

Influence of hydrological connectivity of riverine wetlands on nitrogen removal via denitrification

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Abstract Wetland ecosystems in agricultural areas often become progressively more isolated from main water bodies. Stagnation favors the accumulation of organic matter as the supply of electron acceptors with water renewal is limited. In this context it is expected that nitrogen recycling prevails over nitrogen dissipation. To test this hypothesis, denitrification rates, fluxes of dissolved oxygen (SOD), inorganic carbon (DIC) and nitrogen and sediment features were measured in winter and summer 2007 on 22 shallow riverine wetlands in the Po River Plain (Northern Italy). Fluxes were determined from incubations of intact cores by measurement of concentration changes or isotope pairing in the case of denitrification. Sampled sites were eutrophic to hypertrophic; 10 were connected and 12 were isolated from the adjacent rivers, resulting in large differences in nitrate concentrations in the water column (from <5 to $1,133 \mu\text{M}$). Benthic metabolism and denitrification rates were investigated by two overarching factors: season and hydrological

connectivity. SOD and DIC fluxes resulted in respiratory quotients greater than one at most sampling sites. Sediment respiration was coupled to both ammonium efflux, which increased from winter to summer, and nitrate consumption, with higher rates in river-connected wetlands. Denitrification rates measured in river-connected wetlands ($35\text{--}1,888 \mu\text{mol N m}^{-2} \text{h}^{-1}$) were up to two orders of magnitude higher than rates measured in isolated wetlands ($2\text{--}231 \mu\text{mol N m}^{-2} \text{h}^{-1}$), suggesting a strong regulation of the process by nitrate availability. These rates were also significantly higher in summer ($9\text{--}1,888 \mu\text{mol N m}^{-2} \text{h}^{-1}$) than in winter ($2\text{--}365 \mu\text{mol N m}^{-2} \text{h}^{-1}$). Denitrification supported by water column nitrate (D_W) accounted for 60–100% of total denitrification (D_{tot}); denitrification coupled to nitrification (D_N) was probably controlled by limited oxygen availability within sediments. Denitrification efficiency, calculated as the ratio between N removal via denitrification and N regeneration, and the relative role of denitrification for organic matter oxidation, were high in connected wetlands but not in isolated sites. This study confirms the importance of restoring hydraulic connectivity of riverine wetlands for the maintenance of important biogeochemical functions such as nitrogen removal via denitrification.

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Introduction

Coastal eutrophication and dystrophy have become common globally as nutrient inputs from rivers, particularly nitrogen loads, result in profound modification of coastal biogeochemistry (Nixon 1995; Smith et al. 2006; Diaz and Rosenberg 2008). Thus, both the scientific and environmental management communities interested in nutrient loadings have increased focus on those inland areas where nutrients are generated and on nutrient transport and transformations in freshwater bodies (Schaller et al. 2004; Arango et al. 2006; Boyer et al. 2006). In northern Italy nitrate contamination of surface and groundwater is particularly relevant in the Po River Plain, and the potential for removal of nitrogen has significant consequences to eutrophication of receiving coastal ecosystems. The Po River is one of the most significant freshwater source to the Mediterranean basin and affects the trophic state of the shallow northern Adriatic Sea (Provini and Binelli 2006).

Nitrogen transformations result from different biogeochemical processes including biological uptake, abiotic sedimentation, nitrification, ammonification, nitrate reduction to ammonium, denitrification and anammox, the anaerobic oxidation of ammonium (Brandes et al. 2007; Burgin and Hamilton 2007; Jetten 2008). Among these, microbially mediated denitrification and anammox are the only processes that remove inorganic nitrogen from land and water and displace it into the atmosphere (Schubert et al. 2006; Seitzinger et al. 2006). Studies reporting simultaneous measurements of denitrification and anammox in eutrophic freshwater environments are very limited but indicate that the contribution of denitrification to N_2 fluxes is generally >90% (Trimmer et al. 2003; Schubert et al. 2006; Koop-Jakobsen and Giblin 2009). Among freshwater and marine ecosystems, the most efficient nitrate removal has been measured in wetlands, largely due to the anoxic and organic wetland sediments (Bachand and Horne 2000). But not all wetlands function similarly (Brinson 1993a, b; Brinson et al. 1998; Mitsch and Gosselink 2000; Lin 2006), and there is a need to determine how different classes of wetlands contribute to nitrogen transformations and removal.

Hydrogeomorphic conditions of wetlands affect the physicochemical characteristics of water and sediments which in turn regulate denitrification and

nitrogen removal. Hydrology, climate and basin geomorphology are main components that determine establishment and maintenance of a wetland's hydrogeomorphology (Brinson 1993a, b; Mitsch and Gosselink 2000). Among hydrogeomorphic conditions, hydrological connectivity is relevant in those heavily exploited geographical areas where (1) the control of water flow in rivers impedes flooding and lateral interaction with riparian areas and (2) land use by agriculture has turned wetlands into isolated patches. In waterlogged soils, nitrate availability, autochthonous organic matter and anaerobic conditions are essential factors for the denitrification process, but they are not sufficient to establish a significant nitrogen removal efficiency. Morphology and topographic position in the landscape, together with the distribution of water sources (groundwater, surface inflow or precipitation), turnover time and hydrodynamics influence the degree to which chemicals are transported to or from wetlands (Brinson 1993a, b; Brinson et al. 1998).

Understanding how denitrification is regulated in wetlands is a difficult target as environmental features often interact dynamically, making it difficult to identify a prevailing factor, if indeed one exists. Nitrate is supplied to sediments from nitrification or by diffusion from the water column and its concentration is in general positively correlated with denitrification rates (Reinhardt et al. 2006; Sirivedhin and Gray 2006). In anaerobic wetland sediments, coupled nitrification–denitrification is limited, so the anaerobic respiration may be supported by external nitrate input—e.g. by agricultural runoff or previously nitrified sewage effluent (Verhoeven et al. 2006). Nitrate advected through the sediment from groundwater is potentially an important source, especially in areas with contaminated aquifers (Seitzinger et al. 2006). Co-occurring processes often make it difficult to separate the effect of temperature on denitrification rates alone. Increasing temperatures stimulate ammonification, nitrification and benthic respiration that could promote denitrification process (Bachand and Horne 2000; Sirivedhin and Gray 2006; Hernandez and Mitsch 2007). Simultaneously, at elevated temperatures, low oxygen solubility and high rates of aerobic respiration result in oxygen deficits and enhanced anaerobic mineralization processes, such as sulfate reduction, that may inhibit denitrification and favor DNRA, the

dissimilatory reduction of nitrate to ammonium (Brunet and Garcia-Gil 1996; Piña-Ochoa and Álvarez-Cobelas 2006). Increasing temperatures enhance also macrophytic or microphytobenthic N uptake, a process that competes with nitrification and denitrification (Sundbäck et al. 2000; Bartoli et al. 2003; Risgaard-Petersen 2004). Nitrate removal in wetlands is also regulated by the availability of organic matter and its macromolecular quality (Bastviken et al. 2005; Sirivedhin and Gray 2006). Further, nitrate removal efficiency is inversely correlated with the ratio between litter C and NO_3^- concentration in the water column, while other pathways, such as DNRA, are favored (Tiedje 1988; Ingersoll and Baker 1998; Gardner and McCarthy 2009). Water pH is rarely examined as it is usually not perceived as detrimental to denitrifying metabolism (Kadlec and Knight 1996).

The overall aim of this study was to evaluate the factors regulating denitrification and assess the importance of hydrological connectivity of riverine wetlands on nitrogen removal efficiency. We hypothesize that wetlands with limited hydrological connectivity with adjacent rivers are characterized by low nitrogen removal via denitrification. We measured nitrate removal via denitrification in conjunction with benthic respiration, inorganic nitrogen exchange and, potentially regulating, environmental factors. Denitrification was measured consistently by the isotope pairing technique (IPT) in a number of shallow riverine wetlands located in the Po River Plain (Northern Italy). The wetlands were

characterized by degree of connectivity with the adjacent water bodies (the Po, the Oglio and the Mincio Rivers). To our knowledge, no studies have reported denitrification and benthic flux measurements in shallow freshwater wetlands of the Po Plain, despite their recognized importance as nitrogen receivers and transformers. This study represents one of the most spatially extensive investigation of nitrogen cycling within the wetlands of a large watershed using the same methodological approach.

Materials and methods

Study area

All 22 sites from this study are small-sized and shallow aquatic wetlands located in the Po River Plain, within the basins of the Po, Oglio and Mincio Rivers, in the provinces of Cremona and Mantova, Northern Italy (Fig. 1; Table 1). The Po River Plain is characterised by intensive agricultural practices, livestock farming and breeding (Marchetti 1993). The studied wetlands are eutrophic to hypertrophic and are undergoing rapid infilling; most of them are included in the Oglio Nord, Oglio Sud, and Mincio Natural Parks. We sampled seven oxbow lakes or old meanders, six ponds, four freshwater marshes of which three are old peat bogs, two riverine wetlands and three shallow eutrophic lakes. All are colonized by aquatic plants (emergent, submersed, floating macrophytes and pleustophytes) and have permanent

Fig. 1 Location of the 22 sampling sites in the Po River Plain (Northern Italy)

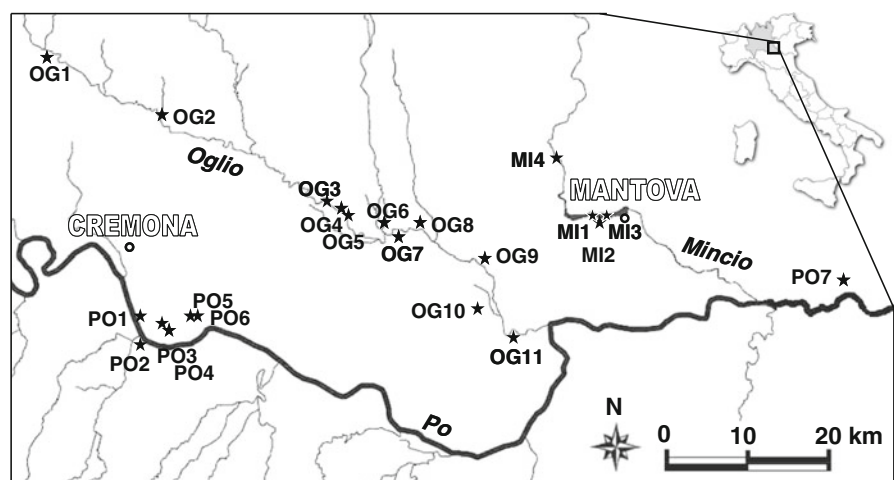


Table 1 Main features of the sampling sites; in the second column, C and I indicate connected and isolated wetlands

Location (code)	Hydrological connectivity and water source	Typology	Geographical coordinate (Gauss–Boaga)		Surface (m ²)	Mean depth (m)	Dominant macrophytes
			Latitude	Longitude			
Gabbioneta (OG1)	C Oglio River, surface inflow	Oxbow lake	5008085	1595982	80,700	0.5	<i>Phragmites australis</i> , <i>Typha latifolia</i> , <i>Carex</i> spp.
Gambara (OG2)	C Oglio and Gambara Rivers, surface inflow	Pond	5004875	1602192	4,100	0.5	<i>Typha latifolia</i> , <i>Carex</i> spp.
Gerre Gavazzi (OG3)	C Oglio River, surface inflow	Oxbow lake	5002880	1605969	38,500	1.5	<i>Phragmites australis</i>
Runate Dx (OG4)	C Oglio River, surface inflow	Oxbow lake	5002809	1606494	1,800	0.8	<i>Nuphar luteum</i>
Runate Sn (OG5)	C Oglio River, surface inflow	Oxbow lake	5002813	1606636	2,900	0.9	<i>Nuphar luteum</i>
Cà Nuove Bernini (OG6)	C Oglio River, surface inflow	Pond	5003055	1610524	6,600	1.2	<i>Ceratophyllum demersum</i> , <i>Nuphar luteum</i> , <i>Phragmites australis</i>
Lago di Mezzo1 (MI1)	C Mincio River, surface inflow	Shallow eutrophic lake	5003314	1640814	12,400	1.5	<i>Nuphar luteum</i> , <i>Nymphaea alba</i> , <i>Trapa natans</i>
Lago di Mezzo2 (MI2)	C Mincio River, surface inflow	Shallow eutrophic lake	5002980	1641376	16,800	2.5	–
Lago di Mezzo3 (MI3)	C Mincio River, surface inflow	Shallow eutrophic lake	5002742	1641882	25,000	1.5	<i>Trapa natans</i>
Goito (MI4)	C Mincio River, surface inflow	Riverine wetland	5015602	1634774	5,500	0.6	<i>Vallisneria spiralis</i>
Bine (OG7)	I Groundwater discharge	Oxbow lake	4999079	1612876	120,800	1	<i>Phragmites australis</i> , <i>Carex</i> spp.

Table 1 continued

Location (code)	Hydrological connectivity and water source	Typology	Geographical coordinate (Gauss–Boaga)		Surface (m ²)	Mean depth (m)	Dominant macrophytes
			Latitude	Longitude			
Mosio (OG8)	I Groundwater discharge	Marsh (old peat bog)	4999276	1617484	2,300	0.3	<i>Phragmites australis</i> , <i>Typha latifolia</i> , <i>Carex</i> spp.
Marcaria (OG9)	I Groundwater discharge	Marsh (old peat bog)	4997260	1621140	10,900	0.5	<i>Phragmites australis</i> , <i>Carex</i> spp.
Belforte (OG10)	I Groundwater discharge	Marsh (old peat bog)	4992786	1621023	3,900	0.3	<i>Nuphar luteum</i> , <i>Nymphaea alba</i> , <i>Phragmites australis</i> , <i>Typha latifolia</i> , <i>Carex</i> spp.
Bogina (OG11)	I Groundwater discharge	Riverine wetland	4986548	1625592	66,200	0.5	<i>Phragmites australis</i> , <i>Typha latifolia</i> , <i>Carex</i> spp.
Gerre de' Caprioli (PO1)	I Groundwater discharge	Oxbow lake	4992209	1582474	1,500	0.5	<i>Phragmites australis</i>
Lancone (PO2)	I Groundwater discharge	Oxbow lake	4986916	1582437	61,900	0.4	<i>Trapa natans</i>
Lazzaretto (PO3)	I Groundwater discharge	Pond	4991424	1584958	12,500	1.5	<i>Spirodela polyrrhiza</i> , <i>Lemna gibba</i> , <i>Wolffia arrhiza</i>
Gerre Ugolani (PO4)	I Groundwater discharge	Pond	4989539	1584997	3,900	0.8	<i>Ceratophyllum demersum</i> , <i>Myriophyllum spicatum</i> , <i>Potamogeton crispus</i>
Cà de' Gatti (PO5)	I Groundwater discharge	Pond	4991353	1589282	2,800	1.4	<i>Spirodela polyrrhiza</i> , <i>Lemna minor</i> , <i>Lemna gibba</i> , <i>Wolffia arrhiza</i>
Cascina S. Margherita (PO6)	I Groundwater discharge	Pond	4991 274	1590295	2,000	0.7	<i>Nuphar luteum</i> , <i>Ceratophyllum demersum</i>
Ostiglia (PO7)	I Groundwater discharge	Marsh	4996157	1665868	14,800	0.9	<i>Phragmites australis</i> , <i>Typha</i> spp., <i>Carex</i> spp.

standing water (depth >0.25 m). We categorized the 22 wetland sites by both Hydrogeomorphic Classification (Brinson et al. 1998; Mitsch and Gosselink 2000; Lin 2006) and Functional Classification of European Wetlands (Simpson 2002). The wetlands exhibit different degrees of hydraulic connectivity with the adjacent rivers (Table 1). Connectivity was assessed from map elevation and hydrological data (water levels) of the considered rivers (Technical Regional Maps of Lombardy Region <http://www.cartografia.regione.lombardia.it>; AdBPo 2001–2008). All wetlands were also directly surveyed in order to detect interventions/alterations, e.g. embankments, levees or berms. Ten sites are permanently connected: OG1–OG6 and MI4 have an inlet river channel while MI1–MI3 represent a river digression with unidirectional water movement. These ten wetlands are classified as “Riverine wetland with surface inflow and unidirectional flow” (Brinson et al. 1998; Mitsch and Gosselink 2000; Lin 2006) or as “Eastern Continental River Marginal Wetlands belonging to unconfined channel sub-type—ECRM2H3P” (Simpson 2002). The remaining 12 wetlands are isolated because of canal infilling, weirs and embankments. The primary water sources in the isolated wetlands are groundwater, precipitation and runoff, with the water movement due largely to vertical fluctuation. They are classified as “Isolated depression supplied by groundwater discharge and characterized by vertical fluctuation” (Brinson et al. 1998; Mitsch and Gosselink 2000; Lin 2006) or as “Eastern Continental River Marginal Wetlands belonging to separated channel sub-type—ECRM3H2P” (Simpson 2002).

Sampling program

We sampled water and sediments during winter (January–March) and summer (June–August) 2007. Intact sediments were collected at each site with transparent Plexiglas cores with different dimensions (diameter × length) for sediment characterization (5 × 30 cm, $n = 3$) and flux measurements (8 × 40 cm, $n = 4$). All cores were transferred in a box with cooled site water and brought to the laboratory within a few hours of sampling for further processing and incubation. Also, on each sampling date, approximately 100 l of water were collected and brought to the laboratory for cores maintenance and incubations.

During sampling, site water was characterized for temperature, pH, dissolved oxygen with a YSI Multi-Probe (model 556, Yellow Springs, OH, USA) and dissolved inorganic carbon and nitrogen (see later for analytical methods).

Sediment characteristics

Benthic microalgal biomass was measured as chlorophyll-*a* (Chl-*a*) concentration in the top 0.5 cm of sediment and determined spectrophotometrically after extraction with 90% acetone (Lorenzen 1967). Bulk density was determined as the ratio between wet weight and volume (typically 5 ml) of sediment. Organic matter content (OM) was measured as percentage of weight loss by ignition (450°C, 2 h) from dried sediment. Total C and N content were determined from dried sediments with a Carlo Erba elemental analyzer (CHNS-O EA 1108).

Dissolved oxygen, inorganic carbon and nutrient flux measurements

Intact cores preincubation and incubation procedures followed the standardized protocol described by Dalsgaard et al. (2000). In the laboratory, all cores were immediately submersed with the top open in a tank containing in situ aerated and well mixed water at ambient temperature. During preincubation, water was stirred inside the tubes and headwater exchange with the tank water was ensured by Teflon-coated magnetic bars suspended 5 cm above the sediment–water interface to minimize particle resuspension and driven by an external motor at 40 rpm.

The day after the sampling, the water in the tank was exchanged and the cores were incubated for flux measurements of O₂ (or sediment oxygen demand, SOD), dissolved inorganic carbon (DIC), and dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$). Incubations were started by lowering the water in the tank just below the top of the cores and by sealing the cores with floating lids provided with a sampling port. The cores were incubated in the dark at field temperature for 2–5 h with continuous stirring. Incubation time was set to keep oxygen concentration at the end to within 20% of the initial value.

Water samples (60 ml) were collected at regular time intervals using plastic syringes from the water phase (750 ml). Samples for O_2 determinations were transferred to glass vials (Exetainer, Labco, High Wycombe, UK) and Winkler reagents were added immediately (Strickland and Parsons 1972). Samples for DIC were also transferred in glass vials and immediately titrated with 0.1 N HCl (Anderson et al. 1986). Samples for NH_4^+ , NO_3^- and NO_2^- determinations were filtered through Whatman GF/F glass fiber filters, transferred to plastic vials and frozen. NH_4^+ was determined spectrophotometrically using salicylate and hypochlorite in the presence of sodium nitroprussiate (Bower and Holm-Hansen 1980). NO_3^- was determined after reduction to NO_2^- in the presence of cadmium, and NO_2^- was determined spectrophotometrically using sulphanilamide and *N*-(1-naphthyl)ethylenediamine (Golterman et al. 1978). Fluxes of O_2 , DIC, NH_4^+ , NO_2^- and NO_3^- were calculated from the changes in concentrations in the cores with time by linear regression and expressed as rate per square meter. Negative fluxes indicate flux from the water column to the sediment while positive fluxes indicate effluxes from sediment to the water column.

From dark fluxes of inorganic carbon and oxygen we calculated the respiratory quotient RQ (Dilly 2003; Hargrave et al. 2008), according to the following equation:

$$RQ = \frac{CO_2 \text{ flux}}{|O_2 \text{ flux}|}.$$

Denitrification measurements

The isotope pairing technique (Nielsen 1992) was used to measure denitrification (dark rates) on the same set of cores used for solute fluxes. Incubations for solute fluxes and denitrification were sequential and both performed the day after the sampling. The water in the tank was renewed between the two incubations, and the open cores were submersed for a couple of hours. The IPT allows for differentiation of total denitrification (D_{tot}), denitrification of nitrate diffusing to the anoxic sediment from the water column (D_w) and denitrification of nitrate produced within the sediment due to nitrification (D_N).

Methodological concerns have been raised about the IPT, mainly due to the concurrence of anammox which cannot be discriminated from denitrification as

a source of N_2 and makes invalid the assumptions on which IPT calculations are based. Recent studies showed that anammox can contribute half or more of the N_2 production in coastal shelves and deep seas (Dalsgaard et al. 2005; Trimmer et al. 2006), while it represents a minor fraction of N_2 production in eutrophic and organic-rich freshwater ecosystems (Burgin and Hamilton 2007; Koop-Jakobsen and Giblin 2009). Therefore, we adopted the IPT as we considered this method as accurate for freshwater eutrophic sites, assuming that the anammox contribution was negligible to N_2 fluxes (Risgaard-Petersen et al. 2003; Trimmer et al. 2003; Schubert et al. 2006; Koop-Jakobsen and Giblin 2009).

At the beginning of the experiment, the water in the tank was lowered just below the top of the cores; and a water subsample (5 ml) was taken from each core for NO_3^- concentration measurement. Different amounts of $^{15}NO_3^-$ from a 15 mM $Na^{15}NO_3$ solution were then added to the water column of each of the 4 replicate cores to perform a concentration series experiment (see Dalsgaard et al. 2000). Labelled nitrate was added to the cores to have a final ^{15}N atom% of at least 30%. Within 5 min of the addition of $^{15}NO_3^-$ another water sample was collected from each core to calculate the $^{14}N/^{15}N$ ratio in the NO_3^- pool; the cores were then closed with floating lids and the incubation started (3–5 h). At the end of the incubation, 5–10 ml of $ZnCl_2$ (7 M) was added to the water phase, and then sediment and water were mixed. An aliquot of the slurry was transferred to a 12.5 ml gas-tight vial; $^{14}N^{15}N$ and $^{15}N^{15}N$ abundance in N_2 were analyzed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark (Risgaard-Petersen and Rysgaard 1995). The denitrification rates were calculated according to the equations and assumptions of Nielsen (1992).

Denitrification rates were also estimated from sediment oxygen demand and concentrations of nitrate and dissolved oxygen with the model by Christensen et al. (1990):

$$D_w = F_{O_2} \cdot \alpha \cdot \left[\sqrt{\left(1 + \frac{D_{NO_3^-}}{D_{O_2}} \cdot \frac{C_{NO_3^-}}{C_{O_2}} \cdot \frac{1}{\alpha} \right)} - 1 \right]$$

where F_{O_2} is the dark sediment oxygen demand, α is the ratio between depth-specific denitrification and O_2 consumption activity (~ 0.8 , Christensen et al. 1990), $D_{NO_3^-}$ and D_{O_2} are the diffusion coefficients of

nitrate and oxygen, and $C_{\text{NO}_3^-}$ and C_{O_2} are the water column nitrate and oxygen concentrations, respectively. Modeled rates represent denitrification of nitrate diffusing into anoxic sediments from the water column.

Statistical analyses

Statistical analyses were performed with SPSS (ver. 16.0) to test for significant differences between seasons and between hydrological connectivity of wetlands. Inferential testing was done for every measured variable (temperature, sedimentary oxygen demand, dissolved inorganic carbon fluxes, respiratory quotient, ammonium fluxes, oxidized forms of inorganic nitrogen fluxes, denitrification rates, organic matter content, benthic microalgal biomass, and bulk density).

We did not use parametric statistics because of violations of assumptions of normality (Kolmogorov–Smirnov test with Lilliefors significance correction) and homogeneity of variance (Levene test). Even after the application of the Cox–Box method, for log transformations, normality was not verified, largely as a result of frequent outliers.

Therefore the non-parametric Wilcoxon paired-sample T test was used to test the differences between winter and summer distributions ($n_1 = n_2 = 22$), and the Mann–Whitney U test was used to test for differences between isolated and connected wetlands ($n_1 = 24$ and $n_2 = 20$). When the Wilcoxon test was significant, we analyzed the hydrological connectivity after separating the winter and the summer data ($n_1 = 12$ and $n_2 = 10$). We identified the correlative relationship among variables with the Spearman's rank correlation coefficient (ρ). Differences were considered non-significant if $P > 0.05$.

Results

General features of water column and surface sediments

Environmental conditions in both the water column and sediments were measured in association with rate measurements (Tables 2, 3). Water temperature medians were 10°C (range 5–13°C) in winter and 25°C (range 22–27°C) in summer. Water column pH

was alkaline in both seasons and ranged between 7.21 and 8.57. DIC concentrations ranged from 1.90 to 7.07 mM in winter and from 2.07 to 6.63 mM in summer; no significant differences were found between seasons and between connected and isolated sites. Dissolved CO_2 saturation, calculated from temperature, pH and DIC data, indicated a general supersaturation in the water column with significant differences between winter and summer periods (median 217%, range 147–464% and median 581%, range 155–4,173%, respectively; Wilcoxon T test, $P < 0.001$). In particular, for summer, we found higher percentages of CO_2 saturation values in isolated than in connected sites (Mann–Whitney U test, $P < 0.05$).

Dissolved O_2 concentrations were significantly higher in winter than in summer (Wilcoxon T test, $P < 0.01$), while percentages of dissolved O_2 saturation were similar between seasons. In winter, median values of % O_2 saturation were close to 100% for both wetland types (97% in isolated and 85% in connected sites). In summer, % O_2 saturation tended to be lower at isolated sites (median value 63%) and hypoxia established in wetlands colonized by Lemnaceae (Table 2).

Nitrate concentrations were extremely variable and were not significantly different between seasons (Wilcoxon T test, $P = 0.3$) (Table 2). In wetlands permanently connected with the river, median values of nitrate concentrations were not different between seasons (360 μM in winter and 367 μM in summer), but were significantly higher than those measured at isolated sites (Mann–Whitney U tests, $P < 0.001$). Here, nitrate contents were significantly greater in winter (median 10 μM) than in summer (median 2 μM). According to the predictions of the model proposed by Christensen et al. (1990), the $\text{NO}_3^-:\text{O}_2$ ratios of connected sites were theoretically favorable to denitrification, with similar or higher availability of nitrate compared to oxygen in bottom waters (Table 2).

Sediment OM content, Chl- a concentrations and bulk density were not statistically different between seasons (Table 3). OM content and bulk density ranges were typical of environments characterized by elevated sedimentation rates, soft and reducing sediment, and anaerobic metabolism. Sediment OM content was significantly higher and bulk density was significantly lower in isolated compared to connected

Table 2 Hydrochemical variables measured in water samples collected in winter and summer 2007 from the 22 sampling sites are reported; in the second column, C and I indicate connected and isolated wetlands

Sites	Hydrological connectivity	pH		[DIC] (mM)		CO ₂ sat (%)		[O ₂] (μM)		O ₂ sat (%)		[NO ₃ ⁻] (μM)		[NO ₃ ⁻]:[O ₂]	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
OG1	C	8.36	8.12	6.07	6.10	326	791	377	119	107	45	355	363	1.34	3.05
OG2	C	8.29	8.38	6.14	7.03	388	520	408	147	116	56	364	479	1.29	3.26
OG3	C	8.35	8.01	6.52	6.39	328	1011	161	87	46	33	1133	681	7.04	8.23
OG4	C	8.20	8.25	5.21	5.29	352	516	365	317	103	120	819	370	2.24	1.17
OG5	C	8.13	8.36	6.16	5.08	464	909	300	602	85	227	821	689	3.14	1.14
OG6	C	8.35	8.04	6.09	4.54	325	756	289	252	82	95	571	960	2.38	4.21
MI1	C	8.25	8.57	3.03	3.32	178	155	304	297	86	112	60	51	0.20	0.17
MI2	C	8.26	8.54	3.25	3.31	187	164	302	289	86	109	60	58	0.20	0.20
MI3	C	8.23	8.51	3.03	3.26	182	173	301	292	85	110	57	28	0.19	0.10
MI4	C	8.19	8.12	2.24	2.31	263	330	320	219	91	83	71	111	0.22	0.51
OG7	I	8.57	8.26	7.03	6.21	213	642	181	192	51	73	3	1	0.02	0.01
OG8	I	8.36	7.21	5.38	4.07	276	4173	323	136	92	51	6	10	0.02	0.07
OG9	I	8.34	8.24	7.07	4.38	387	419	353	141	100	53	13	113	0.04	1.20
OG10	I	8.43	8.09	5.08	4.39	212	652	310	240	88	91	13	1	0.04	0.00
OG11	I	8.46	7.31	6.06	3.16	221	2299	224	545	64	206	80	9	0.36	0.02
PO1	I	8.20	8.29	3.15	3.05	259	1071	401	245	114	93	31	1	0.08	0.00
PO2	I	8.13	8.13	2.30	3.33	163	1035	344	108	98	41	4	2	0.01	0.02
PO3	I	8.44	8.01	4.28	4.01	163	754	500	71	142	27	3	1	0.01	0.01
PO4	I	8.37	8.26	3.37	2.07	147	197	482	291	137	110	13	1	0.03	0.00
PO5	I	8.36	8.08	3.01	3.20	153	461	485	63	137	24	4	21	0.01	0.33
PO6	I	8.13	8.06	3.01	6.38	201	264	343	115	97	43	6	2	0.02	0.02
PO7	I	8.20	8.26	3.04	3.16	198	331	300	247	85	93	114	9	0.38	0.04

All samples were collected close to the sediment surface

Table 3 Median and range (in parenthesis) are reported for organic matter content (OM), bulk density (d), chlorophyll-*a* concentrations (Chl-*a*) and C:N molar ratios in surface sediments (0–1 cm)

Variable	Riverine connected wetlands		Isolated wetlands	
	Winter	Summer	Winter	Summer
OM (%)	10.4 (2.3–14.1)	10.6 (1.5–16.9)	15.9 (5.2–34.5)	17.8 (5.8–34.8)
d (g cm ⁻³)	1.2 (1.0–1.5)	1.1 (1–1.7)	1.0 (0.9–1.4)	0.9 (0.9–1.2)
Chl- <i>a</i> (µg cm ⁻³)	24.7 (22.9–48.9)	25.6 (14.4–32.5)	23.75 (11.8–37.2)	23.15 (13.1–35.9)
C:N (mol:mol)	22.4 (10.7–27)	20.8 (10.9–25.7)	14.9 (10.9–21)	15.4 (11–24.8)

n = 10 for riverine connected wetlands and *n* = 12 for isolated wetlands in each season

wetlands (Mann–Whitney *U* tests, $P < 0.01$ and $P < 0.001$, respectively), likely due to their pensile and stagnant condition. Higher values of OM were observed in marshes (old peat bogs) and in ponds characterized by pleustonic communities. Chl-*a* concentrations were high and similar among sites and seasons. Sediment C:N ratio displayed a large variability among sites and no statistical differences were found between wetland types in both winter and summer.

Benthic processes

As expected, season had effects on benthic processes, and these are likely related to temperature differences. SOD was significantly correlated with water temperature (Spearman $\rho = -0.379$, $P < 0.05$) and with bulk sediment density (Spearman $\rho = 0.332$, $P < 0.05$) but not with OM content in sediments (Spearman $\rho = -0.162$, $P = 0.3$); SOD increased from winter to summer (median values -1.14 and -2.54 mmol O₂ m⁻² h⁻¹, respectively) (Wilcoxon *T* test, $P < 0.05$) (Fig. 2). Significant differences of SOD rates were found between isolated and connected wetlands only in winter (Mann–Whitney *U* tests, $P < 0.01$) whilst in summer no statistical differences resulted (Mann–Whitney *U* tests, $P = 0.1$) (Fig. 2). In a number of sites, DIC effluxes tended to increase from winter to summer, but they were not significantly correlated with water temperature (Spearman $\rho = 0.193$, $P = 0.3$). Differences between seasons were also not significant (Wilcoxon *T* test, $P = 0.2$) with median values of 1.95 (range 0.64–16.70) and 3.61 (range 0.41–11.06) mmol DIC m⁻² h⁻¹ in winter and in summer, respectively. Inorganic carbon effluxes corresponding to SOD rates in place and time resulted in variable respiratory

quotients in both seasons (RQ) (Fig. 2). RQ medians were slightly above 1 in winter and in summer, with values of 1.21 (range 0.52–11.33) and 1.29 (range 0.11–6.95), respectively (Wilcoxon *T* test, $P = 0.5$).

The inorganic nitrogen fluxes were evaluated for patterns associated with season and connectivity (Fig. 3). Ammonium fluxes underwent a great spatial and seasonal variability. Differences between seasons were statistically significant (Wilcoxon *T* test, $P < 0.05$) with lower values in winter (median -5 , range -462 to 864 µmol N m⁻² h⁻¹) than in summer (median 345, -676 to $2,670$ µmol N m⁻² h⁻¹) when fluxes were mainly directed from sediment to the water column (Fig. 3). No statistical differences were identified among wetland types, although during winter in connected wetlands ammonium flux tended to be directed from the water to the sediment (Mann–Whitney *U* test, $P = 0.1$). A significant correlation was found between ammonium fluxes and SOD (Spearman $\rho = 0.480$, $P < 0.01$), temperature (Spearman $\rho = 0.452$, $P < 0.01$) and bulk sediment density (Spearman $\rho = -0.386$, $P < 0.05$). As a general tendency, the fluxes of the oxidized forms of inorganic nitrogen were directed from the water column to the sediment surface. Nitrite fluxes (from -131 to 188 µmol N m⁻² h⁻¹, median -1 µmol N m⁻² h⁻¹) comprised between 0 and 42% (average of the whole dataset 5%) of NO_x⁻ benthic exchanges (=NO₂⁻ + NO₃⁻). Median values of NO_x⁻ fluxes in winter and in summer were respectively -44 µmol N m⁻² h⁻¹, range $-1,616$ to 608 µmol N m⁻² h⁻¹, and -68 µmol N m⁻² h⁻¹, range $-4,062$ to $2,014$ µmol N m⁻² h⁻¹ (Fig. 3). NO_x⁻ sedimentary demand did not demonstrate a statistically significant seasonal difference and increased in summer relative to winter in only 14 out of 22 sampling sites (Mann–Whitney *U* test,

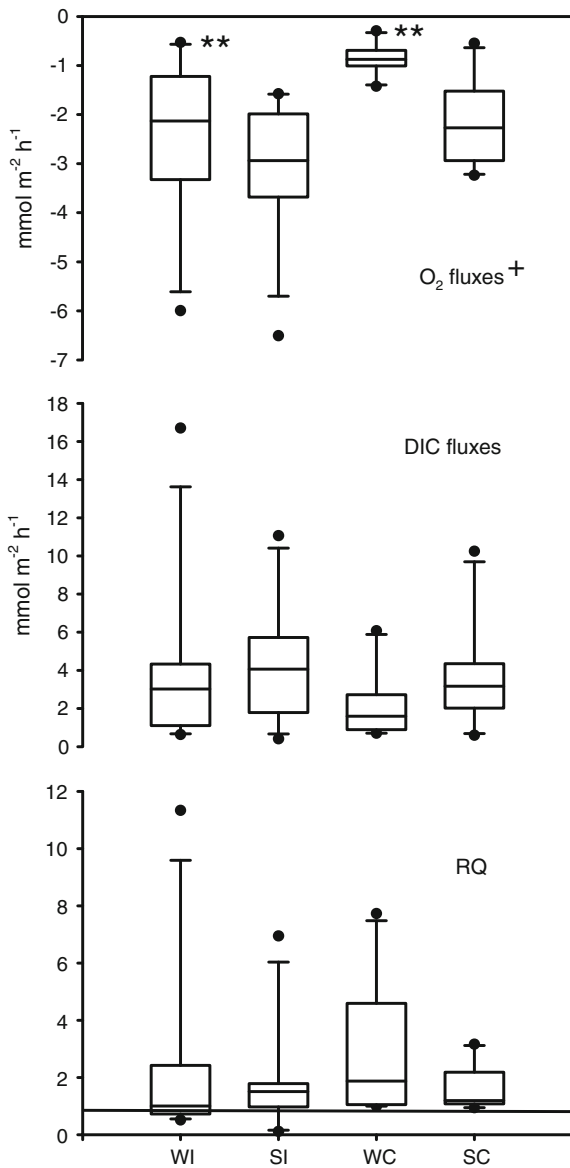


Fig. 2 Dark sediment oxygen demand (SOD), dissolved inorganic carbon fluxes (DIC) and respiratory quotient (RQ) measured at the 22 sampling sites and split up considering winter (W) and summer (S) seasons and connected (C) and isolated (I) wetland types. Seasonal differences (+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$) were tested with the Wilcoxon T test; hydrogeomorphic differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) were tested with the Mann–Whitney U test

$P = 0.1$). Wetland type affected NO_x^- fluxes (Mann–Whitney U test, $P < 0.05$) (Fig. 3). Influx rates were higher in environments connected to rivers in summer (Mann–Whitney U test, $P < 0.05$), but no effects of connectivity were found in winter (Mann–Whitney U

test, $P = 0.5$). A positive correlation was found between NO_x^- sedimentary demand and NO_3^- concentration (Spearman $\rho = 0.575$, $P < 0.001$) and between NO_x^- sedimentary demand and percentage of dissolved CO_2 saturation in the water column (Spearman $\rho = 0.323$, $P < 0.05$). Results of ammonium and NO_x^- flux measurements were combined. No significant differences were found between seasons or between wetland types for DIN ($\text{NH}_4^+ + \text{NO}_x^-$) fluxes (Fig. 3).

Denitrification rates

Nitrogen removal via denitrification was responsive to season. Total denitrification rates ($\text{Dt}_{\text{tot}} = \text{D}_{\text{W}} + \text{D}_{\text{N}}$) were extremely variable and ranged between <5 and $\sim 2,000 \mu\text{mol N m}^{-2} \text{ h}^{-1}$; N_2 effluxes were significantly higher in summer than in winter (Wilcoxon T test, $P < 0.01$) (Fig. 4). Dt_{tot} was mostly supported by water column nitrate (D_{W}) and exhibited the highest values in connected wetlands in both seasons (Mann–Whitney U tests, $P < 0.001$ and $P < 0.001$ in summer and winter, respectively). Both D_{W} and D_{N} were lower in winter than in summer (Wilcoxon T tests, $P < 0.05$). D_{W} ranged between 2 and $300 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ in winter and between 3 and $1,888 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ in summer supporting about 90 ± 18 and $71 \pm 26\%$ of Dt_{tot} in the two periods, respectively. D_{N} ranged from 0 to 67 and from 0 to $205 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ in winter and summer, respectively.

Overall, denitrification activity was affected by wetland type and correlated with several environmental factors. Comparing rates among wetland types, we found lower D_{W} rates in isolated sites both in winter and in summer (Mann–Whitney U tests, $P < 0.01$ and $P < 0.001$, respectively). D_{N} rates were only significantly lower in winter (Mann–Whitney U test, $P < 0.05$). Dt_{tot} rates correlated positively with NO_3^- concentrations (Spearman $\rho = 0.748$, $P < 0.001$), sedimentary NO_x^- demand (Spearman $\rho = 0.691$, $P < 0.001$), dissolved inorganic carbon concentration (Spearman $\rho = 0.339$, $P < 0.05$) and sediment bulk density (Spearman $\rho = 0.341$, $P < 0.05$). Dt_{tot} was not correlated with sediment OM content. Similar relationships resulted for D_{W} , reflecting the higher importance of this fraction on total denitrification. D_{N} was correlated only with temperature (Spearman $\rho = 0.368$, $P < 0.05$). Increasing amounts of added $^{15}\text{NO}_3^-$ in the cores'

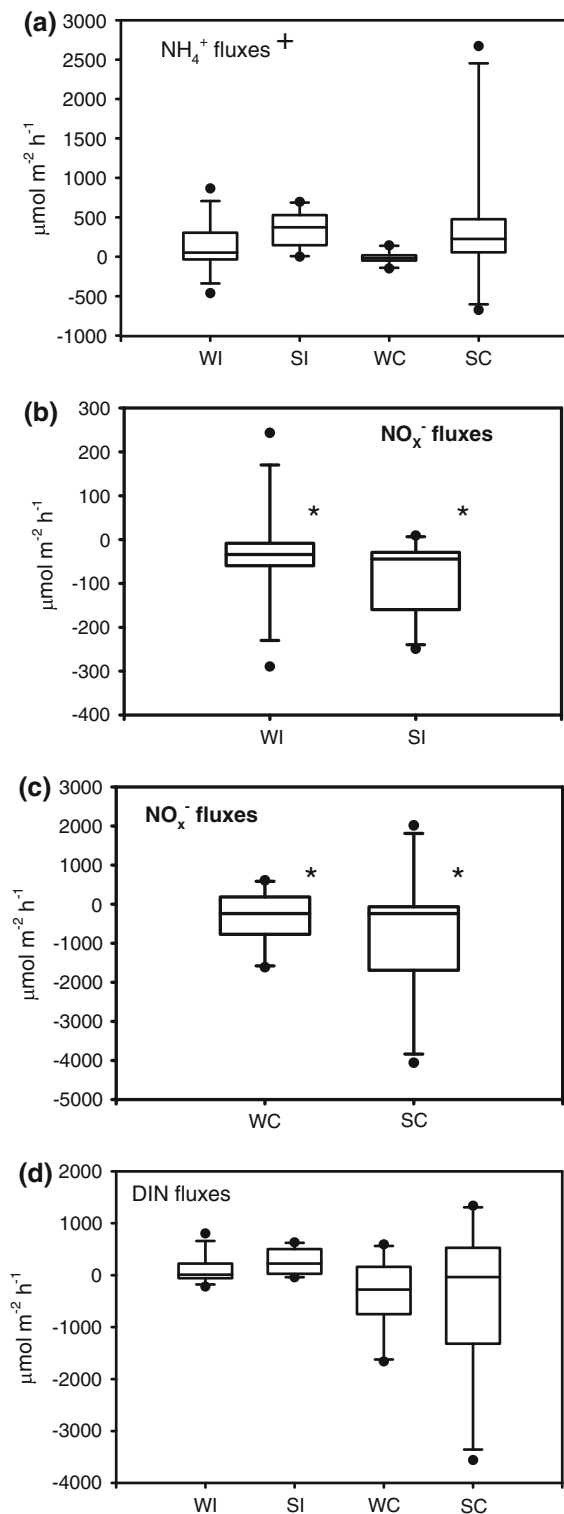


Fig. 3 NH_4^+ (a), NO_x^- ($=\text{NO}_2^- + \text{NO}_3^-$) (b, c) and DIN (d) dark fluxes measured via intact core incubations at the 22 sampling sites and split up considering winter (W) and summer (S) seasons and connected (C) and isolated (I) wetland types. NO_x^- fluxes are shown in two graphs with different scales. Seasonal differences ($^+ P < 0.05$, $^{++} P < 0.01$, $^{+++} P < 0.001$) were tested with the Wilcoxon T test; hydrogeomorphic differences ($* P < 0.05$, $** P < 0.01$, $*** P < 0.001$) were tested with the Mann–Whitney U test

water phase resulted in an immediate stimulation of denitrification rates as D_{15} (i.e. denitrification of $^{15}\text{NO}_3^-$ added to the water column). The increases in rate were linear with the nitrate enrichment, both in some isolated wetlands with low nitrate concentration and in a nitrate-rich, connected wetland (Fig. 5).

Finally, we applied the model of Christensen et al. (1990) using as data input NO_3^- and O_2 concentration in the water (Table 2) and dark O_2 fluxes measured via cores incubation. The model is unidimensional, works in sediments that are neither bioturbated nor colonized by phanerogams and predicts only rates of denitrification supported by water column nitrate (D_W). Theoretical D_W values calculated for the 22 wetlands (pooled winter and summer data) showed a very good fitting with D_W measured via the IPT (Spearman $\rho = 0.847$, $P < 0.01$) only in a limited range of rates ($0\text{--}140 \mu\text{mol N m}^{-2} \text{h}^{-1}$) (Fig. 6). Outputs of the model agree well with experimental results from wetlands characterized by low NO_3^- in the water; on the contrary predicted rates are much higher than those measured at sites with elevated nitrate concentrations.

Discussion

Denitrification in shallow eutrophic wetlands compared to other ecosystems and methodological considerations

The removal of nitrogen in freshwater wetlands is widely reported in the literature (Piña-Ochoa and Álvarez-Cobelas 2006). However, compared to other aquatic environments, few direct measurements of denitrification rates have been performed. A summary

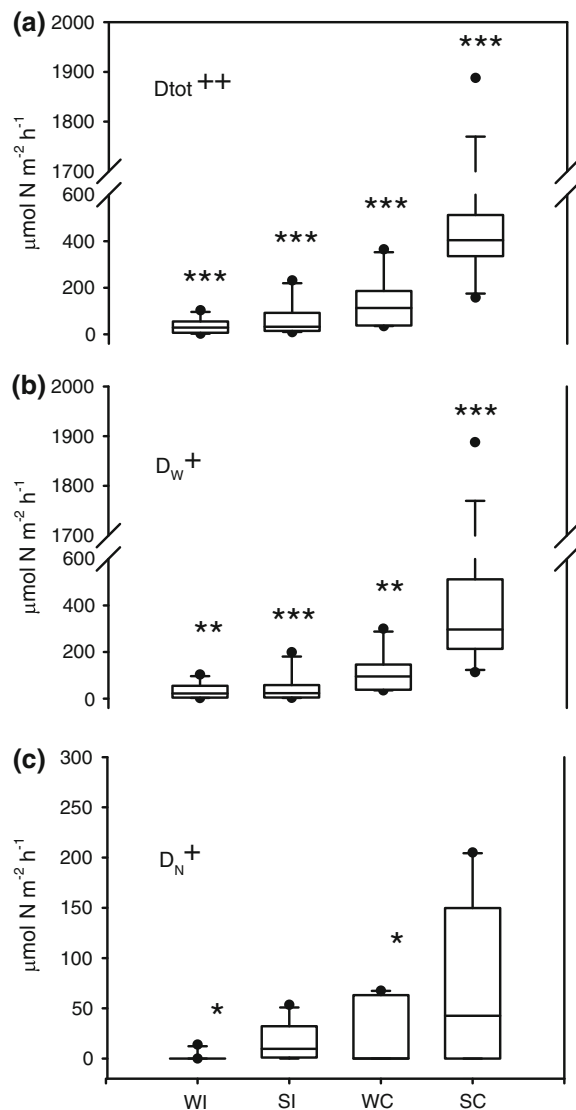


Fig. 4 Rates of denitrification measured with the isotope pairing technique at the 22 sites. Data are grouped in winter (W) and summer (S) values of connected (C) and isolated (I) wetland types and show total denitrification rates (D_{tot} , **a**), denitrification of nitrate diffusing to anoxic sediments from the water column (D_w , **b**) and denitrification of nitrate produced within sediments by nitrification (D_N , **c**). Seasonal differences (+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$) were tested with the Wilcoxon T test; hydrogeomorphic differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) were tested with the Mann–Whitney U test

of recent reports of denitrification measurements is presented in Table 4. A direct comparison with our data is somewhat limited by the fact that experimental designs and methods are different among the studies. Reported rates range between 1 and

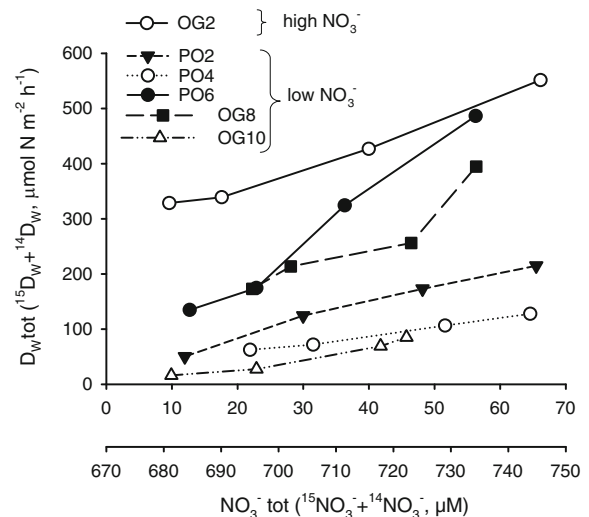


Fig. 5 Results from a concentration series experiment in which increasing amounts of labelled nitrate were added to the water phase of intact sediment cores. Sites where increasing $^{15}\text{NO}_3^-$ concentrations resulted in higher denitrification rates are reported. Denitrification of nitrate diffusing to anoxic sediments from the water column (D_w) was generally stimulated meaning that, at sites with low nitrate concentration in the water column, the denitrification potential was elevated but only partially expressed

$3,188 \mu\text{mol N m}^{-2} \text{h}^{-1}$ and bracket our measured averages both in isolated, $49 \mu\text{mol N m}^{-2} \text{h}^{-1}$, and in connected, $340 \mu\text{mol N m}^{-2} \text{h}^{-1}$, wetlands (medians 30 and $286 \mu\text{mol N m}^{-2} \text{h}^{-1}$, respectively). The range of isolated wetlands within the Po watershed compares well with other riparian wetlands and with some constructed wetlands (Table 4). River-connected sites have higher rates and are within the reported ranges for constructed wetlands in California, Sweden and Arizona. These all receive pulsing floods by river and hydraulic connectivity may explain the higher rates in literature ($12\text{--}3,188 \mu\text{mol N m}^{-2} \text{h}^{-1}$).

The connected riverine wetlands that we studied are among the ecosystems with highest denitrification rates. Reported values are higher than those found in marine environments and comparable to freshwater ecosystems, in particular with rivers. Much lower denitrification rates measured in isolated wetlands are similar to those reported for marine areas (Piña-Ochoa and Álvarez-Cobelas 2006).

In the recent literature a novel microbial process, the anaerobic oxidation of ammonium, has been found to be responsible for a major fraction of N_2

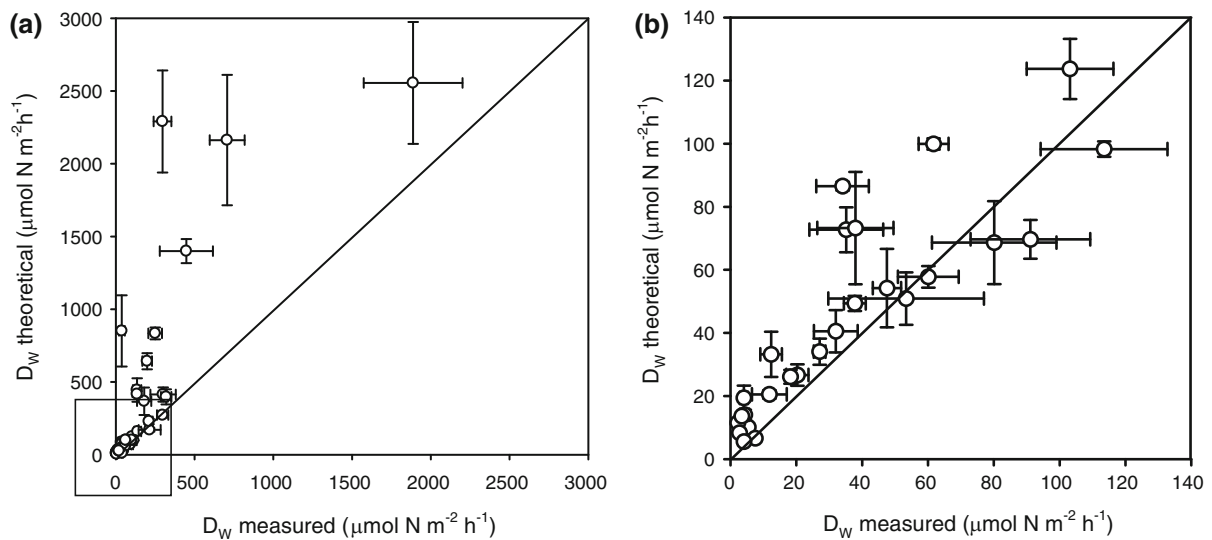


Fig. 6 Denitrification rates of nitrate diffusing to anoxic sediments from the water column (D_w), calculated according to the model of Christensen et al. (1990), are plotted versus rates measured at all sites (a) and at sites with low water column nitrate content (b)

flux, in particular in deep marine environments (Thamdrup and Dalsgaard 2002). Apart from the relevance of anammox for the nitrogen cycle, this finding poses serious methodological concerns on the assumptions on which the IPT is built (Risgaard-Petersen et al. 2003; Trimmer et al. 2006). In particular, as the relevance of anammox to N_2 total flux (r_a) increases, the risk of overestimating true denitrification rates by IPT increases (Risgaard-Petersen et al. 2003). There are only a few papers, mostly dealing with deep marine ecosystems, where anammox has been measured and rates compared with those of denitrification. The available measurements performed in eutrophic brackish or freshwater environments indicate a minor relevance of anammox to the overall N_2 fluxes, but more investigation is needed (Trimmer et al. 2003; Schubert et al. 2006; Koop-Jakobsen and Giblin 2009). Using the equations reported in Risgaard-Petersen et al. (2003) and Trimmer et al. (2006), we calculated from our experiments the risk of overestimating denitrification rates by combining r_a and r_{14} (the ratio between $^{14}\text{NO}_x^-$ and $^{15}\text{NO}_x^-$ in the nitrate reduction zone). Even if not properly designed for such calculations, our denitrification experiment, based on increasing additions of $^{15}\text{NO}_3^-$ to the water phase, allows such estimates. Our results indicate that r_a is low at both connected and isolated sites and during both sampling seasons (0–16%, median value 6%), while r_{14} is high

at connected sites (>3) and variable ($0.1 < r_{14} < 3$) at isolated sites. The combination of these percentages gives a theoretical overestimation of N_2 production via denitrification between 0 and 26% (median values of pooled data 7%). We recognize that further experimental work should be addressed to evaluate more accurately anammox rates and the regulation of this process in freshwater wetlands.

Regulation of denitrification

In this study, denitrification was positively correlated with nitrate concentration and was favored in connected wetlands where we found higher concentrations and availability of nitrate in both seasons (Fig. 4; Table 2). NO_3^- concentrations in the water column of connected sites were comparable to those in the adjoining rivers (Mincio River, about 150 μM , and in the Oglio River, about 400 μM), while isolated wetlands had much lower concentrations. Rates measured in environments hydraulically connected to rivers were up to 1 or 2 orders of magnitude higher than rates measured in isolated wetlands (Fig. 4). As widely reported in the literature, water column nitrate concentration is the main factor controlling the kinetics of denitrification in many ecosystems (Piña-Ochoa and Álvarez-Cobelas 2006; Seitzinger et al. 2006). As a general rule we found that D_w contributed mostly to D_{tot} . Nitrification in organically

Table 4 Denitrification rates measured in different types of shallow eutrophic freshwater environments, including results from the present study

Type of wetland	Location	Method for measuring denitrification rates	Denitrification rate ($\mu\text{mol N m}^{-2} \text{h}^{-1}$)	References
Riparian wetlands	New Jersey (USA)	N_2 fluxes	<20–260	Seitzinger (1994)
Constructed wetlands receiving river water	California (USA)	Mass balance	12–3,188	Reilly et al. (2000)
Wetland system treating effluent from a sewage treatment plant	Netherlands	Acetylene inhibition	1–360	Toet et al. (2003)
Wastewater treatment wetland	Sweden	Acetylene inhibition	714–1,786	Bastviken et al. (2005)
Riparian buffer zone	Estonia	N_2 fluxes	14–571	Teiter and Mander (2005)
Constructed wetland	Switzerland	Isotope mass balance	3–661	Reinhardt et al. (2006)
Marsh receiving water from river	Louisiana (USA)	Isotope ratio mass spectrometry (IRMS)	179–679	Yu et al. (2006)
Constructed wetlands receiving river water	Ohio (USA)	Acetylene inhibition	14–129	Hernandez and Mitsch (2007)
Constructed marsh receiving water pumped from river	Texas (USA)	N_2/Ar (MIMS)	54–278	Scott et al. (2008)
Constructed wetland receiving water from drainage ditch	Indiana (USA)	Isotope pairing	40–175	Herrman and White (2008) (in press)
Constructed wetlands	Arizona (USA)	Mass balance	677–1,248	Kadlec (2008)
Constructed wetlands receiving river water	Japan	Acetylene inhibition	149–185	Zhou and Hosomi (2008)
Isolated wetlands	Italy	Isotope pairing	2–231	This study
Riverine connected wetlands	Italy	Isotope pairing	35–1,888	This study

Apart from Seitzinger (1994), reported values were calculated from original data and expressed in $\mu\text{mol N m}^{-2} \text{h}^{-1}$

loaded wetlands was severely limited by oxygen availability and was a minor source of NO_3^- for coupled denitrification. The exceptions were a few isolated environments with little to no nitrate in the water column during summer months and with a sufficient amount of oxygen for nitrification to proceed. Similar trends, reported by Piña-Ochoa and Álvarez-Cobelas (2006), confirm that water column nitrate regulates the ratio between D_W and D_N .

Our experiments using a concentration series (increasing amounts of $^{15}\text{NO}_3^-$ added in cores) demonstrated that most of the sites exhibited high denitrification potential (D_{15}), and thus the process was not saturated. This was especially true for almost all isolated wetlands in which the in situ process was probably nitrogen-limited by stagnant conditions and by limited nutrient recharge from flooding by river pulses. In connected environments with extremely high nitrate concentration ($>500 \mu\text{M}$), we did not measure a

corresponding increase of D_{15} , which is indicative of substrate saturation, with only one exception (Fig. 5). The kinetics of denitrification are more uncertain when nitrate concentrations are saturating and other factors can limit the reaction, e.g. availability of labile organic carbon (Piña-Ochoa and Álvarez-Cobelas 2006). Also, in those environments where nitrate concentrations did not change significantly in the two sampling periods, denitrification rates increased from the winter to the summer, indicating that water temperature was an important regulating factor. Many studies reported a breakpoint of temperature response of denitrification that reflects the fact that rates fall non-linearly at lower temperatures (Focht and Verstraete 1977; Hénault and Germon 2000).

There are several potential links between denitrification and other metabolisms in sediments, including SOD. SOD in this study was in the higher range of rates reported in the literature for wetlands,

eutrophic lakes, and rivers (Christensen et al. 1990; Nielsen et al. 1990; Seitzinger 1994; Scott et al. 2008). Seasonal temperature was a key factor for SOD, but unlike other reports we did not find a significant correlation between SOD and D_{tot} (Seitzinger 1994; Eyre and Ferguson 2002). According to the simple but robust model proposed by Christensen et al. (1990), denitrification can be predicted by a combination of SOD and $\text{NO}_3^-:\text{O}_2$ ratio. Shallow water environments with high SOD, high nitrate concentrations and anoxia should have a great potential for nitrogen removal via denitrification. The model was originally developed for Northern European sites characterized by lower SOD and lower $\text{NO}_3^-:\text{O}_2$, but appears to be more generally applicable to our systems. However, in some connected wetlands where nitrate concentrations were high, we found much higher predicted than measured rates. This could result from saturation of denitrification, nonlinearity of the model at extremes SOD rates or $\text{NO}_3^-:\text{O}_2$ ratios, occurrence of other more favorable microbial transformation of NO_3^- as DNRA or underestimation of real rates due to insufficient $^{15}\text{NO}_3^-$ labeling (Brunet and Garcia-Gil 1996; Gardner and McCarthy 2009).

Denitrification rates were not correlated with the large pool of sedimentary OM content ($\sim 10\%$ or more), even though this variable is widely recognized as an important regulator of the process (Ingersoll and Baker 1998; Piña-Ochoa and Álvarez-Cobelas 2006; Seitzinger et al. 2006). Nitrogen removal was also not correlated with Chl-*a* content in sediments, even if this represented a large pool of labile, easily degradable organic carbon. Excess organic matter availability at the study sites can mask the effect of these factors.

Sensitivity of denitrification to nitrate concentration has significant implications to water quality and management of wetlands. N removal appears to be highly sensitive to both the availability of nitrate and to temperature. The connected wetlands showed highest denitrification rates due to regular nitrate recharge by water bodies. The potential for increased denitrification in isolated wetlands was demonstrated by our series of increasing nitrate additions. If these environments were connected and received pulsing floods of river water, they should be able to remove high amounts of nitrate from the water column and serve an important role in nutrient removal.

Denitrification efficiency in shallow eutrophic wetland sediments: N sink or sources?

Hydrological connectivity of riverine wetlands influences nitrogen removal efficiency in more ways than simply through increasing nitrate concentration. Denitrification efficiency is evaluated as the ratio between denitrification rates and inorganic nitrogen effluxes across the sediment–water interface ($D_{\text{tot}}/(D_{\text{tot}} + \text{DIN})$). It represents the percentage of the total processed inorganic nitrogen during organic matter decomposition released as N_2 (Eyre and Ferguson 2002). According to Eyre and Ferguson (2002), efficiencies close to 1 mean that net loss of nitrogen is in gaseous form from the system, while values close to 0 mean that inorganic nitrogen ions are recycled to the water column and may sustain new primary production. Calculated denitrification efficiencies for the 10 connected and 12 isolated wetlands studied in this work are reported in Fig. 7. In both seasons, denitrification efficiency in isolated wetlands was below 0.2, suggesting that these environments tended to be sources of inorganic nitrogen with ammonification (or dissimilative reduction of nitrate to ammonium) prevailing over denitrification (Gardner and McCarthy 2009). Low denitrification efficiency at isolated wetlands means that these sites tended to maintain eutrophic–hypereutrophic conditions with cycling between planktonic or macrophytic primary production, organic matter settling, fast regeneration of the labile fraction and burial of the recalcitrant litter, resulting in rapid infilling. Benthic nitrogen cycling in these environments appears to be simplified due to negligible nitrification/denitrification processes and limited availability of oxidized inorganic nitrogen forms. Connected wetlands tended to be sources of DIN to the water column, with ratios below 0.5 in winter, while in summer they tended to be nitrogen sinks (denitrification efficiency median 0.65, range 0.4–0.8). Hydraulic connectivity likely maintains oxidized conditions in the water and in the upper sediment layers and low ratios of sedimentary organic carbon to nitrate availability, enhancing permanent N removal.

The fraction of total NO_3^- uptake undergoing denitrification was calculated dividing N_2 production (from D_{w}) by NO_3^- fluxes (only negative values). At connected sites the ratio was 0.5 and 1.0 in winter and

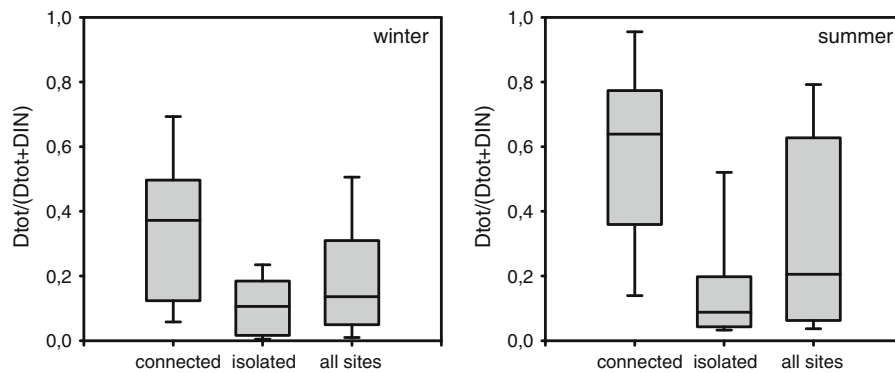


Fig. 7 Denitrification efficiencies calculated according to Eyre and Ferguson (2002) (see the text for major details)

summer, respectively (median values). These numbers suggest that in winter only half of the nitrate uptake was due to denitrification while in summer most of the nitrate was removed via this process. At isolated sites the calculated D_w to NO_3^- flux ratio was 0.6 and 0.5 in winter and summer, respectively (median values), suggesting that at both sampling seasons other processes besides denitrification were responsible for an important fraction of NO_3^- uptake. Dissimilative nitrate reduction to ammonium, dark incorporation in microphytobenthos and bacteria or other microbially mediated or chemical processes could be relevant processes in the studied wetlands.

Denitrification rates in freshwater wetlands could be addressed in a mass balance context to assess the relevance of the process at a basin scale. We calculated the N budget according to the “Soil System Budget” (Oenema et al. 2003) for the lower Oglio River basin, where most of the studied wetlands are located. For this 3,700 km² watershed we estimated an annual nitrogen surplus of 32,633 metric ton (t) N and we calculated, assuming highest measured denitrification rates (1,260 kg N ha⁻¹ year⁻¹) and hydraulic connection for all wetlands in the basin, a potential N removal of only 250 t N year⁻¹. In fact even if maximum denitrification rates on areal basis are about nine-folds higher than average N surplus (143 kg N ha⁻¹ AL year⁻¹; AL = Agricultural Land), total riverine wetlands area (~200 ha) is three orders of magnitude smaller than agricultural lands (~230,000 ha) within the catchment. According to Verhoeven et al. (2006), wetland environments contribute significantly to water quality improvement if they account for at least 10% of the watershed area, while in the Oglio

basin and likely in the Po River Plain the potential of wetlands to remove excess N is at present irrelevant due to their overall negligible surface (<<1%).

Benthic respiration and denitrification in shallow eutrophic wetlands

This last section intends to (1) clarify the role of denitrification in organic matter mineralization in light of measured benthic metabolism and (2) quantify the links among metabolic rates, connectivity and N removal. The elevated rates of oxygen consumption and the low oxygen reserve in the water column measured in the shallow wetlands of the Po River Plain are typical of environments that risk anoxia. We calculated that in summer isolated wetlands can turn hypoxic to anoxic in less than 10 h, mainly in the night, without the offset of photosynthesis. The ratio between SOD and DIC fluxes enabled us to evaluate the importance of anaerobic metabolism. SOD, corrected for oxygen demand due to nitrification, was stoichiometrically comparable or significantly below DIC fluxes. On average, about 77 and 59% of measured DIC fluxes were uncoupled from corresponding O₂ uptake rates in summer and winter, respectively. Imbalances in SOD and DIC effluxes are typical of eutrophic to dystrophic aquatic environments and are associated with accumulation of reduced compounds in pore water (Ingvorsen and Brock 1982; Capone and Kiene 1988). Denitrification in isolated wetlands represented molar equivalents on average 9 and 12% of SOD in winter and summer, respectively. In connected environments, these percentages increased to 19 and 25%, and at some sites the moles of N₂ produced via denitrification were

comparable to the moles of O_2 respired. Oxygen consumed via nitrification, calculated as the sum of $2D_N + 2NO_x^-$ efflux, was a minor fraction of SOD. It averaged 11 and 3% of SOD in winter and summer, respectively, and represented only 2 and 14% of SOD for isolated and connected sites. Oxygen consumption was thus mainly due to aerobic carbon mineralization and to the reoxidation of anaerobic metabolism end products.

We estimated carbon oxidation by denitrification assuming 1.25 C:1 N (mol:mol) (Richards 1965) to evaluate the contribution of denitrification to organic matter mineralization. Total denitrification rates were responsible for an extremely variable production of inorganic carbon, varying between <5 and 450 $\mu\text{mol C m}^{-2} \text{ h}^{-1}$ in winter (average of pooled data $98 \pm 23 \mu\text{mol C m}^{-2} \text{ h}^{-1}$) and between 11 and 2,369 $\mu\text{mol C m}^{-2} \text{ h}^{-1}$ in summer (average of pooled data $355 \pm 108 \mu\text{mol C m}^{-2} \text{ h}^{-1}$). Assuming that DIC fluxes were a proxy for organic carbon mineralization, we estimated that the fraction of carbon oxidation due to denitrification ranged from <1 to 83%, with winter and summer averages of 7.7 and 13.0%, respectively. In connected sites, this fraction was generally ecologically significant (10–83%), while in isolated sites it was <5%. We thus demonstrated that in riverine connected wetlands denitrification was significant (1) for the removal of nitrogen from NO_3^- -polluted waters and (2) as a respiratory process responsible for a significant fraction of carbon mineralization. Similar results were reported for estuarine and shallow marine environments by Yoon and Benner (1992) and Laursen and Seitzinger (2002) and for riparian freshwater wetlands (Seitzinger 1994).

Concluding remarks

This study demonstrates that connectivity of eutrophic wetlands with rivers is a primary factor in controlling denitrification and nitrogen cycling. At connected sites, denitrification can be an important pathway for nitrogen cycling and a significant component of benthic respiration. Here, denitrification was mostly sustained by nitrate diffusing to anoxic sediments from the water column, which was primarily regulated by nitrate availability and temperature. Measured values were within those reported in the literature and, for a wide range of rates (from <5 to

140 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$), can be generally predicted by a simple diffusion–reaction model. Denitrification coupled to nitrification was generally low, probably due to limited oxygen availability within sediments.

At hydrologically isolated sites, denitrification was a negligible process with ammonium regeneration largely prevailing over nitrate removal. This can enhance primary productivity, infilling and, perhaps ultimately, the shift of these temporary environments towards more terrestrial conditions, as export of newly formed organic matter is very low. Therefore, the imbalance of nitrogen pathways could also generate feedbacks with a detrimental loss of riparian aquatic habitats which act as regulators of the whole river ecosystem.

Pulses of nitrate immediately stimulated denitrification meaning that all the studied environments had a potential capacity for greater nitrogen removal. This feature has to be taken into account as a key element in the management of the hydrographic network of the Po River Plain, and similar impacted rivers, where nitrate contamination of surface and ground waters is a critical issue. There is evidence from this study, that actions for rehabilitating the lateral connectivity between rivers and riverine aquatic habitats could greatly improve nitrogen removal via denitrification with beneficial effects on water quality and persistence of habitats themselves.

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