

Denitrification in aquatic environments: a cross-system analysis

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Abstract A meta-analysis was conducted on 136 data sets of denitrification rates (DR) recorded both during the period of highest water temperature and monthly in five types of aquatic ecosystems: oceans, coastal environments, estuaries, lakes and rivers. There was a gradual increase of DR from the ocean to rivers and lakes at both scales, with the rivers showing the highest DR variability. Denitrification peaked during summertime and showed highest seasonal variability in lakes and rivers. High concentrations of nitrate and interstitially-dissolved organic carbon as well as low oxygen concentration in the overlying water enhanced DR both during summer and at a seasonal scale whereas total phosphorus did at the seasonal scale only. There was a positive linear relationship between overlying nitrate and DR over the range of 1–970 $\mu\text{mol NO}_3$ ($r^2 = 0.86$, $P = 0.001$). DR in lakes and rivers might reach values doubling those in the more denitrifying terrestrial ecosystems (e.g. agrosystems). Discrepancies in DR and its controlling factors between site-specific studies and this meta-analysis may arise from environmental variability at two, often confounded, scales of observation: the

habitat and the ecosystem level. Future studies on denitrification in aquatic environments should address the topic of spatial heterogeneity more thoroughly.

Keywords Controlling factors · Meta-analysis · Seasonality · Spatial heterogeneity · Temporal scales

Introduction

Human activities have dramatically affected the global nitrogen cycle. Extensive agricultural fertilization has increased the supply of nitrogen to freshwater and marine ecosystems during the past decades worldwide (Vitousek et al. 1997). Elevated concentrations of nitrogen compounds has a negative impact on the aquatic systems and the importance of denitrification in the N cycle in different aquatic ecosystems has been an expanding topic in aquatic sciences throughout the past 25 years (Steingruber et al. 2001; Galloway et al. 2004). Removal of nitrogen by denitrification is important in acting as a sink in the global marine nitrogen budget thereby regulating the amount of primary production in waters with high concentrations of anthropogenic nutrients (Seitzinger 1988) and increased N export downstream results in global fertilization of inland and coastal waters, alteration of food webs (Carpenter

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et al. 1998) and even human disease (Townsend et al. 2003).

Most studies on denitrification rates (DR hereafter) in aquatic environments have been carried out either as ecosystem studies based upon mass balances of N budgets (Mengis et al. 1997; Jensen et al. 1992) or as studies in local habitats (Risgaard-Petersen et al. 1998; Wang et al. 2003). In the latter case, DR have been measured without assessing the variability among habitats within a given ecosystem and the different approaches of measuring denitrification have resulted in data with poor comparability.

Factors influencing denitrification in aquatic systems include among other the supply of nitrate, concentration of oxygen, dissolved organic carbon and phosphorus, temperature, light, water residence time and the presence of plants (Golterman 2004). Plants may strongly influence denitrification rates (Cornwell et al. 1999), either directly through enhancement of nitrification by increased of O₂ inputs into the sediment or indirectly through increased metabolic rates (Christensen and Sørensen 1986; Caffrey and Kemp 1990, 1992). Benthic microalgae can decrease rates of coupled nitrification/denitrification but may decrease rates of denitrification of water column nitrate (Risgaard-Petersen, 2003). Most of the factors mentioned have been addressed in site-specific studies, but their importance in the global control of the denitrification process has still to be revealed which precludes sound calculations of global estimations of the biosphere N sink (see the Final Report of the Workshop on Advanced Approaches to Quantify Denitrification, held in Woods Hole, May 2004; <http://www.whrc.org/nitrogen/>).

Some studies have reviewed denitrification activity in wetlands, lakes, rivers and coastal ecosystems (Seitzinger 1988; Groffman 1994; Cornwell et al. 1999; Steingruber et al. 2001; Saunders and Kalff 2001; Golterman 2004). However, most of them do not compare DR and the potential controlling factors across different aquatic ecosystems and temporal scales and little knowledge is therefore available on DR across waterscapes.

In this study, we made a cross-system study to compile quantitative estimates of DR among

aquatic environments (coastal ecosystems, estuaries, lakes, rivers and oceans) and to provide insights into factors controlling DR across a broad range of ecosystems. To undertake those goals, the meta-analysis approach was used since it will enabled us to summarize research findings across different studies and to give a quantitative synthesis of the ecological information about the process as well as to explore the general patterns within the aquatic ecosystems studied.

Materials and methods

The data collection

We searched the literature for studies quantifying DR in aquatic ecosystems and divided the ecosystems into five categories: lakes, rivers, estuaries, coastal ecosystems and oceans. Data were collected from text, tables and figures, gathering information on the method of analysis, the measurement site (sediment/water) and DR. Furthermore, data on control variables of denitrification activity such as concentrations of nitrate, oxygen-, total phosphorus, and interstitially-dissolved organic carbon was collected as well as data on plant occurrence and the light/dark regime during the measurements. All potential denitrification rates were excluded. Since water residence time has already been considered in another study of freshwater denitrification (Saunders and Kalff 2001) and since data for water residence time in marine environments are poor, that variable has not been included in this study.

We wanted to tackle different time scales that might be important for the denitrification process (Golterman 2000; García-Ruiz et al. 1998; Risgaard-Petersen 2003; El Harb and Golterman 1990), and hence we divided the studies into two groups. The first group was comprised of those conducted over an annual cycle, with at least a monthly sampling (Database 1, Table 1), and it was used to study seasonality in different systems and to compare their DR with those of terrestrial systems on an annual basis. The second data set contained studies conducted during the month of highest water temperature in the year (Database 2, Table 2). Seasonal studies on denitrification in

Table 1 Database 1. Sources of annual data on denitrification rates

Aquatic ecosystem	Name	Year	Method of analysis	Measurement site	Denitrification rate (mol N m ⁻² y ⁻¹)	Data source
Coastal ecosystem	Boston Harbour (USA)	1992	N ₂ flux	Sediment	0.67	Nowicki et al. (1997)
Coastal ecosystem	Boston Harbour (USA)	1993	N ₂ flux	Sediment	0.62	Nowicki et al. (1997)
Coastal ecosystem	Boston Harbour (USA)	1994	N ₂ flux	Sediment	0.35	Nowicki et al. (1997)
Coastal ecosystem	Coastal North Sea	1977	Acetylene inhibition	Sediment	0.87	Billén (1978)
Coastal ecosystem	Great Bay (USA)	1986	Acetylene inhibition	Sediment	0.09	Slater and Capone (1987)
Coastal ecosystem	Massachusetts Bay (USA)	1992	N ₂ flux	Sediment	0.34	Nowicki et al. (1997)
Coastal ecosystem	Massachusetts Bay (USA)	1993	N ₂ flux	Sediment	0.25	Nowicki et al. (1997)
Coastal ecosystem	Massachusetts Bay (USA)	1994	N ₂ flux	Sediment	0.28	Nowicki et al. (1997)
Coastal ecosystem	Ochlockonee Bay (USA)	1984	N ₂ flux	Sediment	1.07	Seitzinger (1987)
Estuary	Apalachicola Bay (Mexico)	1995	N mass balance	Sediment	0.26	Mortazavi et al. (2000)
Estuary	Culvert Creek (USA)	1995	Acetylene inhibition	Sediment	0.84	Thompson et al. (2000)
Estuary	Lawrence (Canada)	1997	Isotope pairing	Sediment	0.02	Wang et al. (2003)
Estuary	Newport River (USA)	1992	Acetylene inhibition	Sediment	0.12	Thompson et al. (1995)
Estuary	Pawcatuck River (USA)	1991	N ₂ flux	Sediment	0.37	Nowicki (1994)
Estuary	Randers Fjord (Denmark)	1995	¹⁵ N-labelled nitrate	Sediment	1.09	Nielsen et al. (2001)
Lake	Bryrup Lang (Denmark)	1972	N mass balance	Water	2.92	Andersen (1974)
Lake	Bryrup Lang (Denmark)	1973	N mass balance	Water	2.93	Andersen (1974)
Lake	Kul (Denmark)	1972	N mass balance	Water	1.42	Andersen (1974)
Lake	Kul (Denmark)	1973	N mass balance	Water	1.71	Andersen (1974)
Lake	Kvind (Denmark)	1973	N mass balance	Sediment	2.18	Andersen (1974)
Lake	Kvind (Denmark)	1972	N mass balance	Sediment	2.31	Andersen (1974)
Lake	Okechobee (USA)	1978	Acetylene inhibition	Sediment	0.10	Messer and Brezonik (1983)
Lake	Salten Lang (Denmark)	1973	N mass balance	Water	0.15	Andersen (1974)
Lake	Sobygard (Denmark)	1986	N mass balance	Water	2.81	Jensen et al. (1992)
River	Childs River (USA)	1992	N ₂ flux	Sediment	1.10	LaMontagne et al. (2002)
River	Childs River (USA)	1993	N ₂ flux	Sediment	1.27	LaMontagne et al. (2002)
River	Gelbæk (Denmark)	1987	Acetylene inhibition	Sediment	3.50	Christensen et al. (1990)
River	Gelbæk (Denmark)	1985	Acetylene inhibition	Sediment	2.93	Christensen and Sørensen (1988)
River	Rabis Bæk (Denmark)	1985	Acetylene inhibition	Sediment	1.70	Christensen and Sørensen (1988)

aquatic ecosystems with monthly sampling have been undertaken in 10 coastal systems, 5 estuaries, 9 lakes and 5 rivers (Table 1), but no annual data are available for oceans. Estuaries were considered different from coastal ecosystems when freshwater inputs were suspected to occur. Single data in the season of highest water temperature have been collected from 13 studies of oceans, 25 coastal ecosystems, 24 estuaries, 21 lakes and 24 rivers (Table 2). In the ocean, stations were generally sampled during June–September cruises.

Methods of denitrification measurement: advantages and disadvantages

Different methods for measuring denitrification have been developed: the acetylene inhibition technique (Sørensen 1978), the measurement of nitrate disappearance (Andersen 1977), the calculation of nitrate flux between sediment and water (Mengis et al. 1997), the nitrogen mass balance approach (Ahlgren 1967), the direct measurement of N_2 production (Seitzinger et al. 1980), the ^{15}N isotope pairing technique (IPT, Nielsen 1992), the determination of N_2/Ar ratio via Membrane Introduction Mass Spectrometry (MIMS) (Kana et al. 1998) and the whole-system scale of ^{15}N addition (Mulholland et al. 2004). In recent years, there has been considerable discussion regarding the reliability of all these techniques, because they all show advantages but also potential problems, doubtful underlying assumptions and limitations that have been dealt with in some studies (Seitzinger et al. 1993; Cornwell et al. 1999; Steingruber et al. 2001, Final Report of the Workshop on Denitrification 2004), why they will not be repeated here. However, most methodological studies have produced good agreement between the ^{15}N isotope pairing technique and other methods (Steingruber et al. 2001).

Seitzinger et al. (1993) and Lohse et al. (1996) have compared the acetylene inhibition technique with ^{15}N isotope tracer and IPT and both showed that acetylene-based DR is roughly 50% of IPT-based DR. They have also observed that DR measured with IPT (both flow-through system and batch mode assays) is lower by a factor of 2.7 than that estimated with the N_2 flux method.

However, Risgaard-Petersen et al. (1998) have observed good agreement between both methods and they attributed the before mentioned results to the longer preincubation time necessary for the N_2 flux method which can cause accumulation of nitrate and ammonia and thus an overestimation of denitrification.

Nitrogen mass balance studies have shown a good agreement with the N_2 flux method in the lakes studied (van Luijn et al. 1996; Mengis et al. 1997). Mengis et al. (1997) have also compared the application of IPT to a benthic chamber with the batch-mode assay, concluding that two methods yield similar results that correspond well with the results from mass balance calculations. Similar results have been reported by Nielsen et al. (1995), finding good agreement between the nitrogen mass balance and the IPT batch-mode assay. On the contrary, Risgaard-Petersen et al. (1999) have found a significant difference between DR measured with the IPT in the batch-mode assay in laboratory-incubated sediments and estimates from the nitrogen mass balance; IPT rates are 30% of the calculated mass balance.

We can conclude that different methods may produce significantly different results sometimes, mainly due to its own errors and limitations in DR measurement but also to environmental heterogeneity. Most techniques have been assessed via replicated incubations of unreplicated habitat (either water or sediment) samples taken from the environment and, afterwards, the measurements were extrapolated to natural environments. It still remains unclear how we can extrapolate these measurements to the natural environment (Peterson, personal communication). However, the compiled results will be considered jointly, albeit with caution. The most frequently used method for measuring denitrification in aquatic environments (see Tables 1, 2) has been the acetylene inhibition technique (36%), followed by IPT (29%), the direct measurement of N_2 production (20%), the mass balance approach (10%) and other techniques (5%).

Data analysis and meta-analysis

There is an enormous quantity of published information on DR in aquatic ecosystems; however,

Table 2 Database 2. Single data on denitrification rates measured when the water temperature was the highest of the year

Aquatic ecosystem	Name	Year	Method of analysis	Measurement site	Denitrification rate ($\mu\text{mol N m}^{-2} \text{h}^{-1}$)	Data source
Coastal ecosystem	Amazon Shelf	1996	N_2 flux/indirect calculation	Sediment	24.58	Seitzinger and Giblin (1996)
Coastal ecosystem	African Shelf	1996	N_2 flux/indirect calculation	Sediment	36.25	Seitzinger and Giblin (1996)
Coastal ecosystem	Bassholmen (Sweden)	2001	Isotope pairing	Water column	54	Sundbäck and Miles (2002)
Coastal ecosystem	Boston Harbor (USA)	1992	N_2 flux	Sediment	3.75	Nowicki et al. (1997)
Coastal ecosystem	Boston Harbor (USA)	1993	N_2 flux	Sediment	8.33	Nowicki et al. (1997)
Coastal ecosystem	Boston Harbor (USA)	1994	N_2 flux	Sediment	5.83	Nowicki et al. (1997)
Coastal ecosystem	Central and S. America shelf	1996	N_2 flux/indirect calculation	Sediment	38.75	Seitzinger and Giblin (1996)
Coastal ecosystem	Chesapeake Bay (USA)	1986	Acetylene inhibition	Sediment	17.80	Kemp et al. (1990)
Coastal ecosystem	Chesapeake Bay (USA)	1987	Acetylene inhibition	Sediment	8.80	Kemp et al. (1990)
Coastal ecosystem	Great Bay (USA)	1986	Acetylene inhibition	Sediment	16.66	Slater and Capone (1987)
Coastal ecosystem	Gulf of Maine	1982	NO_3^- fluxes	Sediment	0.05	Christensen et al. (1987)
Coastal ecosystem	Gullmar Fjord (Sweden)	2001	Isotope pairing	Sediment	6.60	Sundbäck et al. (2004)
Coastal ecosystem	Massachusetts Bay (USA)	1992	N_2 flux	Sediment	42	Nowicki et al. (1997)
Coastal ecosystem	Massachusetts Bay (USA)	1993	N_2 flux	Sediment	53	Nowicki et al. (1997)
Coastal ecosystem	Massachusetts Bay (USA)	1994	N_2 flux	Sediment	38	Nowicki et al. (1997)
Coastal ecosystem	Midbay-Narragansett Bay (USA)	1979	N_2 flux	Sediment	115	Seitzinger et al. (1984)
Coastal ecosystem	Mexican Shelf	1979	NO_3^- fluxes	Sediment	0.06	Christensen et al. (1987)
Coastal ecosystem	Narragansett Bay (USA)	1990	N_2 flux	Sediment	94	Nowicki (1994)
Coastal ecosystem	Odawa Bay (Japan)	1980	^{15}N -labelled nitrate	Sediment	37	Nishio et al. (1983)
Coastal ecosystem	Offshore-Narragansett Bay (USA)	1979	N_2 flux	Sediment	114	Seitzinger et al. (1984)
Coastal ecosystem	Caribbean Islands	1996	N_2 flux/indirect calculation	Sediment	15.83	Seitzinger and Giblin (1996)
Coastal ecosystem	Rågårdsvik (Sweden)	2000	Isotope pairing	Water column	33	Sundbäck and Miles (2002)
Coastal ecosystem	Svalbard island (Norway)	1991	Isotope pairing	Sediment	0.58	Blackburn et al. (1996)
Coastal ecosystem	Tokyo Bay (Japan)	1980	^{15}N -labelled nitrate	Sediment	33	Nishio et al. (1982)
Coastal ecosystem	West Coast (N. Zealand)	1984	Acetylene inhibition	Sediment	141	Kaspar et al. (1985)
Estuary	Young Sound (Greenland)	2002	Isotope pairing	Ice	1.87	Rysgaard and Glud (2004)
Estuary	Apalachicola Bay (Mexico)	1996	N mass balance	Sediment	143	Mortazavi et al. (2000)
Estuary	Apalachicola Bay (Mexico)	1994	N mass balance	Sediment	67.55	Mortazavi et al. (2000)
Estuary	Apalachicola Bay (Mexico)	1995	N mass balance	Sediment	100.89	Mortazavi et al. (2000)
Estuary	Corpus Christi Bay (USA)	2002	MIMS	Sediment	8	Bernot et al. (2003)
Estuary	Corpus Christi Bay (USA)	2002	Acetylene inhibition	Sediment	30	Bernot et al. (2003)
Estuary	Culvert Creek (USA)	1995	Acetylene inhibition	Sediment	25	Thompson et al. (2000)
Estuary	Four League Bay (USA)	1983	Acetylene inhibition	Sediment	74	Smith et al. (1985)
Estuary	Great Ouse (England)	1994	Acetylene inhibition	Sediment	228	Trimmer et al. (1998)
Estuary	Kysing Fjord (Denmark)	1979	Acetylene inhibition	Sediment	257	Sørensen et al. (1979)
Estuary	Lendrup Vig (Denmark)	1983	Acetylene inhibition	Sediment	213	Andersen et al. (1984)
Estuary	Mashapaquit Marsh (USA)	2005	N_2 flux	Sediment	141	Hammersley and Howes (2005)
Estuary	MERL mesocosms (USA)	1984	N_2 flux	Sediment	596	Seitzinger and Nixon (1985)
Estuary	Newport River (USA)	1992	Acetylene inhibition	Sediment	25	Thompson et al. (1995)

Table 2 continued

Aquatic ecosystem	Name	Year	Method of analysis	Measurement site	Denitrification rate ($\mu\text{mol N m}^{-2} \text{h}^{-1}$)	Data source
Estuary	Norsminde Fjord (Denmark)	1989	^{15}N -labelled nitrate isotope pairing	Sediment	412	Binnerup et al. (1992)
Estuary	Nueces River (USA)	2002	Acetylene inhibition	Sediment	27	Bernot et al. (2003)
Estuary	Nueces River (USA)	2002	MIMS	Sediment	27	Bernot et al. (2003)
Estuary	Patuxent River (UK)	1981	^{15}N -isotopic tracer	Sediment	89	Jenkins and Kemp (1984)
Estuary	Pawcatuck River (USA)	1991	N_2 flux	Sediment	20	Nowicki (1994)
Estuary	Providence River-Narragansett Bay (USA)	1979	N_2 flux	Sediment	107	Seitzinger et al. (1984)
Estuary	Randers Fjord (Denmark)	1995	^{15}N -labelled nitrate isotope pairing	Sediment	237	Nielsen et al. (2001)
Estuary	San Francisco Bay (USA)	1981	Acetylene inhibition	Sediment	1.20	Oremland et al. (1984)
Estuary	St. Lawrence (Canada)	1997	Isotope pairing	Sediment	3.3	Wang et al. (2003)
Estuary	Tama (Japan)	1980	^{15}N -labelled nitrate	Sediment	594	Nishio et al. (1983)
Lake	Almind (Denmark)	1975	NO_3^- changes	Sediment	208.33	Andersen (1977)
Lake	Baldegg (Switzerland)	1995	^{15}N -labelled nitrate	Water	254	Mengis et al. (1997)
Lake	Bariri (Brazil)	1999	Acetylene inhibition	Water	15.89	Abe et al. (2002)
Lake	Barra Bonita (Brazil)	1999	Acetylene inhibition	Water	50.32	Abe et al. (2002)
Lake	Ernest (USA)	1986	N_2 flux	Sediment	56	Seitzinger (1988)
Lake	Frederiksborg Slot (Denmark)	1975	NO_3^- changes	Sediment	297.60	Andersen (1977)
Lake	Hampen (Denmark)	1983	Acetylene inhibition	Sediment	52	Christensen and Sørensen (1986)
Lake	Kalgaard (Denmark)	1975	NO_3^- changes	Sediment	312.48	Andersen (1977)
Lake	Kvie (Denmark)	1989	N mass balance	Sediment–water interface	57.71	Olsen and Andersen (1974)
Lake	Lacawak (USA)	1985	N_2 flux	Sediment	50	Seitzinger (1988)
Lake	Mendota (USA)	1970	^{15}N -labelled nitrate	Sediment	10	Keeney et al. (1971)
Lake	Michigan (USA)	1985	N_2 flux	Sediment	40	Gardner et al. (1987)
Lake	Naivasha (Kenya)	1982	Acetylene inhibition	Sediment	51.70	Viner (1982)
Lake	Nuldernaau (The Netherlands)	1994	N_2 flux	Sediment	154	van Luijn et al. (1996)
Lake	Nuldernaau (The Netherlands)	1992	N_2 flux	Sediment	132	van Luijn et al. (1996)
Lake	Okaro (New Zealand)	1981	Acetylene inhibition	Sediment	59.42	Downes (1991)
Lake	Ringsjön (Sweden)	1996	Isotope pairing	Sediment	30	Svensson et al. (2001)
Lake	Sonachi (Kenya)	1982	N_2 flux	Sediment	1.48	Viner (1982)
Lake	Vilhelmsborg (Denmark)	1990	Acetylene inhibition	Sediment	2	Seitzinger et al. (1993)
Lake	Vilhelmsborg (Denmark)	1990	N_2 flux method	Sediment	260	Seitzinger et al. (1993)
Lake	Zug (Switzerland)	1995	^{15}N -labelled nitrate	Water	45.80	Mengis et al. (1997)
Ocean	Arctic Sea (Norway)	1991	Isotope pairing	Sediment	5.83	Blackburn et al. (1996)
Ocean	Baltic Sea (Gulf of Finland)	1994	Isotope pairing	Sediment	16.25	Tuominen et al. (1998)
Ocean	Baltic Sea (Gulf of Finland)	1995	Isotope pairing	Sediment	22.08	Tuominen et al. (1998)

Table 2 continued

Aquatic ecosystem	Name	Year	Method of analysis	Measurement site	Denitrification rate ($\mu\text{mol N m}^{-2} \text{h}^{-1}$)	Data source
Ocean	Baltic Sea (Gulf of Finland)	1996	Isotope pairing	Sediment	26.66	Tuominen et al. (1998)
Ocean	Baltic Sea (Gulf of Finland)	1997	Isotope pairing	Sediment	15.41	Tuominen et al. (1998)
Ocean	Bering Sea	1980	Isotope pairing	Sediment	1.80	Haines et al. (1981)
Ocean	Bothnian Sea	1996	Isotope pairing	Sediment	12.5	Tuominen et al. (1998)
Ocean	East China Sea	1984	Acetylene inhibition	Sediment	1.40	Aller et al. (1985)
Ocean	North Sea	1994	Isotope pairing	Sediment	13.21	Lohse et al. (1996)
Ocean	Northern Baltic	1996	Isotope pairing	Sediment	12.50	Tuominen et al. (1998)
Ocean	Pacific Ocean	1985	NO ₃ changes	Sediment	5.40	Christensen et al. (1987)
Ocean	Wadden Sea (Germany)	1993	Isotope pairing	Sediment	6	Jensen et al. (1996)
Ocean	Wadden Sea (Germany)	1994	Isotope pairing	Sediment	60	Jensen et al. (1996)
River	Big Ditch (USA)	2002	Acetylene inhibition	Sediment	714	Schaller et al. (2004)
River	Childs River (USA)	1992	N ₂ flux	Sediment	430	LaMontagne et al. (2002)
River	Childs River (USA)	1993	N ₂ flux	Sediment	300	LaMontagne et al. (2002)
River	Childs River (USA)	1994	N ₂ flux	Sediment	190	LaMontagne et al. (2002)
River	Delaware (USA)	1984	Acetylene inhibition	Sediment	345	Seitzinger (1988)
River	Embaras (USA)	2001	Acetylene inhibition	Sediment	970	Royer et al. (2004)
River	Garonne (France)	1999	Acetylene inhibition	Sediment	577	Teisser and Torre (2002)
River	Gelbæk (Denmark)	1987	Acetylene inhibition	Sediment	404	Christensen et al. (1990)
River	Green Creek (Antarctica)	1999	Acetylene inhibition	Sediment	15	Gooseff et al. (2004)
River	Iroquois River (USA)	1999	Whole-stream ¹⁵ N exp	Water	3400	Laursen and Seitzinger (2002)
River	Kaskaskia (USA)	2001	MIMS	Sediment	1069	Royer et al. (2004)
River	Little Lost Man Creek (USA)	1984	Acetylene inhibition	Sediment	0	Duff et al. (1984)
River	Millstone (USA)	2001	MIMS	Water	2500	Laursen and Seitzinger (2002)
River	Ouse (England)	1995	Acetylene inhibition	Sediment	700	Pattinson et al. (1998)
River	Potomac (USA)	1985	N ₂ flux	Sediment	235	Seitzinger (1988)
River	Raan (Sweden)	1988	Acetylene inhibition	Sediment	3277	Jansson et al. (1994)
River	Rabisbaek	1987	Acetylene inhibition	Sediment	166	Christensen and Sørensen (1988)
River	Sangamon (USA)	2001	Acetylene inhibition	Sediment	969	Royer et al. (2004)
River	San Francisco Creek (USA)	1984	Acetylene inhibition	Sediment	27	Duff et al. (1984)
River	Sugar Creek (USA)	2000	MIMS	Water	270	Laursen and Seitzinger (2002)
River	Sugar Creek (USA)	2001	Whole-stream ¹⁵ N exp	Sediment	120	Böhlke et al. (2004)
River	Swale River (England)	1995	Acetylene inhibition	Sediment	250	Pattinson et al. (1998)
River	Walker Branch (USA)	2002	Whole-stream ¹⁵ N exp	Sediment	12	Mulholland et al. (2004)
River	Wiske (England)	1995	Acetylene inhibition	Sediment	850	Pattinson et al. (1998)

drawbacks of DR data for cross-system analysis sensu Hedges and Gurevitch (1999) are missing data, data poorly reported and the lack of statistical power or incomplete statistics in some data. Even more common are the failures to report standard deviations, means or correlation coefficients and missing data on the control variables studied, especially on a yearly basis. Also, teasing data out of published papers is not an easy task because peer-reviewed paper do not usually supply all amount of data necessary to be useful for others. Therefore, although it would have been desirable to use other possible options when available data are poorly reported (Gurevitch et al. 2001), such as randomization methods, we have rather selected the most complete studies, from which we have been able to extract a high number of categorical controlling factors, studies that reported basic statistical parameters and studies made over an annual basis or over the most favourable period, i.e. that of highest water temperature to diminish the temperature control of the biogeochemical process as much as possible.

Statistical analysis has been performed using STATISTICA 6.1 (Statsoft Inc. 1997). Due to the lack of a Gaussian distribution and homogeneity of variances for most of the variables of the data sets, non-parametric tests have been undertaken to compare data sets. A Kruskal–Wallis test has been used to assess differences among DR in the five categorized aquatic ecosystems, whereas a Mann–Whitney test has been employed to compare DR data sets with different levels (high or low) of a given controlling factor. These tests have been applied at the yearly- and during the water highest temperature of the year scale. We have converted N release rates from $\mu\text{mol N m}^{-2} \text{h}^{-1}$ into $\text{mol N m}^{-2} \text{y}^{-1}$ (by using the integration algorithm) to diminish the existing variability in denitrification measurements (CV 28–20%). The Kavvas and Delleur (1975) method to visualize periodicities in a time series was used with the annual data set to study seasonality in different aquatic ecosystems and those environments with high and low total phosphorus content. Seasonal dynamics related to other controlling factors could not be attempted because of paucity of data. We calculated a mean over a constant time unit (year) and formed a new series as the difference

between the original series and the mean. For any denitrification time series, a scaled difference, DN_t , was calculated to represent the observed rate, X_t , on day t

$$\text{DN}_t = 100 * [(X_t - X)/X] \quad (1)$$

where X is the yearly mean calculated for that time series. This procedure was repeated for each time series in all aquatic ecosystems from the annual database (Table 1). Furthermore, to compare the strength of seasonality among ecosystem types the max:min ratios of DR were calculated and averaged for each type of aquatic ecosystem.

At the highest water temperature of the year, linear correlation was employed to study the relationship between DR and either latitude or pore-water nitrate concentration (Steingruber et al. 2001), but we had to use nitrate concentration in overlying waters as a surrogate of the latter because data on pore-water nitrate were very scarce. Furthermore, a multiple regression model of DR and controlling factors for which quantitative data existed was attempted to ascertain the percentage of variation in DR that could be explained by each variable previously recognized to control denitrification rate (nitrate, total phosphorus, sedimentary organic carbon, dissolved oxygen; Golterman 2004). No linear or multiple regression models were attempted at the annual scale because of paucity of field data.

Controlling factors

Factors selected that likely affect DR are the following: concentrations of oxygen, nitrate, interstitially-dissolved organic carbon and total phosphorus, light regime and plant occurrence. Denitrification data gathered both during highest water temperature and during the entire season was divided using two levels of each controlling factor, depending on the high or low content of the factor. Oxygen must be lower than 0.5 mg l^{-1} for denitrification to occur although below that concentration it may have been masked by overlapping with nitrification (Golterman 2004). We have therefore, considered the concentration of

0.5 mg l⁻¹ to be the boundary between low and high oxygen content. Fifty $\mu\text{mol NO}_3^-$ has been used as the boundary between denitrification data of high- and low nitrate sets, assuming that denitrification follows Michaelis–Menten kinetics (Golterman 2004) and that the half saturation constant is approximately 50 $\mu\text{mol NO}_3^-$ (Seitzinger 1988; Oremland et al. 1984). The boundaries for high interstitially-dissolved organic carbon content and high total phosphorus content are 10 mg C g⁻¹ and 0.03 mg P l⁻¹, respectively. Those values are usually considered as boundaries between oligo- and eutrophic systems in freshwater environments (Mackereth 1966; OCDE 1982). Light/dark cycles and the occurrence of submerged vegetation have further been used to divide denitrification data as both light/dark cycles (Laursen and Seitzinger 2004) and the presence/absence of aquatic vegetation (Risgaard-Petersen 2003) is affecting DR in freshwaters and marine sediments.

Results

Annual DR in aquatic environments was quite different among ecosystem types, as judged by a Kruskal–Wallis test (Fig. 1; $P = 0.0004$). DR varied widely being highest in lakes (0.10–3.72 mol N m⁻² y⁻¹) and rivers (1.10–3.54 mol N m⁻² y⁻¹) compared to coastal ecosystems

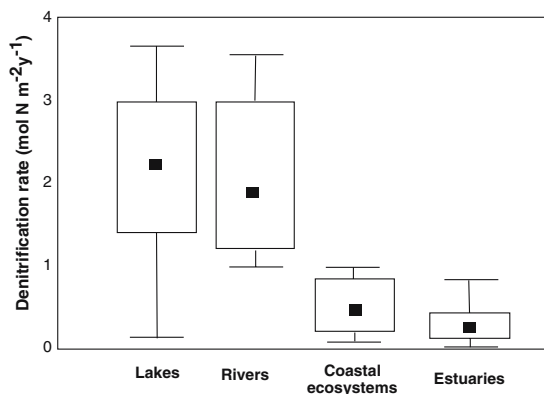


Fig. 1 Box-whisker plot on annual denitrification rates recorded in different aquatic ecosystems. No annual data are available for ocean sites. Boxes include 25–75% of overall data, black squares are median values and whiskers comprise the whole range of data

(0.09–1.10 mol N m⁻² y⁻¹) and estuaries (0.02–0.84 mol N m⁻² y⁻¹). On the average, the lowest DR was found in estuaries (mean \pm SD: 0.32 ± 0.31 mol N m⁻² y⁻¹), followed by coastal ecosystems, lakes and rivers (0.53 ± 0.36 , 2.03 ± 1.19 , 2.11 ± 1.07 mol N m⁻² y⁻¹, respectively). Such a DR increase from estuaries to rivers was considerable because medians were statistically different among ecosystem types (Mann–Whitney tests, $P < 0.01$).

DR measured during the month of highest water temperatures also showed statistical differences among ecosystem types (Fig. 2; Kruskal–Wallis test, $P = 0.00001$). On the average, highest DR was found in rivers and lakes (289 ± 252 and 126 ± 105 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, respectively), followed by estuaries, coastal ecosystems and the ocean (109 ± 177 , 63 ± 73 and 15 ± 15 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, respectively). The oceanic areas were lower than those recorded in the remaining aquatic ecosystems ($P < 0.001$, Kruskal–Wallis test). Such a DR increase from oceans and coastal ecosystems to lakes and rivers was considerable because medians were statistically different between ecosystem types (Mann–Whitney tests, $P < 0.04$).

Box-whisker plots of annual DR related to different controlling factors showed that high concentrations of nitrate and interstitially-dissolved organic carbon, stimulated DR as well as low concentrations of phosphorus and oxygen; a

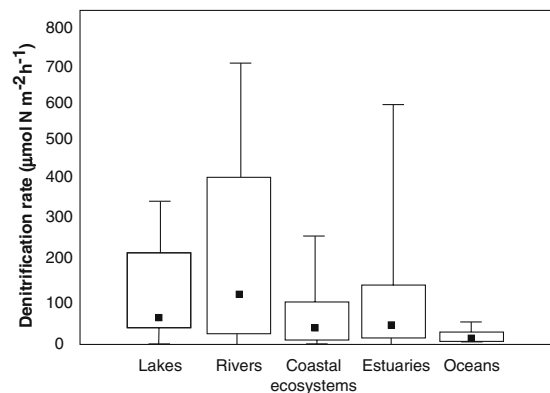
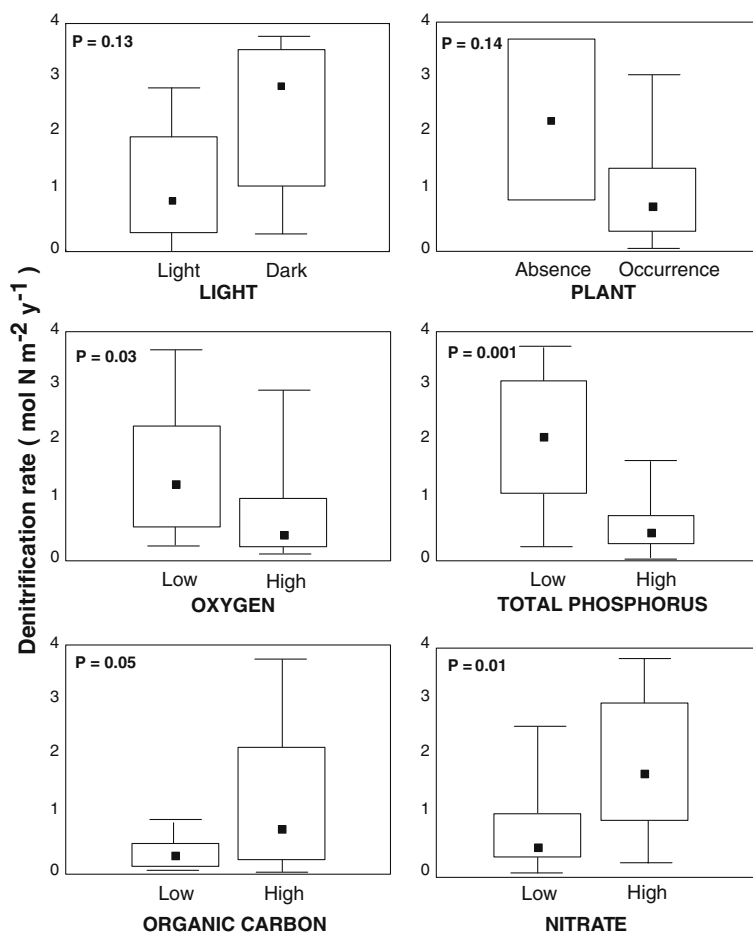


Fig. 2 Box-whisker plots of denitrification rates from single data measured during the month of highest temperature of the year in aquatic ecosystems. All plots as in Fig. 1

result supported by the statistical differences observed ($P < 0.05$, Mann–Whitney tests; Fig. 3). DR measured in ecosystems with high nitrate and high interstitially-dissolved organic carbon concentrations ranged between 0.16–3.72 mol N m⁻² y⁻¹ and 0.02–3.72 mol N m⁻² y⁻¹, respectively. DR in systems with low phosphorus and oxygen concentrations ranged between 0.18–3.72 and 0.02–3.72 mol N m⁻² y⁻¹, respectively. The variability of DR was much greater in systems with high nitrate and interstitially-dissolved organic carbon contents compared to systems with low concentrations of nitrate and interstitially-dissolved organic carbon (SD: 1.18 vs. 0.81 and 1.14 vs. 0.05, respectively). Similar results were found in systems with lower phosphorus and oxygen content (1.28 vs. 0.28 and 1.10 vs. 0.85, respectively).

The most pronounced seasonal variation in DR was found in lakes, followed by rivers, coastal ecosystems and estuaries (Fig. 4), the average max-to-min DR ratios being 2.10, 1.67, 1.63 and 1.58, respectively. Most systems seemed to experience highest denitrification rates during summer and the summertime seemed thus to be the most favourable period for the denitrification process. Mean DR values increased significantly ($P < 0.001$, Mann–Whitney U test) during summertime in all aquatic ecosystems (except for coastal ecosystems, although an increased in mean values (7–19%) could be observed). A clear decrease in activity was observed at the onset of autumn in all environments. The Kavvas and Delleur method (1975) corroborated those analyses (Fig. 5), highlighting the strong seasonality found in all aquatic ecosystems.

Fig. 3 Box-whisker plots of annual denitrification rates and qualitative controlling factors in aquatic ecosystems. Boundaries splitting DR data sets for each factor are the following: dissolved oxygen >0.5 mg l⁻¹, total phosphorus >0.03 mg l⁻¹, interstitially-dissolved organic carbon >10 mg g⁻¹, nitrate >50 μmol. Box-whisker plots as in Fig. 1. Probabilities of Mann–Whitney tests comparing data sets for each factor are also shown



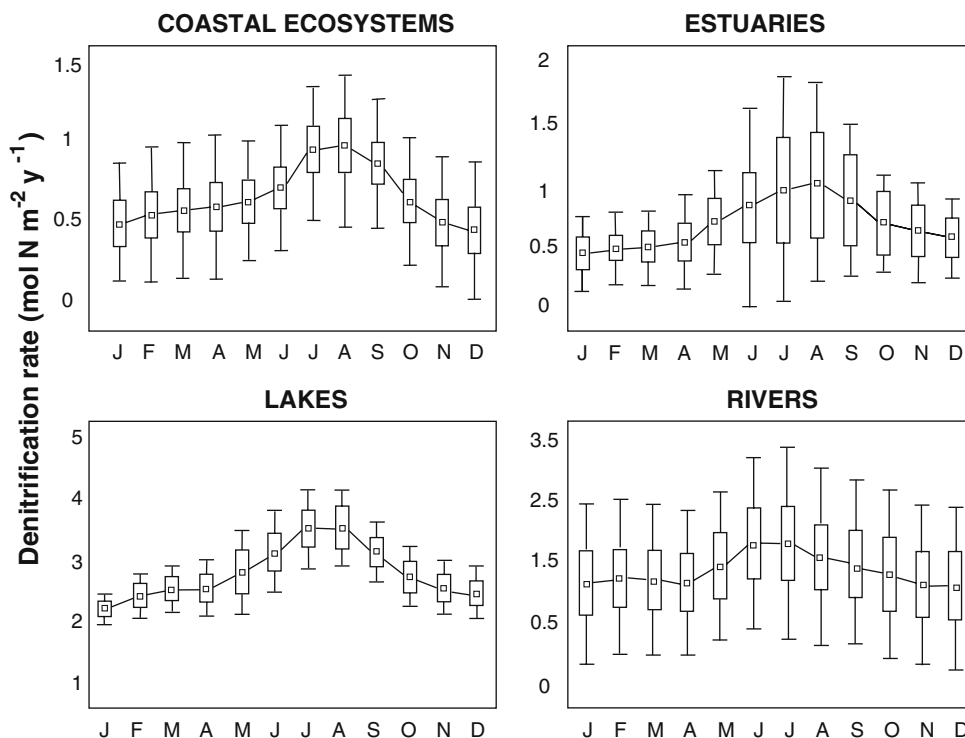


Fig. 4 Seasonal denitrification rates in aquatic ecosystems. Box-whisker plots as in Fig. 1, except that open squares represent average values

Box-whisker plots of DR in the month with highest water temperatures showed statistically significant results when related to controlling factors when separating data of high and low nitrate, interstitially-dissolved organic carbon and oxygen concentrations (Fig. 6, $P < 0.05$, Mann-Whitney test). The remaining factors, including the occurrence of micro- and macroplants and total phosphorus, did not show statistically significant differences in DR.

Most studies on DR were located at 25–60°N; very few data from both tropical- (but see Abe et al. 2002; Esteves et al. 2001; Viner 1982; Gianotti and Santos 1997) and polar regions (see Blackburn et al. 1996; Rysgaard and Glud 2004; Rysgaard et al. 2004; Gooseff et al. 2004) were reported. Most data came from Europe (47%) or from America (35%), but there were few from Asia (10%) and Oceania (8%) and very few from Africa and Antarctica. Therefore, most denitrification measurements were confined to temperate zones in the Northern Hemisphere and many areas of the earth lacked aquatic denitrification

data. There was no statistically significant relationship between latitude and DR ($P < 0.05$) in the highest water temperature season (Fig. 7, upper panel). We found a linear relationship between nitrate concentration in overlying waters and DR (Fig. 7 middle panel, $r^2=0.86$, $n = 60$, $P = 0.001$) over the range of 1–970 $\mu\text{mol NO}_3^-$.

A multiple regression model of denitrification rates vs. nitrate, dissolved oxygen, interstitially-dissolved organic carbon and phosphorus content was established for 26 aquatic ecosystems where data were available (Table 3). Only nitrate (μmol) and dissolved oxygen (mg l^{-1}) significantly explained DR ($\mu\text{mol N m}^{-2} \text{h}^{-1}$) variability (Table 3), nitrate being the highest explaining factor of denitrification variability (70%). The multiple regression equation was

$$\text{DR} = -2.17(\pm 31.08) + 0.97(\pm 0.19) * [\text{NO}_3^-] - 1.09(\pm 7.10) * [\text{O}_2].$$

That equation explained 76% of observed variability of DR (Fig. 7 lower panel).

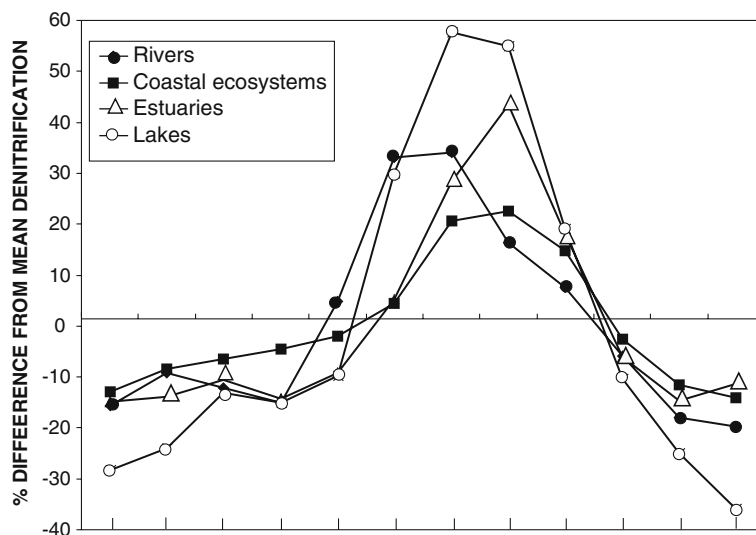


Fig. 5 Seasonality of denitrification in aquatic ecosystems, as shown by the Kavvas and Delleur (1975) approach (see text). All time series for each ecosystem-year have been scaled by expressing denitrification as the percent difference from the annual mean. All ecosystems are located in the Northern Hemisphere since no data sets with that

temporal resolution are available for the Southern Hemisphere. The horizontal line represents the mean. The area above that line indicates denitrification greater than the mean, whereas denitrification lower than the mean falls below the line

Discussion

Variability of denitrification rates in aquatic ecosystems

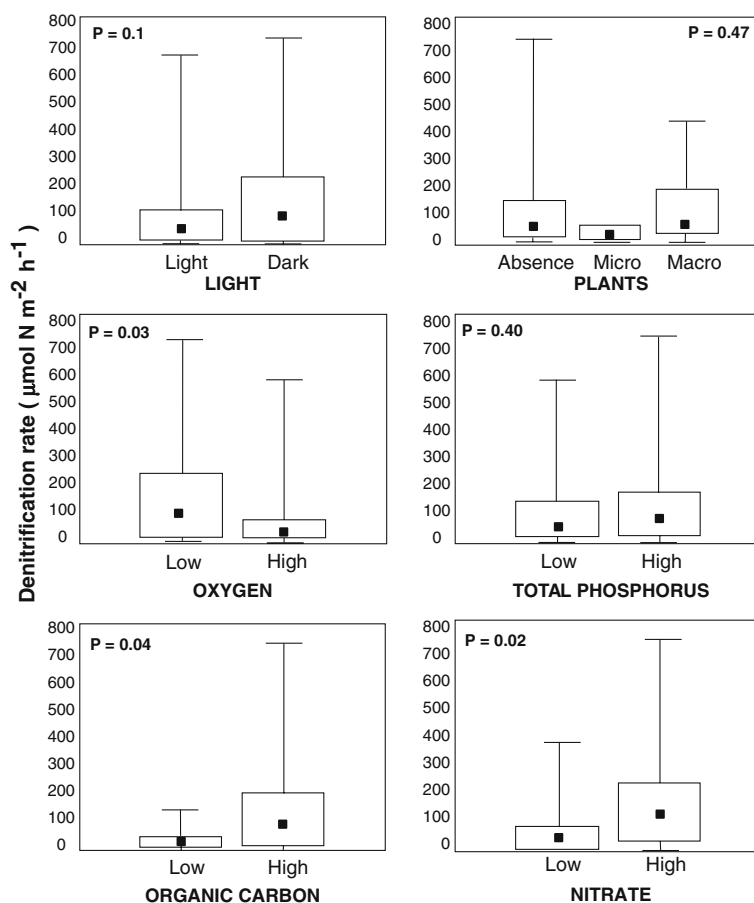
Agricultural and aquatic systems have denitrification rates of the same order of magnitude. However, denitrification rates in forest and pasture are much lower. In terrestrial systems, highest rates occur in irrigated, nitrogen fertilized soils (Barton et al. 1999). The range of DR in agricultural soils ($0.001\text{--}1.46 \text{ mol N m}^{-2} \text{ y}^{-1}$) compares well with that of estuarine and coastal waters ($0.02\text{--}1.10 \text{ mol N m}^{-2} \text{ y}^{-1}$), but its highest rate is still lower than those observed for lakes and rivers ($3.52\text{--}3.72 \text{ mol N m}^{-2} \text{ y}^{-1}$).

Worldwide, rivers showed the highest denitrification rates up to $3.72 \text{ mol N m}^{-2} \text{ y}^{-1}$ or $700 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ (Figs. 1, 2). The activity within the rivers also showed the highest variability, probably reflecting strong fluctuations of stream discharge (Saunders and Kalff 2001). The highest denitrification rates were found in some agricultural rivers of the midwestern USA; the Sangamon, Embarras, Kaskaskia and Big Ditch ($715\text{--}1,128 \mu\text{mol N m}^{-2} \text{ h}^{-1}$, Royer et al. 2004;

Schaller et al. 2004). Further, estimates of three small rivers in northeast Illinois, demonstrated rates of up to $8,400 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ in Iroquois river (Laursen and Seitzinger 2002), which are the highest reported rates. Denitrification rates within these ranges suggested that might be typical of low gradient, agricultural streams and particularly of those in which discharge and nitrate loads are closely linked to precipitation and tile drainage (Royer et al. 2004; Jansson et al. 1994; Schaller et al. 2004).

As a mean, coastal ecosystems and lakes demonstrated highest seasonal variability in denitrification rates and also showed highest activities during summer (Figs. 4, 5) a feature that was already pointed out by Kemp et al. (1990) and Windolf et al. (1996). This seasonal pattern may reflect influence of nitrate availability, which was usually higher after springtime peak flows (Kana et al. 1998), and the temperature increase in summer (Seitzinger 1988; Golterman 2004). Therefore, high nitrate concentrations, low redox conditions and reduced oxygen concentrations in all aquatic ecosystems during summer may increase denitrification rates (Figs. 4, 5). However, there are also other systems characterized

Fig. 6 Box-whisker plots of denitrification rates from single data during the month of highest water temperature of the year and qualitative controlling factors in aquatic ecosystems. Criteria as in Fig. 3. Probabilities of Mann–Whitney tests comparing data sets for each factor are also shown



by reduced midsummer activities, in particular marine systems, where ambient nitrate concentrations are very low during summer (Seitzinger et al. 1984; Jenkins and Kemp 1984; Kemp et al. 1990; Nielsen et al. 1995; Sørensen et al. 1979).

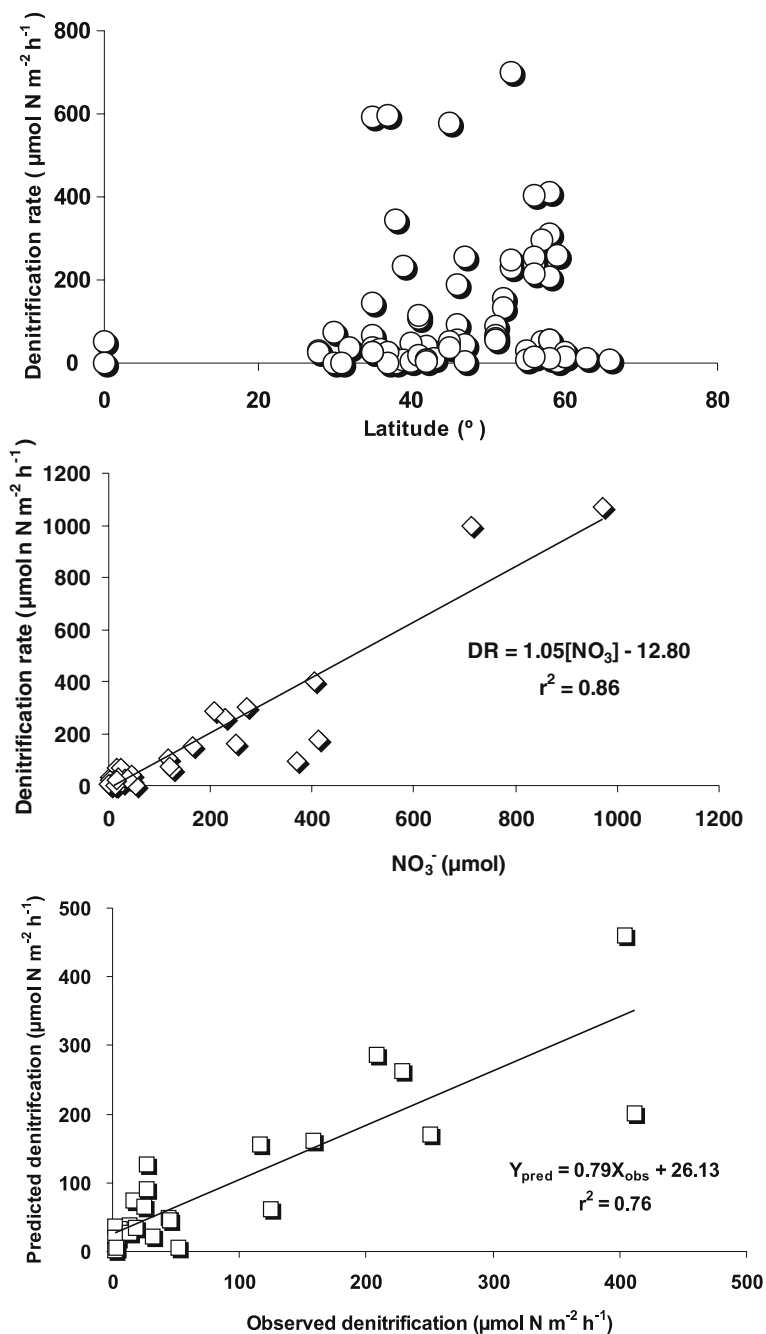
Environmental controls of denitrification

Availability of nitrate, interstitially-dissolved organic carbon supply, concentrations of oxygen and phosphorus all influenced the patterns of DR at both temporal scales tested in this study (Figs. 3, 6). This is consistent with previous reports (i.e. Koike and Hattori 1978; Oremland et al. 1984; Thompson et al. 1995; Nowicki et al. 1997; García-Ruiz et al. 1998; Nielsen et al. 2001).

Nitrate availability at the physical place for denitrification may be driven by water column concentration, by sediment nitrification and through groundwaters. (Seitzinger 1988, Jenkins

and Kemp 1984; Nielsen et al. 1995; Cornwell et al. 1999). There are not many studies of the latter although it may be very important for areas where groundwater is contaminated with nitrate from agricultural or industrial sources. Nitrate produced in the sediment via nitrification of ammonia released from benthic oxidation of organic matter appears to be the major substrate source for denitrification in most aquatic sediments (Seitzinger 1988). However Christensen et al. (1990) pointed that for systems with significant nitrate concentration in the overlying water, this statement could be dubious. When water column nitrate is the main nitrate source, Christensen et al. (1990) have reported that DR is inversely proportional to the thickness of the oxic surface layer, as nitrate has to diffuse through this layer, and proportional to the nitrate concentration in the overlying water. Moreover, Kana et al. (1998) suggested that the in situ denitrifying

Fig. 7 Relationships between denitrification rates ($\mu\text{mol N m}^{-2} \text{h}^{-1}$) measured during the month of highest water temperature of the year in aquatic ecosystems and either geographical latitude (upper panel) or nitrate concentration in the overlying water (middle panel). Measured denitrification rate vs. predicted rate according to the multiple regression model (see text) for nitrate- and oxygen content in 26 aquatic ecosystems where data are recorded when water temperature was the highest (lower panel)



bacteria respond rapidly to increases in nitrate concentration in the overlying water. In this study, the data analysis demonstrated a positive linear relationship between DR and the nitrate concentration in the overlying water (range of 1–970 $\mu\text{mol NO}_3^-$, Fig. 7 middle panel, Table 3) likewise many site-specific studies (Kana et al.

1998; Nielsen et al. 1995; Pelegrí et al. 1994; Rysgaard et al. 1995; García-Ruiz et al. 1998; El-Harb and Golterman 1990; Royer et al. 2004). However, there are still uncertainties on the kinetics of the process at very high (>970 μmol) nitrate concentrations (Seitzinger 1994), where either saturation by nitrate or limitation by other

Table 3 Multiple regression model of denitrification rate vs. nitrate, dissolved oxygen, sedimentary organic carbon and phosphorus content. r^2 expresses the proportion of variation in denitrification rate that can be accounted for by each variable. $n = 26$

	P	r^2
Nitrate content (μmol)	0.001	0.70
Oxygen content (mg l^{-1})	0.05	0.18
Organic carbon content (mg g^{-1})	0.33	0.05
Phosphorus content (mg l^{-1})	0.42	0.07

controlling factors (e.g. organic carbon; Andersen 1985) might be occurring.

Environmental controls are often different for coupled nitrification/denitrification than for denitrification supported by the overlying water NO_3^- (Cornwell et al. 1999), as well as the before mentioned importance of the thickness of the oxic zone. This fact helps explain the small number of statistically significant variables in multiple regression analysis (Table 3), where roughly one fifth of overall variability of DR remains unexplained.

The reducing power of organic carbon compounds is a very important factor controlling the activity of the majority of denitrifying bacteria and the importance of this substrate was also reflected in denitrification activities across the ecosystems (Figs. 3, 6). The carbon supply stimulates denitrification activity directly by supplying the necessary substrate for growth and indirectly as the oxygen consumption is increased by the supply of carbon, thereby decreasing the thickness of the oxic zone (Chalamet 1985; Goering 1985; Seitzinger 1988). There are several reports within the literature supporting those finding. Mitchell and Baldwin (1999) demonstrated a very strong increase in DR when organic carbon was added to the sediments of Lake Hume. Further, Duff et al. (1984) suggested a supply of nitrate to a pristine stream did not stimulate denitrification due to the low organic carbon content.

The importance of the oxygen concentration in the overlying water on denitrification has been examined both experimentally (Andersen 1985; Rysgaard et al. 1994) and at the whole ecosystem level (Kemp et al. 1990). Analysis of the effect of oxygen concentration in this study indicated that in both freshwater and marine systems, oxygen

concentrations lower than 0.5 mg l^{-1} enhance denitrification rates in the water or sediment (Fig. 3, 6). Andersen 1977 showed that anaerobic water conditions resulted in a high rate of denitrification in six Danish lakes compared with conditions with oxygenated water overlying in the sediment. With oxygen present, the uppermost sediment will be maintained at a high redox level as denitrification will occur below this oxidized zone, a longer diffusion pathway for the nitrate will result in lower denitrification rates.

Low concentrations of total phosphorus were found to enhance DR at the yearly scale (Fig. 3), but not during the period with high water temperature (Fig. 6). A low N:P ratio was found in eutrophic systems, i.e. sites with high TP, might result in an imbalance of the N and P supply to bacterial denitrification metabolism (Knowles 1982). Further a low N:P ratio may lead to a higher competition for nitrogen from i.e. benthic micro algae.

There are only a few studies on the effect of temperature on DR. Most data show increasing rates with increasing temperature (Nowicki 1994; Cavari and Phelps 1977; Chalamet 1985; Sørensen et al. 1979; van Luijn et al. 1999; Seitzinger 1988). However, an inverse relationship between temperature and denitrification has been found in Danish coastal sediments (Sørensen et al. 1979) and in the mesohaline region of Chesapeake Bay (Kemp et al. 1990). In this study, we did not see any latitudinal effect, which is a proxy of the water temperature effect, on denitrification rates (Fig. 7 upper panel). As factors such as nitrification rate, oxygen content and external nutrient loading may also be changing with changing temperatures (Andersen et al. 1977; Seitzinger and Nixon 1985; Nowicki 1994; Seitzinger 1988), it is difficult to isolate the effect of temperature on DR worldwide. The poor relationship between latitude and denitrification is therefore not surprising.

Factors such as the light regime and the occurrence of submerged plants have not affected DR at those levels of analysis, despite some studies reporting true effects of those factors on DR (Andersen et al. 1984; Koike and Hattori 1978; Risgaard-Petersen et al. 1994; Caffrey and Kemp 1992; van Luijn et al. 1996). Its effects are obviously site-specific (Seitzinger 1988; Christensen

and Sørensen 1986; Nielsen et al. 1990; Middelburg et al. 1996), and hence can account for the differences observed.

Conclusions

Discrepancies in DR and its controlling factors between site-specific studies and our study may result from environmental variability at two scales of observation that are often confounded: the habitat and the ecosystem level. Very few spatial data on DR from a given ecosystem are available; i.e. most ecosystems where DR have been measured have certainly been undersampled and so those rates are hardly representative of the whole rate. Those by Hopkinson et al. (2001) for the continental shelf of Eastern Massachusetts, based upon mass balance calculations, suggest ranges of almost an order of magnitude ($0.3\text{--}2.3 \text{ mmol N m}^{-2} \text{ d}^{-1}$) at the ocean mesoscale. However, it is likely that such variability might increase in other aquatic ecosystems with higher environmental variability and sharpest gradients like many wetlands (Groffman 1994). Future studies on denitrification in aquatic environments should address the topic of spatial variability more thoroughly using the approach of landscape ecology (Turner et al. 2001). Novel, much more rapid- and cheaper methodologies must be implemented and cross-comparisons of existing techniques at selected sites must be undertaken to achieve this goal (see Woods-Hole Workshop recommendations; <http://www.whrc.org/nitrogen/>).

Nowadays, there is useful information about denitrification activity and its controlling variables across different ecosystems, which has resulted in a more complex picture than earlier reviews could suggest. However, comparable data on whole-system (i.e. aquatic or terrestrial) studies of denitrification are still lacking. Spatial heterogeneity has also been a neglected topic when addressing denitrification issues. Therefore, an integrated, holistic view combining multiple approaches across systems with recently updated techniques and at different (albeit simultaneously studied) spatial and temporal scales, would broaden our current knowledge of the denitrification process. Such knowledge will increase our

understanding of the anthropogenic altered functioning of aquatic and terrestrial ecosystems, providing more accurate and sound estimates of the global N flux.

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