microbial processes in the river channel itself. Groundwaters and drainage waters from agricultural areas have been reported to contain high amounts of N_2O (Hasegawa et al. 2000, Hiscock et al. 2003). The levels of supersaturation and fluxes of N_2O increased with the presence of agricultural soils and N loads in the catchments, supporting results from earlier studies (McMahon and Dennehy 1999; Reay et al. 2003). However, Cole and Caraco (2001a, b) determined that the Hudson River is not an important source of N_2O at the landscape level. Thus, there are conflicting results regarding N_2O emissions from various rivers.

The gases produced in the catchments were quickly degassed from the river water. For example, the differences in the CO_2 and CH_4 concentrations between sites 1 and 2 were large. Dawson et al. (2004) also reported a sharp decrease in CO_2 and CH_4 concentrations from upstream to downstream in peatland streams. Reay et al. (2003), in turn, reported that most of the dissolved N_2O the river received was degassed from the water within 100 m. For N_2O, we did not find any similar effects, as agricultural soils and thus sources of N_2O were present all along the river course from site 3 to the bay.

The Liminganlahti Bay—a minor source of gases to the atmosphere

Unlike the Temmesjoki River, the open water area of the Liminganlahti Bay was not a significant source of greenhouse gases. The water in the bay exhibits higher pH values and SO_4^{2-} concentrations and lower TOC and nutrient concentrations than the river water (Table 5). The bay mainly consists of river water, already degassed in the river and the estuary, and is therefore CH_4, CO_2 and N_2O depleted (Fig. 5; Table 6) In the bay, the river water is further diluted by water from the Bothnian Bay and therefore, concentrations and fluxes of gases decrease further. Similar results have been found for other river–estuary systems, where gas supersaturations and fluxes from the water to the atmosphere are lower

Table 6 Concentrations of gases and dissolved inorganic carbon (DIC) and partial pressure of CO_2 (pCO_2) in surface water at the study sites

Sites		*N*	DIC (µM)	CO_2 (µM)	pCO2 (µatm)	CH_4 (nM)	N_2O (nM)
A	Mean (SE)	21	737 (122)	215 (44)	3,520 (370)	632 (98)	36 (5)
	Min–max		52–1,610	16–595	340–8,380	122–1,920	10–95
1–8	Mean (SE)	65	217 (24)	74 (12)	1,400 (164)	870 (265)	18 (1.3)
	Min–max		40–850	0.5–530	10–16,080	62–15,360	7–53
River	Mean (SE)	53	228 (53)	87 (14)	1,710 (220)	984 (322)	19 (1.5)
	Min–max		40–820	4.7–531	150–16,080	87–15,360	6.7–53
1	Mean (SE)	11	342 (87)	210 (51)	4,100 (820)	3,450 (1,350)	17 (3)
	Min–max		57–850	24–531	450–16,080	718–15,360	6.7–39
2	Mean (SE)	12	130 (25)	51 (7)	980 (93)	367 (62)	15 (2)
	Min–max		40–338	10–97	180–2,150	139–869	9.6–29
3	Mean (SE)	11	185 (38)	57 (11)	1,070 (140)	210 (22)	18 (2)
	Min–max		42–451	12–125	240–2,940	87–313	9.8–34
4	Mean (SE)	11	172 (48)	60 (11)	1,100 (140)	308 (63)	23 (4)
	Min–max		49–572	7.3–117	150–2,700	106–825	10–47
5	Mean (SE)	8	354 (100)	50 (13)	1,100 (170)	517 (177)	26 (6)
	Min–max		106–763	4.7–114	110–2,700	239–894	11–53
Bay	Mean (SE)	12	166 (11)	15 (34)	660 (93)	353 (41)	11 (0.7)
	Min–max		91–227	0.45–34	10–2,690	62–588	7.3–15
6	Mean (SE)	4	166 (32)	17 (6)	400 (110)	400 (19)	10 (1)
	Min–max		91–227	4.5–34	100–890	358–452	9.2–13
7	Mean (SE)	4	165 (4)	19 (6)	420 (87)	437 (57)	12 (2)
	Min–max		154–173	2.8–28	64–650	335–588	8.5–15
8	Mean (SE)	4	169 (14)	9 (6)	190 (81)	223 (78)	9.6 (1)
	Min–max		133–193	0.45–27.8	10–620	62–406	7.3–13

in estuaries than in rivers (de Angelis and Lilley 1987; Sansone et al. 1998). However, when comparing gas emissions in estuaries and open seas, estuaries are important marine sources of CH_4 (Bange et al. 1994; Upstill-Goddard et al. 2000; Bange 2006), CO_2 (Borges 2005; Borges et al. 2006) and N_2O (Bange et al. 1996; Seitzinger et al. 2000; Bange 2006).

Saturations and fluxes of CO_2 were smaller at the bay sites than at the river sites ($P < 0.01$) (Fig. 4a; Table 6). The calculated fluxes differ substantially from the fluxes measured with the dark floating chambers. The dark chamber may disturb photosynthesis and uptake of CO_2 from the atmosphere, resulting in higher CO_2 fluxes. CO_2 concentrations in surface water were close to atmospheric equilibrium (Fig. 5a; Table 6). According to the calculated flux (Fig. 5d), the bay sites were either small sinks or sources of CO_2. The fluxes measured with the chambers show that the sites are only minor sources of CO_2 (Fig. 4a). CO_2 saturations correlated negatively with pH ($r = -0.811$, $P = 0.001$) (Table 5). At low pH values, below 6.35, most of the inorganic carbon is present as CO_2; at higher pH values, bicarbonates (HCO_3^{-}) and carbonates (CO_3^{2-}) dominate (Buttler 1982). In the bay, the mixing of bay and river water results in higher pH and dissociation of CO_2 to bicarbonates (pH of 7.7 in the bay and 6.5 at the river sites), which could also partly explain smaller CO_2 fluxes in the bay than in the river. The CO_2 fluxes in the Liminganlahti Bay are at the lower end of the range of values found previously (from -16 to 9,100 mg CO_2–C m^{-2} d^{-1}) in inner estuaries around the world (Borges 2005; Abril and Borges 2005; Borges et al. 2006). The organic carbon load to Liminganlahti Bay is probably not as high as in other European estuaries, where high levels of anthropogenic labile carbon have been measured (Abril and Iversen 2002). The average CO_2 fluxes measured

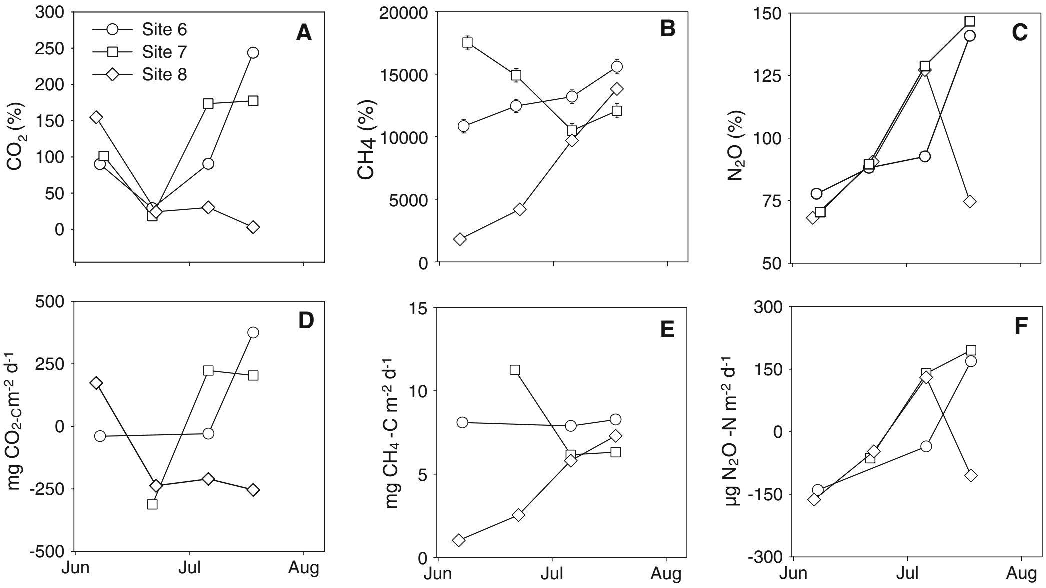

Fig. 5 Saturations of (**a**) CO_2, (**b**) CH_4, and (**c**) N_2O and calculated fluxes (**d**) CO_2, (**e**) CH_4, and (**f**) N_2O at the bay sites (sites 6–8)

from the Liminganlahti, i.e. from the inner estuary, are similar to the average emissions measured from the adjacent northern Bothnian Bay (Algesten et al. 2004). Generally, inner estuaries have shown higher CO_2 emissions than coastal oceans (Borges 2005). The whole northern Bothnian Bay is actually oligohaline rather than brackish water, salinity being <2‰ (Kronholm et al. 2005); thus, most of its water is of riverine origin.

CH_4 fluxes were generally below 40 mg CH_4–C m^{-2} d^{-1} in the bay, but high CH_4 emissions, up to 300 mg CH_4–C m^{-2} d^{-1}, were obtained with chamber measurements at site 7 (Figs. 4b, 5b). Emissions and supersaturations of CH_4 in the Liminganlahti Bay are, on average, within the range previously found for other European estuaries (Upstill-Goddard et al. 2000; Middelburg et al. 2002; Bange 2006). However, the average CH_4 supersaturation of several European estuaries was found to be lower, 3,640% (Bange 2006), than the average CH_4 supersaturation of 11,030% (Fig. 5b) found in Liminganlahti Bay. One reason for this might be the low SO_4^{2-} concentrations in Liminganlahti Bay (Table 5). CH_4 supersaturations correlated negatively with SO_4^{2-} concentrations ($r = -0.795$, $P = 0.002$). Methanogenesis, a thermodynamically less favorable process than sulfate reduction, occurs generally only after the sulfate that is present has been reduced (Capone and Kiene 1988). Sulfate also participates in anaerobic oxidation of CH_4 and therefore reduces CH_4 fluxes (Boetius et al. 2000). Additionally, in the shallow Liminganlahti Bay, a smaller part of the CH_4 is likely to be oxidized than in areas with deeper waters (Abril and Iversen 2002). The volume of water diluting the river water rich in CH_4 is also smaller than the volume of deeper estuaries. A part of the CH_4 in surface waters of the bay may have originated from CH_4 production in anoxic sediments or bottom waters. Although the CH_4 emissions were lower from the open water of the bay than from the river sites, the bay region could be releasing significant amounts of CH_4 from the surrounding wetlands (Heyer and Berger 2000). Average CH_4 emissions from the wetlands of Liminganlahti Bay were found to be 154 mg CH_4–C m^{-2} d^{-1} (Liikanen et al. unpublished data), which is higher than the average emissions of the Temmesjoki River or the open water area of the bay.

The N_2O concentrations in the bay were close to atmospheric equilibrium values and the bay was alternately either a sink or source for N_2O (Figs. 4c, 5c, 5f). The fluxes and concentrations of N_2O were an order of magnitude lower at the bay than at the river sites (Fig. 5c; Table 6) (for concentrations $P = 0.045$). The N_2O fluxes were highest from site 7, which was located near the mouth of the Lumijoki River (Figs. 4c, 5f). At site 7, the levels of inorganic N species were highest (Table 5). The N content controlled N_2O dynamics, and the N_2O saturations correlated positively with $NO_2^- + NO_3^- - N$ ($r = 0.698$, $P = 0.012$). The N_2O concentrations and fluxes measured are within the range previously reported (Robinson et al. 1998; de Wilde and de Bie 2000; LaMontagne et al. 2003; Bange 2006). The average supersaturation of N_2O (136%) in Liminganlahti Bay is lower than the mean for other European estuaries (465%) (Bange 2006). There are some earlier observations that estuarine waters act occasionally as sinks for N_2O (Robinson et al. 1998). Benthic denitrification is a possible sink for N_2O in the bay (LaMontagne et al. 2003). N_2O emitted from the bay can be of riverine origin or produced in situ from nitrification (Barnes and Owens 1998; de Wilde and de Bie 2000) or denitrification (Robinson et al. 1998). Since the emissions of N_2O from Liminganlahti Bay were small, no significant production of N_2O is likely to be occurring in the bay. The wetlands surrounding Liminganlahti Bay are either small sinks or sources of N_2O, like the open water area of the bay (Liikanen et al. unpublished data).

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CHAPTER VI:

GENERAL DISCUSSION

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6.1 High latitude river sediments exhibit low denitrification rates

Temperate river and estuary ecosystems have been reported to exhibit high denitrification rates (Seitzinger et al. 1988, Pfenning & McMahon 1996, García-Ruiz et al. 1998b). The results in this thesis (Chapters II, III, IV) show that boreal rivers discharging to the Bothnian Bay exhibit lower denitrification rates (440-1,718 µmol N m^{-2} d^{-1}) than temperate river (up to 79,000 µmol N m^{-2} d^{-1}) lake (up to 7,500 µmol N m^{-2} d^{-1}) or estuary (up to 14,200 µmol N m^{-2} d^{-1}) sediments (Piña-Ochoa & Álvarez-Cobelas 2006). It is probably the lower nutrient input and lower temperature with substantial temporal variation in boreal rivers that explain the lower denitrification rates in comparison to the lower latitude rivers.

The denitrification rates measured from river estuaries of the northern Baltic Sea varied from 90-910 µmol N m^{-2} d^{-1} (Chapter II), which is generally higher than rates measured in the open sea area of the Bothnian Bay (120–160 µmol N m^{-2} d^{-1})(Stockenberg & Johnstone 1998) and fall into the same range as the denitrification rates measured from the open Gulf of Bothnia (0–940 µmol N m^{-2} d^{-1}) (Stockenberg & Johnstone 1998). Tuominen et al. (1998) measured denitrification rates of 150–650 µmol N m^{-2} d^{-1} from the open sea area of the Gulf of Finland. Higher denitrification rates in the rivers probably result from higher NO_3^- concentrations. In rivers, uncoupled denitrification prevails (Chapters II, III), whereas the open sea areas of the Baltic Sea exhibit lower NO_3^- concentration and denitrification in the sediments is fed by NO_3^- originating from mineralization and nitrification in the upper sediment layers (coupled nitrification) (Tuominen et al. 1998).

6.2 Regulators of denitrification and N_2O production

Nitrate as the substrate of denitrification was the most profound regulator of the denitrification rate. An increase in NO_3^- concentration increased both the denitrification rate and the amount of N_2O produced by denitrification (Chapter III). Both denitrification and N_2O production were also enhanced by lower O_2 concentrations (Chapter IV). Higher temperatures enhanced denitrification but suppressed N_2O production (Chapter IV).

6.2.1 Increases in NO_3^- concentration and temperature enhance denitrification

Denitrification exhibited a strong positive correlation with NO_3^- load (Chapter III), supporting prior results from various aquatic ecosystems. Many studies have demonstrated a positive relationship between denitrification rates and NO_3^- concentration (Anderssen 1977, Oren & Blackburn 1979, Oremland et al. 1984, Nielsen et al. 1995, Kana et al. 1998, Royer et al. 2004). In Norsminde Fjord sediment (Denmark) both total NO_3^- reduction and denitrification peaked in spring and autumn subsequently with increased NO_3^- concentrations. Only when availability of NO_3^- was high enough, denitrification was limited by temperature, O_2 and availability of energy (Jørgensen & Sørensen 1988). Law & Owens (1990) did not observe any significant correlation between water column NO_3^- concentrations and denitrification rates in their study, and argued that rather than the NO_3^- concentration *per se*, the physical transport of NO_3^- to active sites of denitrification regulates the denitrification rate.

The total denitrification in boreal river sediments exhibited an apparent half-saturation concentration (K_m - value) of 20 µM NO_3^- (Chapter III). This is lower than the K_m values measured in marine sediments (27-53 µM NO_3^-, Seitzinger 1988), and falls at the lower end of values reported for temperate rivers (13.1-90.4 µM NO_3^-, Garcia-Ruiz et al.

1998b). The result shows that the denitrifying bacteria in boreal river sediments are well adapted to living under low NO_3^- concentrations, i.e. they have a high affinity for NO_3^-.

Temperature in boreal rivers varies greatly from close to 0 °C to 25 °C in summer. The results in Chapter IV show that there is some increase in denitrification rate with increasing temperature. The positive effect of temperature on denitrification rates is well known from various aquatic ecosystems (Cavari & Phelps 1977, Sørensen et al. 1979, Seitzinger 1988, Nowicki 1994, Pattinson et al. 1998, van Luijn et al. 1996). There are only a few studies reporting decreased denitrification rates as temperature increases (Sørensen et al. 1979).

The impact of temperature on denitrification was studied under different oxygen concentrations (Chapter IV, 6.2.2). The anoxic treatment exhibited the clearest response of denitrification rates to temperature, with a Q_{10} - value of 3.1, indicating that a 10 °C rise in temperature increases the denitrification rates by a factor of three. This result is in good agreement with prior studies from aquatic ecosystems reporting Q_{10} values of between one and four (Seitzinger 1988, and references therein).

6.2.2 Decreased O_2 availability increases denitrification

To the best of my knowledge, the impact of O_2 concentration on denitrification has not been studied in boreal rivers and the data from all aquatic ecosystems are few. Oxygen availability together with NO_3^- availability was among the most important factors affecting denitrification in a cross-ecosystem analysis (Piña-Ochoa & Álvares-Cobelas 2006).

The O_2 concentrations measured from the surface waters of the Temmesjoki River vary from 3 to 14 mg O_2 l^{-1}, being highest in winter and lowest in summer. River water is turbulent and presumably well-aerated, especially in spring and autumn, when flow rates are high. The actual O_2 concentrations close to the sediments (at depth of 5-6 m in the water column) are presumably lower, especially in summer and winter when the flow rates and vertical turbidity are low.

The impact of O_2 on denitrification was measured along with temperature (Chapter IV). Lack of O_2 in the water column clearly enhances denitrification, as denitrification rates measured at the highest temperature were two- and three -fold higher in the anoxic treatment than in the treatments with O_2. However, neither a significant difference between different O_2 treatments nor a significant correlation between O_2 input and denitrification rates was found. In a cross ecosystem analysis (Piña-Ochoa & Álvares-Cobelas 2006) only dissolved oxygen together with NO_3^- concentrations significantly explained the denitrification rates. In aquatic sediments where NO_3^- supply from the overlying water is limited (such as lake and open sea sediments), anaerobic conditions do not enhance denitrification, in contrast to river sediments. In those ecosystems, nitrification, a process suppressed in anaerobic conditions, is the only source of NO_3^- - therefore denitrification is also suppressed (Liikanen et al. 2003)

6.2.3 N_2O production in high latitude river sediments as affected by NO_3^- load, temperature and O_2 concentration.

A high NO_3^- concentration increased the N_2O efflux by a factor of 15, and the N_2O/N_2 ratio increased from 1 % at low NO_3^- concentrations to 3.4 % at the higher NO_3^- concentrations (Chapter III). Several studies in soils and sediments have shown that the high NO_3^- concentrations limit the conversion of N_2O to N_2 by inhibiting the N_2O reductase (Blackmer & Bremmer 1976, Letey et al. 1981, Terry & Tate 1980) which increases the N_2O/N_2 ratio (e.g. Blackmer & Bremner 1978, Weier et al. 1993). Thus, there is a possibility for increased local N_2O emissions from denitrification in boreal eutrophic rivers and estuaries resulting from the increase in the N_2O/N_2 ratios

(Seitzinger et al. 1988, García-Ruiz et al. 1999).

The N_2O effluxes from high latitude river sediments decreased as temperature increased, thus showing a completely opposite temperature response than the overall heterotrophic activity, including denitrification (i.e. production of N_2+N_2O), in the sediment (Chapter IV). A possible explanation for the increased N_2O production is the temperature sensibility of the N_2O reductase enzyme, which catalyzes the last step of denitrification (reduction of N_2O to N_2). Inhibition of this enzyme at low temperatures has been reported for soil ecosystems (Mellin & Nõmmik 1983, Maag & Vinther 1996), but it has never been shown in sediments. García-Ruiz et al. (1998b), however, reported, that N_2O production occasionally exhibited greater variability than denitrification in a river in NE England. They concluded that environmental factors may affect the last step (N_2O reduction) and the initial steps of denitrification differently. In their data, N_2O production was detected at low temperatures (2°C), and at low NO_3^- concentrations, when denitrification activity was already low.

The N_2O effluxes were enhanced by anoxia simultaneously with overall denitrification (Chapter IV) but the response of N_2O to O_2 input was not as clear as the response of denitrification rate.

Although the N_2O/N_2 ratio was affected by the environmental variables, the contribution of N_2O in end products of denitrification did not exceed 4 %. Seitzinger et al. (1988) reported that in eutrophic water ecosystems, up to 5 % of the gases produced in denitrification are released as N_2O. Ratios as high as 80 % have been measured from very eutrophic rivers in NE-England (Garcia-Ruiz et al. 1998b). The measured benthic N_2O fluxes were lower (4-68 µmol N_2O-N m^{-2} d^{-1}) than the fluxes previously measured for temperate rivers (14-497 µmol N_2O-N m^{-2} d^{-1} Garcia-Ruiz et al. 1998b), but were similar to the effluxes for high latitude lakes (Liikanen et al. 2003). It can be concluded that denitrification in boreal river sediments is a minor source of N_2O. Changing climate and land-use patterns may, however, lead to changes in the driving factors (e.g enhanced leaching, changes in temperature) and thereby to increased N_2O production.

6.3. Nitrate removal

The overall denitrification rates (N_2 + N_2O effluxes) were used to estimate the NO_3^- removal during denitrification. In various rivers entering the Bothnian Bay, 18-22 %, of the NO_3^- load was removed in denitrification (Chapter II). In the Temmesjoki River sediments, only up to 8 % of the added NO_3^- was removed by denitrification (Chapter III). Thus, it can be concluded that denitrification in boreal river sediments has a limited capacity to reduce NO_3^- loading (Chapters II, III). At the end of the 1980's it was suggested that in estuarine areas denitrification could remove up to 40-50 % of the N inputs (Seitzinger 1988). Only a few studies have been done in the open sea or estuaries of the Baltic Sea, and in those studies the annual N removal has varied between <1 and 11% (Pind et al. 1997, Savage et al. 2004). In the more northern Simojoki river basin, annual N retention was estimated to be negligible (Lepistö et al. 2001). The N retention in a river basin has been found to be higher, if the catchment contains lakes and peatlands (Lepistö et al. 2001). In boreal lakes the N removal has been found to range from 5 to 11 % (Jonsson & Jansson 1997).

At high NO_3^- load, 96% of the NO_3^- was consumed by processes other than denitrification. The most probable mechanism for the removal was assimilation to microbial biomass, not denitrification (Chapter III). This means that NO_3^- is not permanently removed from the system. Immobilized N can be remineralized, increasing once again the content of DIN in the water.

6.4. Greenhouse gas emissions from a high latitude river-estuary-bay continuum

6.4.1 Spatial variation

The gas concentrations in rivers and gas fluxes strongly reflected the land use in the catchments (Chapter V). Carbon gas supersaturations and fluxes were higher from the upstream sites surrounded by peatlands and forests than from the downstream sites surrounded by agricultural fields. At the sites surrounded by peatlands, river water probably received considerable dissolved CO_2 and CH_4 from ground and surface waters. Organic C, which remained at a similar concentration along the river, was not the limiting factor in the C gas emissions. In small streams and rivers, most of the emitted CO_2 and CH_4 have been shown to originate from terrestrial ecosystems (de Angelis & Lilley 1987, Jones & Mulholland 1998, Mayorga et al. 2005). The results are consistent with the results of Hope et al. (2004), who found high CO_2 and CH_4 fluxes from an upstream site of upland Britain surrounded by drained peatlands.

In contrast to the carbon gases, N_2O supersaturations and fluxes were high at downstream sites, where agricultural soils were present in the catchments. This result is consistent with other studies reporting that groundwater and drainage water from agricultural areas contain extremely high amounts of N_2O (Hasegawa et al. 2000, Hiscock et al. 2003). The land use in the catchments seems to be a better predictor of gas release than any of the river water quality parameters

The open water area of the Liminganlahti Bay was not a significant source of greenhouse gases, in contrast to the Temmesjoki River. The bay mainly consists of river water, already degassed in the river and the estuary, and therefore the concentrations of CH_4, CO_2 and N_2O in the water are below the saturation levels. In the Bay, the river water and gases are further diluted by bay water. Similar results have been found for some other river-estuary systems, where gas supersaturations and emissions from water to the atmosphere were much lower in estuaries than in rivers (de Angelis & Lilley 1987, Sansone et al. 1998).

6.4.2 Seasonal variation

The concentrations of CO_2 and CH_4 in river water were highest in the winter. At least three explanations are possible for high carbon gas supersaturations during winter. I) Surface runoff is limited during wintertime in boreal regions since the soil is mostly frozen. Thus, the water entering the Temmesjoki River in winter is mainly groundwater filtrated through anoxic soil layers, resulting in river water rich in carbon gases produced in terrestrial ecosystems. II) Secondly, parts of the rivers are frozen during winter. Prior studies from lake ecosystems have shown that as ice forms a physical barrier for the gas transfer between water and the atmosphere, high concentrations of CO_2 and CH_4 can accumulate in the water beneath the ice (Striegel & Michmerhuizen 1998, Semiletov 1999, Kortelainen et al. 2000). III) In winter, photosynthesis is suppressed in cold and dark water and is therefore not consuming CO_2 (Semiletov 1999, Dawson et al. 2001). Snow cover on the ice further reduces the penetration of light to river water. Those parts of rivers remaining unfrozen in winter due to high turbulence exhaust dissolved gases accumulated in the river water in ice-covered parts. Previous studies have shown various seasonal patterns in river water CO_2 and CH_4 concentrations. Results from rivers without ice-covered periods are heterogeneous; some rivers exhibiting the highest concentrations of CO_2 in autumn and winter when photosynthesis is reduced (Dawson et al. 2001), some showing the highest CO_2 concentrations in summer when discharge is low and DOC concentrations are high (Raymond et al. 2000) and some without a clear seasonal pattern in CO_2 or CH_4 concentrations (Dawson et al. 2004). CH_4 concentrations in various Pacific Northwest rivers did not show any consistent seasonal trend (Lilley et al. 1996). The accumulation of CO_2 and CH_4 has been well documented in ice-covered lakes (Striegel & Michmerhuizen 1998, Semiletov 1999, Kortelainen et al. 2000), but this is the first time this phenomenon has been shown for boreal rivers.

N_2O can accumulate in waters beneath ice-cover, but not in such quantities as CH_4 and CO_2 (Kortelainen et al. 2000). The N_2O concentration seems to be most related to the amount of flooding and leaching of N and N_2O from the catchments. Rivers in boreal zones receive high amounts of water and nutrients as snow in their catchments melts. Another peak in runoff occurs in late summer and autumn after rainstorms (Hyvärinen 1994, Kortelainen et al. 1997). Especially if catchments contain agricultural areas, runoff water in spring can contain high concentrations of nitrogen, phosphorus and organic matter as floodwater flushes agricultural soils lacking growing crops. In addition to nutrients, rivers may receive some N_2O dissolved in runoff waters from agricultural soils. Agricultural soils are known to have high contents of N_2O in spring during the thawing period (Syväsalo et al. 2004) and in late summer/autumn (Robinson et al. 1998, Cole & Caraco 2001).

6.5 Methodological considerations

6.5.1 Laboratory experiments (Chapters II-IV)

Long-term incubation experiments, such as those documented in this thesis (Chapters II-IV), include a risk of heterotrophic processes becoming limited by the availability of organic carbon. However, it is likely that boreal river sediments contain sufficient carbon to supply the sediment metabolism with labile carbon throughout the four-week incubations used here (Chapter VI).

Determination of ^{15}N in N_2O was based on the label obtained from the N_2 measurements. During the long-term incubation experiments, the added $^{15}NO_3^-$ could have been assimilated to microbial biomass and later on mineralized to $^{15}NH_4^+$, which could be further nitrified. Therefore, N_2O from nitrification could also be enriched with ^{15}N. The $^{15}NO_3^-$ could have been reduced to $^{15}NH_4^+$, so that additional ^{15}N measurements would be needed to trace with certainty whether N_2O was produced in denitrification or in nitrification. The results of the experiment in Chapter IV, show that the greatest N_2O effluxes appeared in the treatment where the O_2 concentration was so low (<0.2 mg l^{-1}), that nitrification was already suppressed. This verifies that denitrification was the main process for N_2O production.

There is a possibility, that anammox causes inaccuracies in the measurements of denitrification rates. The measured $^{15}N_2$ pool, interpretated here as denitrification, might have received part of the label via anammox if the added $^{15}NO_3^-$ was reduced to $^{15}NH_4^+$ via the routes described above. However, anammox has not yet been detected in boreal fresh waters. Mathematical approaches have been developed for separating anammox and denitrification (Thandrup & Dalsgaard 2002, Risgaard-Petersen et al. 2004, Trimmer et al. 2006, Spott & Stange 2007), but as they require accurate time dependant determination of NO_2^-, they are not suitable for this data. The environmental impact of anammox and denitrification in removal of N from water ecosystems is, however, equal, as the end product of both of these processes is N_2.

6.5.2 Field experiments (Chapter V)

Application of floating chambers to gas emission measurements in turbulent ecosystems has been criticized recently (Matthews et al. 2003, Borges et al. 2004), as the chambers create artificial turbulence at the chamber borders, leading to an overestimation of the gas emissions. As an alternative to the chamber measurements, gas emissions from aquatic ecosystems can be calculated from the gas concentrations in the surface water by taking into account the prevailing wind conditions and water current velocities in the river (Jähne et al. 1987). At the Liminganlahti Bay, emissions were determined by applying both floating chambers and calculating the emissions based on the gas concentrations. The results indicated an overestimation of the gas emissions by the floating chamber method.

6.6 Significance of the study and future research perspectives

This thesis provides the first measured data on denitrification and regulatory factors for high latitude rivers, thus filling an existing void in our knowledge of denitrification. Clearly, these results suggest that the denitrification in river sediments is an inefficient filter for removal of nitrate. This study supports the finding of prior studies, using mass balance technique, where nitrogen retention in boreal river channels was estimated negligible (Lepistö et al 2001, Rekolainen et al 1995). Therefore, wise land use in the catchments and water protection, such as buffer zones and water protection control wetlands, should be supported in order to prevent eutrophication of the recipient waterbodies.

These results are valuable for calibrating the N models for rivers. The calibration of currently available models is based on the difference between the N discharging from catchments to the river and the amount of N at the river mouth. The parameters controlling processes in sediments are estimated according to literature values. Data are available for the processes in boreal terrestrial ecosystems for the models, but the regulation of denitrification in the river sediments is modelled according to the literature values of reaction kinetics and temperature response obtained from lower latitude rivers (e.g. Wade et al. 2002). This approach may lead to overestimation of denitrification rates and thus overestimations of N removal in denitrification in boreal rivers.

Denitrification in boreal rivers is also most probably affected by changing climate and land use patterns. Increased knowledge of the controlling parameters enhances our ability to predict impacts of these environmental changes on denitrification and related N_2O effluxes and NO_3^- removal.

There are still open questions regarding the nitrogen cycle in high latitude rivers, quantification of anammox presented above being one of them. The observed increase in assimilation of NO_3^- with increased loading is an interesting finding, which may deserve further studies. At high NO_3^- additions, not only the assimilation of N but the overall sediment metabolism was accelerated: O_2 consumption increased as did the DIC fluxes (Chapter III). Addition of *in situ* concentrations of labile, short chained organic acids (acetate, formate, lactate and oxalate) did not enhance denitrification or respiration in comparison with control cores (no acid treatment) in a four-week experiment (Silvennoinen et al. unpublished data). These results suggest that N rather than C limits the overall microbial activity in boreal river sediments. According to the results of the laboratory study in this thesis, if high latitude rivers were subjected to high N loading, carbon decomposition might be enhanced in the sediments thus increasing CO_2 production and emissions to the atmosphere. This suggests that although the N_2O production in denitrification remained relatively low under high NO_3^- concentrations, increases in NO_3^- loading may also increase emissions of CO_2.

There has been a lack of data on greenhouse gas dynamics of high latitude rivers and estuaries. This study provides the first such data. The results suggest that the land use in the catchments is the main factor determining the gas fluxes from the river. In comparison to other boreal ecosystems, rivers have relatively low emissions of N_2O, but quite high emissions of CH_4. The N_2O emissions (g m^{-2}) from river are less than 1% of the emissions (g m^{-2}) measured from organic agricultural fields that are considered important sources of N_2O (Maljanen et al. 2003) The CH_4 emissions (g m^{-2}) measured from the river sites surrounded by peatlands are 69% of the emissions (g m^{-2}) measured from undrained minerotrophic fens (Nykänen et al. 1995). When the greenhouse gas budgets of terrestrial ecosystems are evaluated, riverine liberation of gases originating from terrestrial sources should also be taken into account.

A great part of the annual CO_2 and CH_4 emissions can be released in winter from the unfrozen parts of the river. In the future studies on annual greenhouse gas budgets of boreal river ecosystems, the winter emissions from unfrozen parts of the river cannot be neglected.

6.7 Conclusions

Based on the results of this thesis the following conclusions were made:

- Denitrification rates in boreal rivers are low compared to temperate rivers, but higher than those in open sea and estuary areas of the Baltic Sea
- Denitrification does not have a great importance in diminishing NO_3^- loading from the boreal rivers. Thus reducing anthropogenic loading of nitrogen from the catchment to the rivers is very important.
- The N_2O/N_2 ratio in denitrification increases with increasing NO_3^- load and decreasing temperature, but the amount of N_2O always remains low.
- A high nitrogen load may enhance nitrate assimilation and organic matter decomposition in river sediments and increase the emissions of CO_2 to the atmosphere, but further studies are needed to confirm and extend this observation
- Among the boreal rivers, those surrounded by agricultural areas are the greatest potential sources of N_2O, as part of the N_2O produced in the catchments leaks into the rivers and is degassed there to the atmosphere
- Overall greenhouse gas dynamics of the high latitude rivers are defined by the characteristics of their catchments: Rivers surrounded by peatlands release CO_2 and CH_4, whereas rivers surrounded by agricultural fields release N_2O.
- A great part of the annual CO_2 and CH_4 emissions can be released in winter from the unfrozen parts of the river.
- Greenhouse gas emissions are higher from boreal rivers than from recipient estuaries

The main conclusion from this thesis is that the nitrate loading from anthropogenic sources enters into the coastal waters of the Bothnian Bay with little retention in the river sediments. Land use in the surrounding catchments not only defines the nitrogen losses to rivers/streams but also determines the greenhouse gas fluxes from the river.

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