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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 (~ 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).

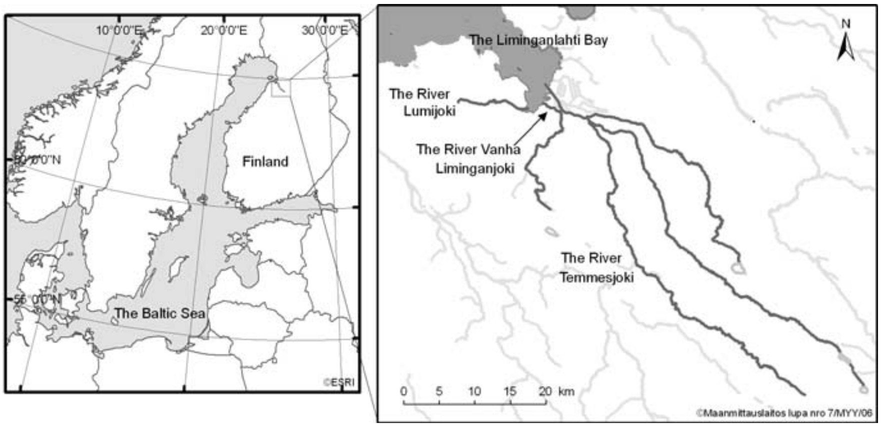
This study provides the first information on the effects of oxygen availability and temperature on denitrification and N_2O efflux rates in boreal river sediments.

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



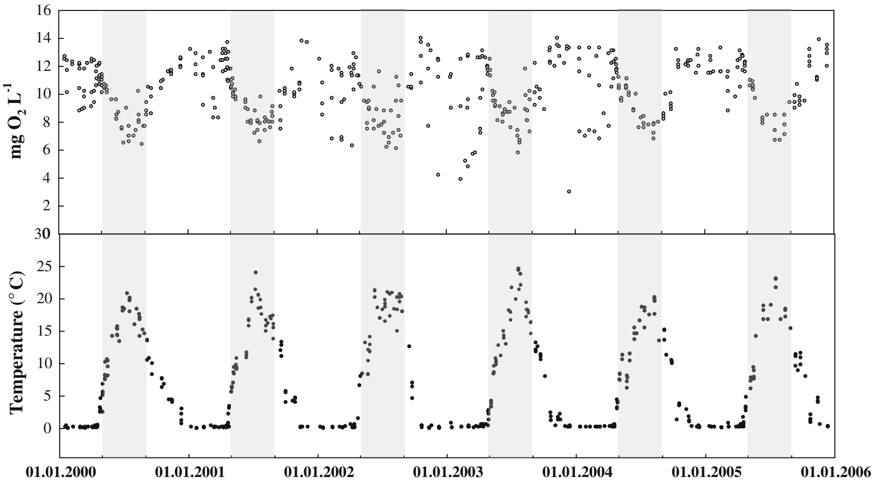
Annual variation in temperatures and oxygen concentrations of the river Temmesjoki

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:

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

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

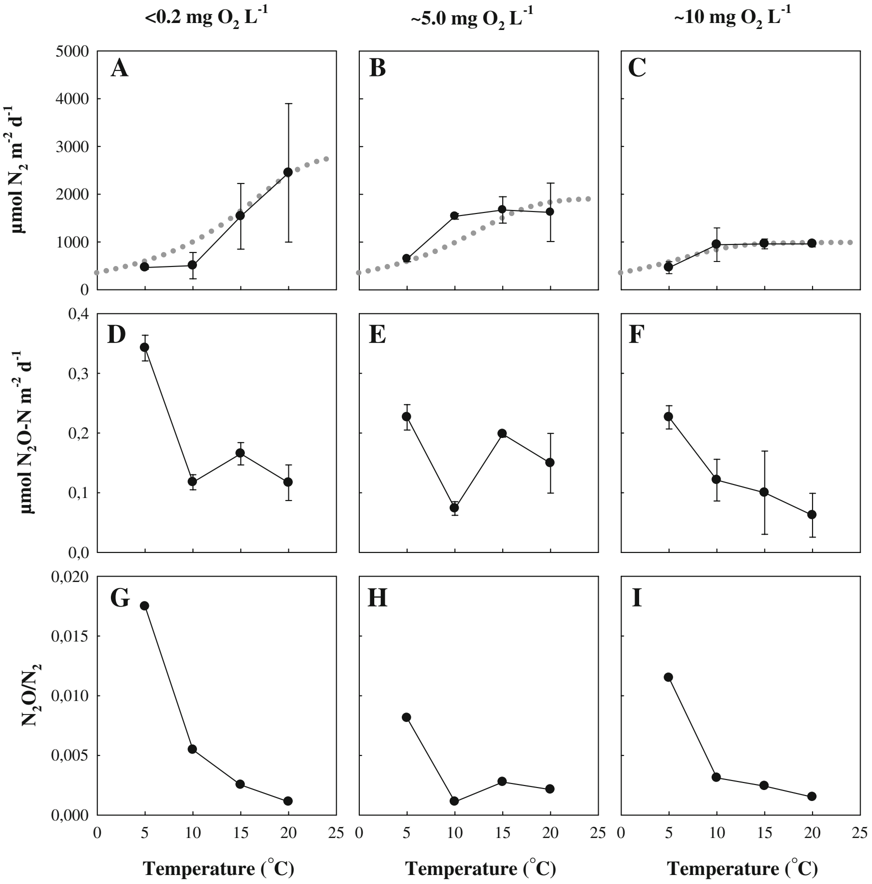


Fig. 3 Denitrification rates (**a–c**), N_2O effluxes (**d–f**) and N_2O/N_2 ratios (**g–i**) for different temperatures and oxygen concentrations. N for each point is 3. The *error bars* represent the standard deviations. The *dotted dark grey lines* in **a–c** represent the results of the combined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

$$f(temp) = \exp(P1 * (temp - 20°C)) \qquad (2)$$

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}:

$$f(O_2) = 1 - P2 * O_2 \qquad (3)$$

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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