Chapter 5

Measured denitrification and nitrous oxide fluxes in intertidal mudflats along the Zeeschelde (Scheldt estuary, Belgium)

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Abstract

Seasonal measurements were carried out of denitrification, using the acetylene inhibition method on laboratory incubated sediment samples, concurrent nitrate consumption rates, and N_2O emission measurements in field and laboratory conditions along a longitudinal and vertical gradient of mudflats in the Scheldt estuary, Belgium. Additional experiments were carried out to determine limitation of nitrate vs. carbon, oxygen consumption and to investigate the role of flooding regime on N_2O emission. Spatial and temporal variation of the gaseous emissions was high and correlation with ambient parameters was generally not significant. Laboratory incubated denitrification values ranged from 0.22 to 6.8 mmol N m^{-2} d^{-1}, concurrent N_2O emissions ranged from 0.005 to 0.48 mmol N m^{-2} d^{-1} and field incubated N_2O emissions at low tide ranged from 0 to 0.49 mmol N m^{-2} d^{-1}. Although nitrate was limiting for denitrification, N_2O emission at 10kPa acetylene incubation and constant nitrate availability decreased with rising tide. Oxygen consumption related with the presence of macrozoobenthos (*Oligochaeta*) was with 1.23 mol O^2 mol C^{-1} d^{-1} higher than previously assumed. It is hypothesised that benthos affects denitrification not only by enhancing nitrate transport from the overlying water into the sediment, but also by reducing the oxygen concentration at the water sediment interface.

5.1 Introduction

Among the numerous goods and services provided by estuaries, nutrient cycling is often prominent. In one case (Shepherd *et al.*, 2007), nitrogen cycling amounted up to nearly the complete estuarine habitat value as given by Costanza *et al.* (1997). Denitrification is the main process that accounts for the ecosystem services of the nitrogen cycle, although it is directly linked with emission of trace gas involved in global warming and stratospheric ozone destruction (Seitzinger *et al.*, 1984; 1988). In many studies on N-cycling in estuarine habitat, denitrification is determined in different indirect ways: as a rest fraction in mass balances (*e.g.* Middelburg *et al.*, 1995a; Van Damme *et al.*, 2009) or isotope budgets (*e.g.* Gribsholt *et al.*, 2005), or through modeling (*e.g.* Soetaert *et al.*, 1995; Vanderborght *et al.*, 2002). Direct measurements of estuarine denitrification, with the acetylene inhibition technique, microsensors, direct measurement of N_2 emission in a He background or isotope measurements of nitrogen gas are less common. For instance, several ecological models, including benthic and pelagic denitrification, are available of the Scheldt estuary (Soetaert *et al.*, 1995; Vanderborght *et al.*, 2002). In contrast, direct denitrification measurements are restricted to one study and one sampling station (Laverman *et al.*, 2007). Such measurements are more important than indirect determinations, as these could be more biased than previously thought. Indeed, in recent years our understanding of the estuarine nitrogen cycle has been amended with several new pathways, including anaerobic ammonium oxidation to N_2 (anammox). Annamox has a well documented importance in marine and coastal environments (Meyer *et al.*, 2005), but its role in estuarine N-cycling is still under investigation. Only a few estuaries have been examined so far, showing *e.g.* that N_2 production due to annamox ranged between 0 and 22% in the Chesapeake Bay (Rich *et al.*, 2008). Annamox could bias nitrification coupled denitrification estimates such as presented by Middelburg *et al.* (1995a). The acetylene inhibition technique, a well established method to measure denitrification (Seitzinger *et al.*, 1993), is unaffected by annamox as this process is almost completely inhibited at an acetylene concentration of 22 μM while effective denitrification inhibition to N_2O requires 4mM (Jensen *et al.*, 2007).

The role of benthic denitrification in the Scheldt is gaining relative importance on pelagic denitrification, as the ecological recovery of the estuary leads to higher oxygen concentrations in the pelagic environment(Cox *et al.*, submitted); the Scheldt estuary is but one of several systems in this situation (*e.g.* Billen *et al.*, 2005).

It is not the aim to assess the influence of new pathways on previous denitrification estimates, but to present data obtained by a reappraised method of a system that still lacks data. We report the first measured denitrification data of mudflat sediments of the Scheldt estuary that cover both the salinity gradient and a vertical gradient in the freshwater part, as measured with the acetylene inhibition technique. N_2O emission rates were recorded additionally. A comparison between the benefits of increasing benthic denitrification and the negative effect of N_2O emission is useful within the framework of ecosystem goods and services, as the greenhouse gas issue is of ever growing importance.

5.2 Material and methods

5.2.1 Study area

The Scheldt estuary is located in Northern Belgium (Flanders) and the Southwest Netherlands (Fig.1). It extends from the mouth at Vlissingen (km 0) till Gent (km 158); there tidal movement is stopped through a complex of sluices. The lower and middle estuary, the Westerschelde (55 km long), situated in the Netherlands, is a well mixed region characterized by a complex morphology with flood and ebb channels surrounding several large intertidal mud and sand flats. The surface area of the Westerschelde is 310 km^2, with the intertidal area accounting for 35% of the area. The Sea Scheldt, situated in Belgium, is single channeled and its surface amounts to only 44 km^2. The water quality of the Scheldt has been heavily impacted, especially in the Sea Scheldt (Van Damme *et al.*, 2005), but is now in a phase of partial recovery (Soetaert *et al.*, 2006). This study is confined to the Sea Scheldt (105 km long).

5.2.2 Sampling sites

Four intertidal mudflat sites were selected along the Sea Scheldt, largely on the basis of salinity and accessibility (Fig. 5.1). Groot Buitenschoor (GB) is situated in the brackish part, Burcht (BU) on the transition zone between the freshwater and the brackish zone, Durme (DU) and Appels (AP) in the freshwater part (Table 5.1). In Appels a lateral gradient of four stations was installed between low water level and the *Scirpus* spp. vegetation bordering the marsh (Table 5.1). Wooden boardwalks were constructed and used to reduce the disturbance caused by visits.

Campaigns were conducted on a monthly basis during 1996 (N_2O emission) and 1997 (N_2O emission and denitrification).

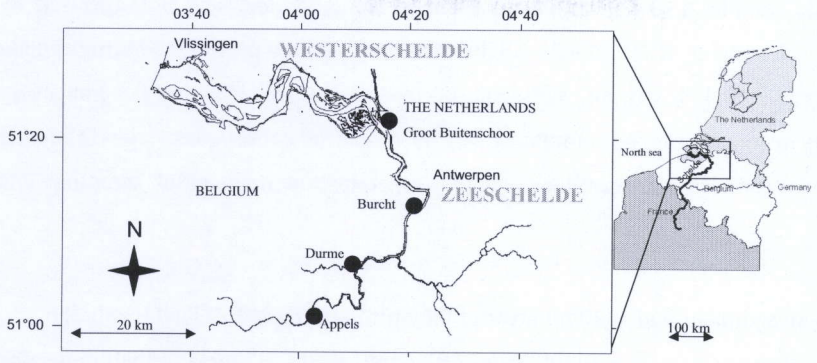

Fig. 5.1: Map of the selected sites

Table 5.1: Site description

Station	Elevation (m TAW)	Moisture (% DW)	Conductivity ($\mu S\ cm^{-1}$)	pH	C tot (% wt)	NH_4 (% wt)	$CaCO_3$ (% wt)	Sand (%)	Loam (%)	Clay (%)
GB	3.9	95 ± 13	15146 ± 9006	7.8 ± 0.3	3.0 ± 0.5	4.7 ± 4.3	17	30	46	25
BU	4.2	48 ± 19	2517 ± 1536	8.0 ± 0.1	1.2 ± 0.2	10.7 ± 5.0	12	60	29	11
DU	4.5	146 ± 22	1504 ± 319	7.8 ± 0.2	3.6 ± 0.8	19.3 ± 10.8	9.9	52	25	23
AP1	3.2	69 ± 20	1002 ± 362	7.5 ± 0.1	1.2 ± 0.6	10.9 ± 10.2	8.4	75	14	11
AP2	3.8	81 ± 22	1028 ± 281	7.5 ± 0.1	1.6 ± 0.2	10.6 ± 10.3	9.0	67	18	15
AP3	4.7	98 ± 30	1138 ± 206	7.3 ± 0.1	2.5 ± 0.8	8.7 ± 10.7	8.3	48	32	21
AP4	5.1	97 ± 37	1042 ± 344	7.5 ± 0.2	4.5 ± 1.2	3.5 ± 5.2	8.7	18	47	35

5.2.3 Field flux measurements

The release of nitrous oxide was measured by sampling accumulated gas beneath chambers placed over the sediment. Before placing them -about 4 cm deep in the sediment- the circular top lid was removed. This allowed chamber ventilation with ambient air while the sediment could re-establish equilibrium after the possible sediment disturbance due to placement of the chamber. The gas chambers had a circular ground surface of 176.7 cm^2, a height of 10cm and were made of non transparent polypropylene. The top lid contained a gastight septum at the top that was used for connection with Teflon vacutainers by means of a double needle, so that headspace samples could be transported and stored for analysis in the lab.

Samples were taken at t0 (*i.e.* immediately after closing the top lid) and half hour intervals during one hour (*i.e.* 3 in total per replicate).

5.2.4 Laboratory flux measurements

Sediment surface samples (~top 3 cm) were taken to the laboratory and homogenised by gentle stirring. Subsamples of 40 g fresh weight were then put into preweighed airtight glass recipients (volume 218.4 ± 0.8 ml, sediment horizontal surface 15.8 cm^2). For every sampling, fluxes were determined simultaneously in 6 sets of subsamples, with following treatments at two controlled temperatures (field temperature at moment of sampling and 25°C):

- N_2O from sediment with a 40 ml layer of 0.66 mM (10 mg N L^{-1}) KNO_3 solution
- N_2O from sediment with a 40 ml layer of 0.66 mM KNO_3 solution and with injection of 10 kPa acetylene in the headspace
- Nitrate water-sediment flux from a 40 ml layer of 0.66 mM KNO_3 solution

As soon as the solution was added, benthic invertebrates emerged from the sediment and showed activity that persisted during the entire flux measurements. Nitrous oxide fluxes under laboratory conditions (3 replicates) were determined by taking samples at t0 and at 24h intervals during 2 days. At this time lag under steady state conditions, all fluxes, calculated by regression analysis from the recorded change of concentration over time in the headspace, were significant ($p < 0.05$). Acetylene (10 kPa) was injected into the headspace to block conversion of N_2O to N_2 during denitrification. Shortcomings of the technique were thus limited by applying the incubations on small sediment volumes (40 g), enhancing the penetration of the acetylene gas, and by providing long incubation times (3d). Nitrate and ammonium fluxes were determined by sampling the overlying water layer in the separate subsamples at four to eight regular time intervals in separate replicates of a two days covering time series.

5.2.5 N vs. C limitation experiment

To investigate whether N or C was a limiting factor for denitrification, nitrate flux and N_2O-emission, laboratory flux measurements using homogenised sediment (as described above) were conducted at four different concentrations of nitrate (0, 1, 5 and 10 mg N L^{-1} as KNO_3) at 25°C. This experiment was carried out twice for sediment op Appels 2, Burcht and Groot

Buitenschoor, and 3 times for Durme. For each of these stations one experiment was carried out with and without addition of 1% glucose in the overlying solution.

5.2.6 Oxygen consumption experiment

In the same setup as the laboratory flux measurements (at 25°C), oxygen consumption from the overlying water was measured with a 'WTW OXI 91' oxygen meter. This was done on sediments of Groot Buitenschoor (samplings of 10 and 20/09/1996), Burcht (11 and 19/09/1996), Durme (3 replicates on sampling of 11/01/1996 and 2 replicates on sampling of 16/03/1996), Appels 2 (10 and 18/09/1996) and Appels 3 (17/09/1996). Sediment of Appels 3, that contained almost no benthic macro-invertebrates, was amended with and without addition of 100 individual Oligochaetes, collected on a 250µ sieve, corresponding with a density of 63290 ind. m^{-2} which is representative for an average site in the oligohaline part of the estuary (Seys *et al.*, 1999).

Oxygen consumption was calculated by determining the slope between -10% and -90% of the total oxygen concentration decrease during the experiment, relative to the initial concentration.

5.2.7 Experimental tidal mudflat

A mixture of (1:4) garden soil and mudflat sediment (Burcht) was mixed and transferred to a container (l x w x h = 2.0 x 0.5 x 0.8 m). The container was placed in a temperature (18±0.5°C), humidity (70±7%) and CO_2 concentration (380±40 ppmv) controlled room. The sediment was allowed to settle for a period of 4 months. After this period sediment height was ~35 cm. Two polypropylene collars with an inner diameter of 21 cm were installed in the centre of the container to ascertain consistent placement of the gas collecting chambers and minimise disturbance during successive emission measurements (Van der Nat & Middelburg, 2000). The flux chambers were 80 cm high (volume 55.4 l). A 16 hours day period with a light intensity of ~0.4mmol photons $m^{-2}.s^{-1}$ at the sediment surface (about 1.6 m below lamps) was set, except during the measurements when light was continuously supplied to avoid interference of the light regime with N_2O emission. Two tidal regimes (6 hours low – 6 hours high tide, and 10 hours low – 2 hours high tide resp.) were installed, each one during one week of which three days were used for allowing the sediment to come into equilibrium with the imposed tidal regime. The tidal range was ~16 cm. A large basin (~500 l) was used as a

reservoir for the overlying water. The nitrate concentration of the overlying water was daily adjusted at 10 mg N L^{-1}.

5.2.8 Analysis

Gas samples were transferred from the vacutainers or laboratory recipients to the detection equipment by means of 'Hamilton-GASTIGHT®' syringes. N_2O was determined using a Chrompack® 437A gas chromatograph, equipped with a stainless steel Altech Chromosorb 102 column of 4.88 m length and 3.175 mm diameter and a ^{63}Ni electron capture detector, under the following conditions: injector temperature 90°C, oven temperature 90°C and detector temperature 300°C. Standard gas of 51.4 ppmv N_2O in helium was used for calibration over a range of 10 – 1000 µL injection volume. For high tide conditions the N_2O concentration of the water phase was taken into account according to Moraghan & Buresh (1977).Nitrate and ammonium in water were determined according to Bremner (1965a).
Total carbon of the sediment was determined on air dry sediment according the method of Walkley & Black (Allison, 1965). Nitrate and ammonium in sediment were determined according Bremner (1965a) on extraction of 40 g sediment, extracted during 1h with 30 ml 3N KCl. Total N was determined after Kjeldahl destruction according Bremner (1965b). Sediment temperature was measured with a sediment thermometer at 2 cm depth. Conductivity of the pore water was measured with a WTW LF 91' conductivity-meter. pH of the sediment was measured in a sediment suspension according to Verloo (1988) with a C 832 Consort. Sediment texture was determined with a Sedigraph 5100. The CaCO3 content of sediment was determined through titration with HCl.

Estimates for annual nitrous oxide emission rates were calculated for all sites by integration of the curves connecting averages of replicate measurements. Months without data were interpolated linearly. Q10 values were determined on pair wise incubations at field temperature and 25 °C, and determined only if this temperature difference was at least 10°C.

5.3 Results

5.3.1 Site characterisation

The annual average sediment conductivity, carbon and nitrogen contents are listed in Table 5.1. The ammonium content of the sediment was in the freshwater stations generally higher

than in the brackish stations of Burcht and Groot Buitenschoor. The vertical gradient in Appels was generally characterised by an increasing total carbon content, clay and loam fraction and moisture content in upward direction. Dissolved nitrate concentrations (data not shown) were an order of magnitude lower than ammonium concentrations.

5.3.2 N_2O-Emission rates

Emissions of nitrous oxide from intertidal sediments of the Schelde estuary, as sampled *in situ* at low tide, and those determined after addition of nitrate solutions in the laboratory were highly variable, both temporal and spatial (Fig. 5.2-5.3). Negative fluxes were not recorded; all fluxes were from the sediment into the atmosphere. Laboratory incubated denitrification ranged from 0.22 to 6.8 mmol N m^{-2} d^{-1} (Fig. 5.2), concurrent N_2O emissions ranged from 0.005 to 0.48 mmol N m^{-2} d^{-1} (Fig. 5.3a) and field incubated N_2O emissions at low tide ranged from 0 to 0.49 mmol N m^{-2} d^{-1} (Fig. 5.3b). N_2O emissions in the field and in the laboratory after addition of nitrate solution thus were situated within the same range. On average, when a 10 mg N L^{-1} nitrate solution was applied at field temperature, N_2O emission with addition of acetylene was on average 51 times higher (3 to 345 times) than without acetylene. These high values indicate that denitrification was effective under ample nitrate provision. Denitrification removed between 3 and 60% (average 21%) of the nitrate in the overlying water.

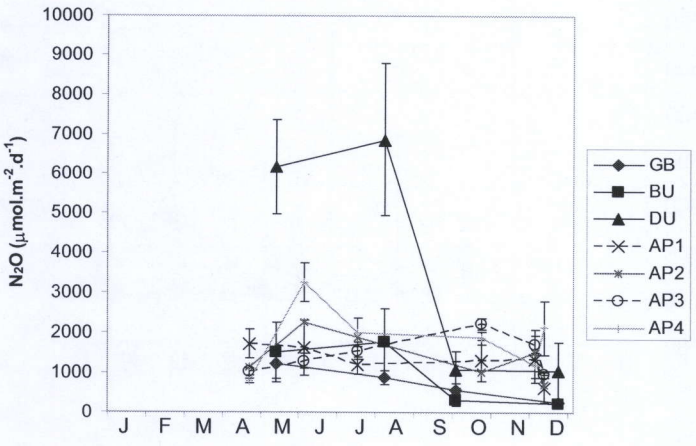

Fig. 5.2: Sediment denitrification; incubation in laboratory at field temperature, after addition of 10 mg N L^{-1} nitrate solution

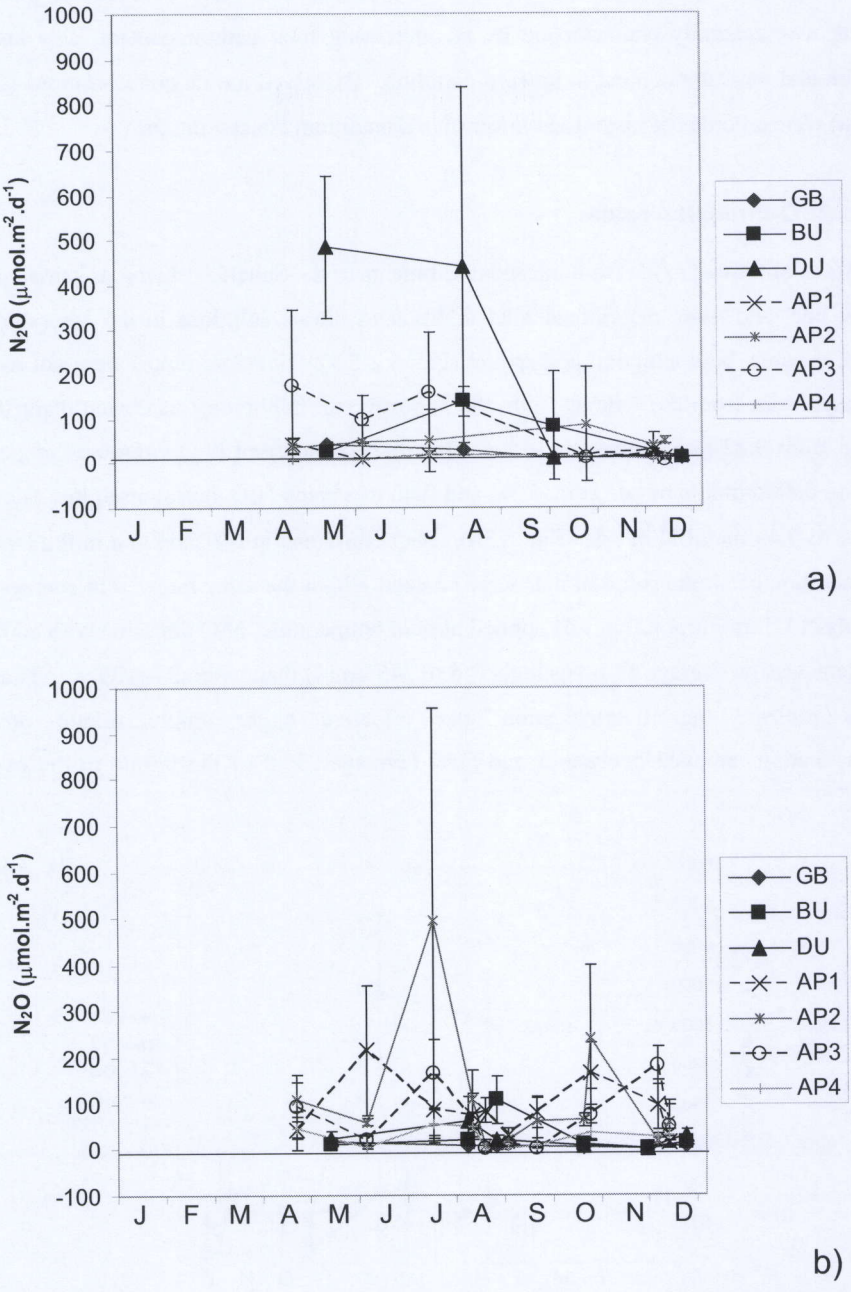

Fig. 3: N_2O emission from sediment; a) incubation in laboratory at field temperature, after addition of 10 mg N L^{-1} nitrate solution (data from 1996); b) *in situ* incubation at low tide (data from 1997)

In general, maximal values were recorded during summer and minima during winter (Fig. 5.2-5.3). Univariate analysis of variance on all replicates of the different sampling stations, with sampling month as a sorting variable, showed for denitrification significant variances ($p<0.05$) between sampling campaigns for all stations except Appels 1 and Appels 2 (Table 5.2). For Appels 3, however, the variance was related with a maximum denitrification value in winter (Fig. 5.2). In Appels, 3 of 4 stations thus showed no seasonality for denitrification. For laboratory incubated N_2O emissions, variances between sampling months were significant for all stations except for Burcht and Appels 2 (Table 5.2), but for Appels 1 and 4 the significant differences were very small. For field incubated N_2O emissions, not one variance between sampling months was significant. It can be concluded that the variance of the data was so large that seasonality was not clearly apparent.

Table 5.2: Univariate analysis of variance on all replicates of the different sampling stations, with sampling month as a sorting variable, for *in situ* and laboratory incubated N_2O emission and denitrification

Station	N_2O *in situ*			N_2O (10 mg NO_3^--N solution)			N_2O (10 mg NO_3^--N solution) (10 kPa acetylene)		
	df	F	p	df	F	p	df	F	p
GB	12	3.1	0.62	12	10.4	0,004	12	8.8	0,006
BU	12	2.2	0.12	12	2.6	0.12	12	5.9	0,02
DU	12	0.7	0.35	12	4.5	0,04	12	20.2	0,00
AP1	18	1.6	0.11	18	6.5	0,004	18	2.0	0.15
AP2	18	0.2	0.23	18	0.3	0.91	18	2.4	0.09
AP3	18	3.9	0.64	18	111	0,000	18	15.4	0,000
AP4	18	0.6	0.54	18	9.3	0,001	18	10.8	0,000

For laboratory incubated N_2O emissions (Fig. 5.4a) and denitrification (Fig. 5.4b), variability on the data was larger in the freshwater zone than at higher conductivity. This statement is, however, biased by the higher number of samples in the freshwater zone when taking also into account the data of the vertical gradient of Appels, as was done in Fig. 5.4. Univariate analysis of variance on data of only Durme, Burcht and Groot Buitenschoor, which are distributed more uniformly along the salinity gradient, showed, with specific conductivity as a sorting variable, a significant effect of specific conductivity between Durme and Burcht (denitrification: $F_{1,36} = 8.0$, $p = 0.001$; N_2O emission: $F_{1,36} = 25$, $p = 0.004$), but not between Burcht and Groot Buitenschoor.

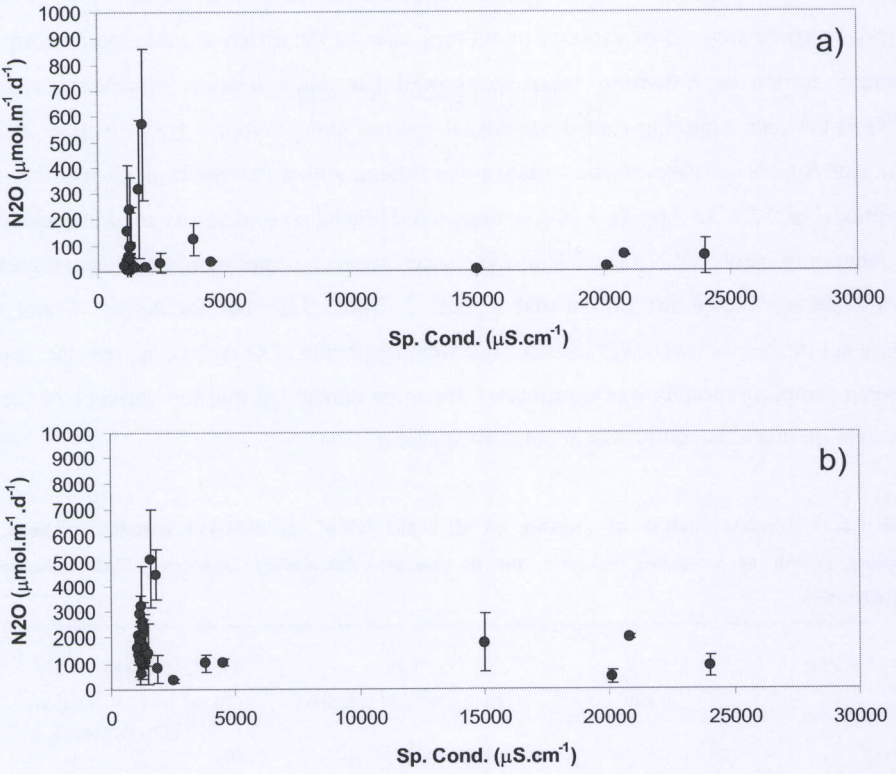

Fig. 5.4: Sediment N_2O emission (a) and denitrification (b) vs. specific conductivity of sediment interstitial water; incubation in laboratory at 25°C, after addition of 10 mg N L^{-1} nitrate solution

Univariate analysis of variance on all replicates, with location as a sorting variable, of the different sampling campaigns of the stations in Appels showed zero significant ($p<0.05$) differences for denitrification (data not shown). As such, for denitrification, no vertical gradient was observed at all.

For laboratory incubated N_2O emissions Appels 1 and Appels 4 showed both significantly lower values than both Appels 2 and Appels 3 (AP1-AP2: $F_{1,18}=6.3$, $p=0.018$; AP1-AP3: $F_{1,18}=35$, $p=0.009$; AP4-AP2: $F_{1,18}=2.3$, $p=0.002$; AP4-AP3: $F_{1,18}=42$, $p=0.012$). All other differences were not significant (data not shown).

For field incubated N_2O emissions, only one station showed consistently lower values. Appels 4 showed significantly lower values than all other stations (AP1: $F_{1,40}=45$, $p=0.008$; AP2: $F_{1,40}=12$, $p=0.013$; AP3: $F_{1,40}=1,8$, $p=0.024$). No other significant differences between stations were observed for field incubated N_2O emissions (data not shown).

Careful examination of the data revealed not a single factor, or any combination of measured variables, that could explain the temporal or spatial variation of the observed fluxes.

Estimates for annual nitrous oxide emission rates were calculated for all sites by integration under the curves that connect monthly averages of replicate measurements (Table 5.3). Months without data were interpolated linearly. Q10 values ranged between 0.7 and 2.6, showing thus high variability but within the range of biological processes.

Table 5.3: Annual integrated N_2O emission rates and Q_{10} values

Station	N_2O *in situ* (mmol $m^{-2} y^{-1}$)	N_2O (10 mg NO_3^--N solution) (mmol $m^{-2} y^{-1}$)	Q10	N_2O (10 mg NO_3^--N solution) (10 kPa acetylene) (mmol $m^{-2} y^{-1}$)	Q10
GB	3.6 ± 1.8	8.7 ± 4.7	1.5 ± 1.2	243 ± 122	2.0 ± 1.4
BU	7.7 ± 6.7	18 ± 15	0.9 ± 0.5	314 ± 232	1.0 ± 0.7
DU	8.9 ± 4.1	84 ± 76	1.2 ± 0.6	1335 ± 911	1.0 ± 0.4
AP1	29 ± 23	7.6 ± 3.4	2.6 ± 1.7	462 ± 116	1.3 ± 0.3
AP2	41 ± 48	19 ± 5.0	0.7 ± 0.3	472 ± 167	1.1 ± 0.5
AP3	26 ± 20	31 ± 21	1.0 ± 0.4	503 ± 154	1.3 ± 0.3
AP4	11 ± 5.5	4.3 ± 1.5	0.8 ± 0.3	538 ± 311	0.9 ± 0.3

5.3.3 N vs. C limitation experiment

As the nitrate concentration increased in the overlying solution (0, 1, 5 and 10 mg N L^{-1}), both the nitrate consumption rate and the denitrification rate increased (Fig. 5.5). Only in one case the increase was not significant (nitrate water-sediment flux Burcht, slope 0.02, R^2 0.01, $F_{1.8}$=8.2, p>0.5). Apart from this case the slope of nitrate water-sediment flux ranged from 0.32 to 2.14 (R^2 ranging from 0.78 to 0.99), and the slope of denitrification ranged from 0.09 to 0.98 (R^2 ranging from 0.82 to 0.99). Nitrate disappeared 2 to 5 times faster from the overlying solution than the according denitrification increase (Fig. 5.5). An additional similar experiment where the sediment was added in different recipients so that the water-sediment surface was 12.6 vs. 315 cm^2, *i.e.* 25 times larger, *ceteris paribus*, showed that the time before the overlying nitrate solution dropped from 10 to 2 mg N L^{-1}, was 4 days resp. 4 hours, *i.e.* 24 times shorter. The concordance between the surface increase and the nitrate consumption rate

decrease indicates that the reactive surface, hence the water sediment transport, was limiting for nitrate removal of the overlying solution.

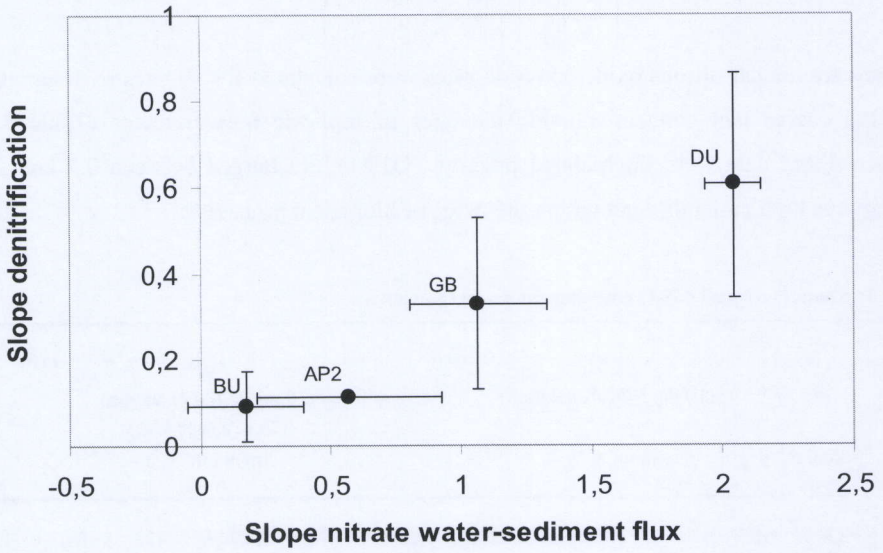

Fig. 5.5: Slope of denitrification vs. nitrate water sediment flux at increasing overlying nitrate solutions (0, 1, 5 and 10 mg N L^{-1}); replicates over the nitrate concentration gradient: $n_{DU} = 3$, $n_{AP2} = 2$, $n_{BU} = 2$, $n_{GB} = 2$. Slopes were determined on individual regression lines of the flux rates (in mmol m^{-2} d^{-1}) at increasing nitrate concentrations and then averaged over the different experiments.

Emission of nitrous dioxide did not show a similar increase as in Fig. 5.5 (data not shown); slopes ranged from -0.03 to 0.16 (R^2 from 0.32 to 0.77).

Amendment with glucose on replicate samples (addition of 10 mg N L^{-1} only) of the N vs. C limitation experiment did not lead to any change in nitrate water-sediment flux rates after the first day of incubation (paired T-test, performed on individual nitrate water-sediment flux rate slopes with and without glu amendment for the stations Durme, Appels 2, Burcht and Groot Buitenschoor, t=0.86, p=0.89, n=4). These results show that nitrate availability, not carbon, was the limiting factor for denitrification in the Scheldt sediments.

5.3.4 Oxygen consumption experiment

Oxygen consumption was highest in sediment of the Durme and lowest in sediment of Appels 3 (Table 5.4). Addition of 100 individual oligochaetes on sediment of AP3 (experimental sediment surface = 15.8 cm^2) resulted in a change from zero order to a polynomial decrease

(Fig. 5.6). Given an oxygen consumption rate increase from 0.48 to 8.33 μg $O_2.cm^{-2}$ h^{-1} (Table 5.4), the oxygen consumption of one average Oligochaete amounted to 1.2 μg O_2 h^{-1}., corresponding with a consumption rate of 1.23 mol O^2.mol C^{-1} d^{-1}.

Table 5.4: Oxygen removal rates of mudflat sediment

Station	Rate (mg O_2 cm^{-2} h^{-1})	n
GB	1.3 ± 0.5	2
BU	2.3 ± 1.2	2
DU	7.9 ± 1.8	5
AP 2	2.0 ± 1.0	2
AP3	0.5	1
AP3+100 Oligochaetes	8.3	1

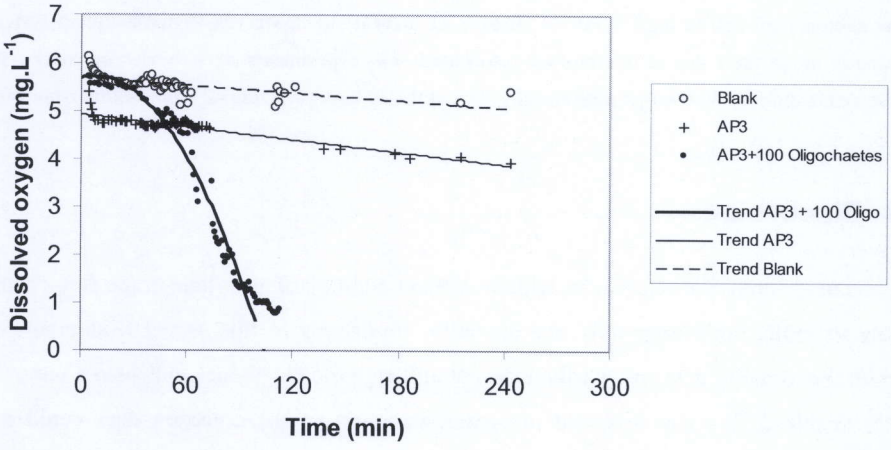

Fig. 5.6: Dissolved oxygen concentration in overlying water of sediment with and without addition of 100 Oligochaetes; blank = water without sediment or Oligochaetes

5.3.5 Experimental tidal mudflat

Denitrification in sediment, exposed after submersion by 10 $mg.L^{-1}$ nitrate solution, and under 10 kPa acetylene incubation, decreased exponentially with time (Fig. 5.7). At a high tide - low tide regime of 2h - 10 h, the measured denitrification rate decreased to zero after 8 to 9 hours, whereas at the 6h-6h regime, the slope of the exponential curve did not reach zero at the end of the low tide. This pattern was repeated over 3 tides (data not shown). Organic

matter was not the limiting factor in the decrease, as the N_2O concentrations in the head space continued to increase after consequent low tide phases (Fig. 5.7).

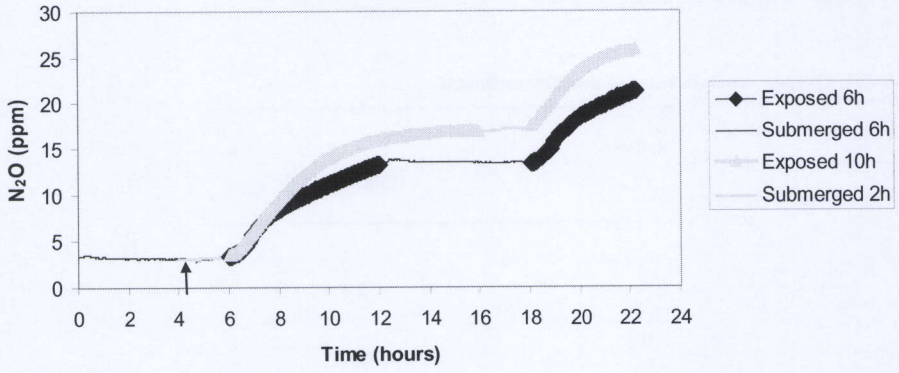

Fig. 5.7: Comparison of N_2O concentrations in the flux chamber of the tidal experiment container under two tidal regimes: 6 hours low tide - 6 hours submersion with 10 mg L^{-1} NO_3^--N, and 10 hours low tide - 2 hours submersion with 10 mg L^{-1} NO_3^--N. Before the measurements were recorded the meso-system was allowed to adapt for 1 day to the imposed conditions. The experiments were carried out under 10 kPa acetylene incubation. The arrow indicates the start of the 10 hours low tide - 2 hours submersion run.

5.4 Discussion

Nitrous oxide emission rates were, with or without addition of acetylene in the flux chambers, highly variable, both temporally and spatially, confirming in this aspect Middelburg *et al.* (1996). Seasonality was only partly apparent although the Q_{10} values indicated a temperature effect (Table 5.2). The temporal or spatial variation of the emission data could not be explained by any combination of variables.

5.4.1 Denitrification

The observed denitrification rates (Table 5.2) were for the corresponding locations (Durme and Burcht) an order of magnitude lower than the denitrification rates estimated by Middelburg *et al.* (1995b). Middelburg *et al.* (*op cit.*) calculated nitrification coupled denitrification at low tide, while in our approach acetylene inhibited nitrification in submerged sediment. According to a review of Seitzinger *et al.* (1988), nitrification in sediments is in most estuaries the major source of nitrate for sediment denitrification; denitrification resulting from overlying nitrate being about ten times lower than nitrate

coupled denitrification. This is confirmed by our denitrification results: they are one order of magnitude lower than the nitrification supported denitrification values of Middelburg *et al.* (1995b), while the agreement with the diffusion supported fraction is good: 0.50 mol m^{-2} y^{-1} for Doel which is comparable with our values of the nearby station of Groot Buitenschoor. There was also agreement with the modelled results of Soetaert & Herman (1995).
Laverman *et al.* (2007), who combined the acetylene technique with microsensor measurements on sediment of Appels, found depth integrated denitrification values of 12 mmol N m^{-2} d^{-1} which is much more than our values of 0.22 to 6.8 mmol N m^{-2} d^{-1}. These values, however, represent potential rates, stimulated by flow through conditions (Laverman *et al.*, 2006). High denitrification values (up to 13.8 mmol N m^{-2} d^{-1}) were recorded for intertidal freshwater sediments of the Yorkshire Ouse, using the acetylene inhibition technique (Garcia-Ruiz *et al.*, 1998). The global averaged system denitrification values (adding up both nitrification and overlying nitrate as sources) of estuaries (about 1 mmol N m^{-2} d^{-1}, excluding fresh water zones) and rivers (about 2 mmol N m^{-2} d^{-1}), as reviewed by Seitzinger *et al.* (2006), indicate that denitrification in the Scheldt, according to any author, is intensive.

The nitrate load leaving the Zeeschelde amounted in the early 1990's to 9000 tons per year (Soetaert & Herman, 1995). Based on our data, extrapolated linearly for the pelagic nitrate concentrations (as recorded by Van Damme *et al.*, 2005) by using the slopes of Fig. 5.5, and by using the compartments proposed by Soetaert & Herman (*op cit.*), the mudflats of the Sea Scheldt could only eliminate 0.6% of this load by diffusion supported denitrification. Despite the intensity of the process, diffusion supported denitrification in mudflats constituted a marginal effect on the nitrogen budget of the estuarine system. Including also nitrification supported denitrification, Middelburg *et al.* (1995b) estimated that intertidal sediments might account for 14% of the total estuarine nitrogen retention, making abstraction of possible annamox bias. The recent evolution of the water quality (Cox *et al.*, 2009), indicates that despite the recovery of the oxygen status, the nitrate concentration continues to decrease so that diffusion supported denitrification will decrease with it, as nitrate was the limiting factor for denitrification. At constant nitrate availability, however, the N_2O emission rate decreased as the inundation period and concurrent tidal submersion level increased (Fig. 5.7), although the headspace concentration continued to increase. The depth profiles of Laverman *et al.* (2007) show similar patterns at some mm in depth, as the pore water concentration increased with inundation time, but at the surface the production rate did not significantly change.

The stimulating influence of benthos, and more specifically of Oligochaetes, on denitrification has already been reported (Chatarpaul *et al.*, 1980; Pelegri and Blackburn 1995). This was explained by enhanced water-sediment transport. The peaking values of denitrification in the sediment of the Durme (Fig. 5.2) were registered when this station was characterised by a massive presence of oligochaetes. On this location, Seys *et al.* (1999) found an average benthos density of 243400 ind. m^{-2} which was about 4 times more than the average density in the entire Zeeschelde. It was noted that the abundant benthos community in the Durme site was removed by local dredging after the month of August, which could explain the lower denitrification values in the Durme in October (Fig. 5.2). In order to further assess the impact of the benthos, comparative oxygen consumption rates were measured. The sediment oxygen consumption increase after addition of 100 individual oligochaetes was of the same range as the difference between the oxygen consumption rates of the Durme and the other stations (Table 5.4). The benthos oxygen consumption recorded in this study (1.23 mol O^2 mol C^{-1} d^{-1}), was more than 3 times larger than given by the benthos respiration formula at 25°C in the model of Soetaert *et al.* (1995). This indicates that the enhanced denitrification rates of the Durme station are probably linked with benthos activity, not only through enhanced exchange of nitrogen, but also because the benthic activity lowered the oxygen content of the sediment surface layer.

5.4.2 Global warming

The *in situ* N_2O emission rates at low tide (Table 5.2) were up to 4 times higher than the corresponding values found by Middelburg *et al.* (1995b): as indicated in their paper, their values could have been higher if they had applied longer accumulation times in the incubation chamber.

The emissions of N_2O were compared with other sources of greenhouse gas emissions in the Schelde. The global warming potential of CO_2, CH_4 and N_2O (ratio 1:23:296), was multiplied with the according annual emission rates, in order to rank the importance of the emissions per surface unit according their contribution to global warming (Fig. 5.8). This comparison revealed that CO_2 is by far the most important contributor the greenhouse problem when compared with CH_4 and N_2O. Only in the freshwater tidal flats, enormous CH_4 emissions that were recorded by Middelburg *et al.* (1996) in the Durme tributary caused CH_4 to be the dominant greenhouse gas (18 times the reference). These high values were not reported

elsewhere in the freshwater part (Siebens, 1997). The peculiar aspect of the Durme station, as described earlier, is likely to be not representative for the freshwater part of the Schelde.

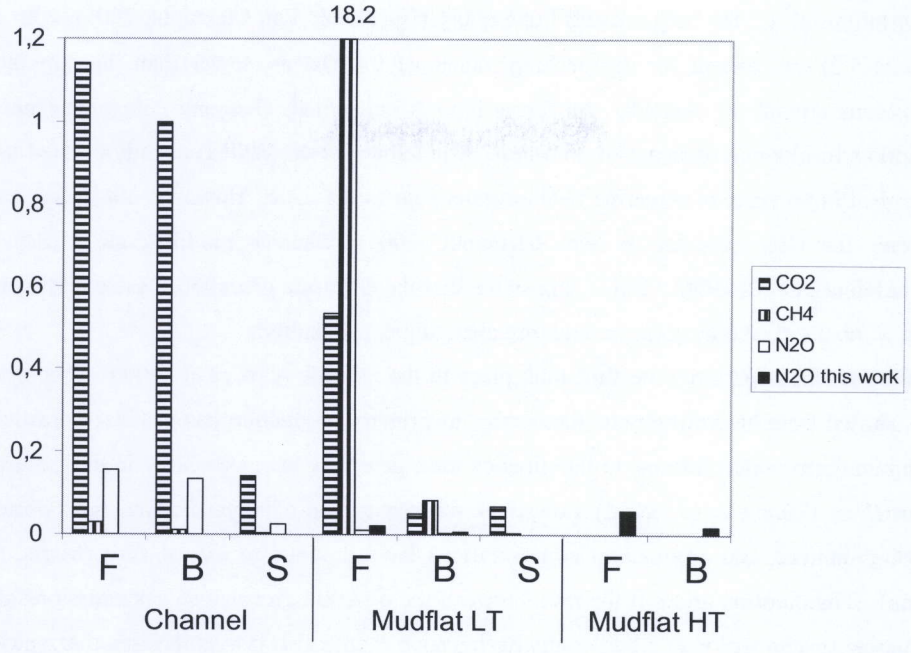

Fig. 5.8: Relative contribution of greenhouse gas emission fluxes of the Schelde to global warming potential per surface unit for different compartments of the Scheldt estuary, compared to the CO_2 GWP of the brackish channel compartment, set as reference value 1, as measured during the period 1993-1997 (channel = pelagic + subtidal sediment); F = salinity 0-2, B = salinity 2-15, S = salinity > 15, LT = low tide, HT = high tide. Sources: CO_2 channel: Frankignoulle *et al.* (1996); CH_4 channel: Middelburg *et al.* (2002), fluxes calculated with piston velocity of 8.4 cm.h^{-1} as determined by Frankignoulle *et al.* (1996); N_2O channel: De Wilde & de Bie (2000); CO_2 and CH_4 mudflat: Middelburg *et al.* (1996); N_2O mudflat: Middelburg *et al.* (1995b) and this work. For Mudflat HT only data of this work (N_2O) are available.

Nitrous oxide emission from the water surface contributed 5 (fresh water part) to 13 times (brackish and saline part) more to global warming than emission of CH_4. In mudflats at low tide, on the contrary, emission of N_2O contributed marginally in comparison to CO_2 and CH_4. For mudflats at high tide, no CO_2 or CH_4 emission data were available to compare with the higher N_2O emissions. Our data indicated that emission of N_2O from exposed tidal flats were an order of magnitude lower than emission from the water surface according to De Wilde &

de Bie (2000). As the mudflat surface becomes submerged, the emission values shifted more to those of the water surface above the subtidal system compartment.

The average N_2O emission from cultivated land is 101 mmol m^{-2} y^{-1} for the Netherlands and 76.4 mmol m^{-2} y^{-1} for Belgium and Luxemburg (Boeckx & Van Cleemput, 2001). Our data (Table 5.2) are, except for the peaking values of the Durme, lower than the agricultural emissions around the Scheldt. As far as N_2O is concerned, changing cultivated land into mudflats results in a decrease of emission. Middelburg *et al.* (1995a) already pointed to the marginal importance of estuarine N_2O emissions on world scale. However, cultivated land is a sink for CH_4 (Boeckx & Van Cleemput, 2001), whereas mudflats are clearly not (Middelburg *et al.*, 1996). But it is possible that the estuarine greenhouse gas emission rates that were observed during the nineties are changing considerably.

During recent years a regime shift took place in the Schelde (Cox *et al.*, 2009). The system has shifted from heterotrophy to autotrophy, as primary production has increased drastically. While oxygen concentrations in the nineties were generally low, especially in the freshwater zone (Van Damme *et al.*, 2005), nowadays supersaturation of oxygen is regularly observed during summer, and ammonium concentrations have dropped to almost zero (Maris, pers. com.). The question arises if the ratio between the different greenhouse gas emissions of the estuarine system will alter significantly during such changes. It is hypothesised that, since the nineties, emission of CO_2 and CH_4 has shifted from the estuarine system to water treatment plants, offering technical possibilities to capture CH_4. If the hypothesis is true it can be concluded that restoring estuarine ecological habitats by creating more intertidal areas cannot be compromised by pointing to the medal backside of enhancing greenhouse gas emission.

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