

## Benthic metabolism and denitrification in a river reach: a comparison between vegetated and bare sediments

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### ABSTRACT

This study aims at comparing biogeochemical processes in a *Vallisneria spiralis* meadow and in unvegetated sediments in the upper reach of the Mincio River (Northern Italy). The main hypothesis of this work is that meadows of rooted macrophytes affect benthic metabolism, enhancing capacity to retain nutrients (assimilation) and dissipate (denitrification) nitrogen loadings. In order to highlight how plants affect benthic processes in the riverbed, oxygen, dissolved inorganic carbon (DIC), soluble reactive phosphorus (SRP) and inorganic nitrogen fluxes, together with denitrification rates, were measured from February to November 2007 in intact cores collected from stands of *V. spiralis* and bare sediments. *V. spiralis* biomass, elemental composition and growth rates were concurrently measured. Macrophyte biomass ranged from 60 to 120 g m<sup>-2</sup> (as dry matter); growth rates followed a seasonal pattern from 0.001 in winter up to 0.080 d<sup>-1</sup> in summer. On an annual basis, the macrophyte meadow was autotrophic with net O<sub>2</sub> production and dissolved inorganic carbon uptake, while the bare sediment was net heterotrophic. The concurrent N assimilation by macrophytes and losses through denitrification led to similar N uptake/dissipation rates, up to 2500 mmol m<sup>-2</sup> y<sup>-1</sup>. Under the very high NO<sub>3</sub><sup>-</sup> concentrations of the Mincio River, the competition between primary production and denitrification processes was also avoided. A significant ammonium regeneration from sediments to the water column occurred in the *V. spiralis* meadow, where plant debris and particulate matter accumulated. Here, SRP was also released into the water column, whilst in the bare sediment SRP fluxes were close to zero. Overall, *V. spiralis* affected the benthic metabolism enhancing the ecosystem capacity to control nitrogen contamination. However, the actual N removal rates were not sufficient to mitigate the pollution discharge.

Key words: *Vallisneria spiralis*, oxygen and carbon dioxide fluxes, phosphorus and nitrogen cycling, denitrification

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### 1. INTRODUCTION

Eutrophication processes and the associated changes in community composition from submerged phanerogams to planktonic, macroalgal, or floating forms have been a central subject of recent research in shallow aquatic environments (Harlin & Rines 1993; Schramm 1999; Scheffer *et al.* 2003; Viaroli *et al.* 2008 and references therein). The loss of rhizophyte meadows is a consequence of excess organic matter and nutrient inputs, water turbidity and reducing conditions at the sediment level (Hauxwell & Valiela 2004). Overall, the shift of benthic vegetation resulting from rhizophytes displacement causes the loss or change of important ecosystem processes, i.e. nitrification coupled denitrification. Rooted phanerogams have a well-recognised role as benthic regulators of biogeochemical cycles with implications also for benthic fauna and fish communities (Schramm 1999; Hemminga & Duarte 2000; Heck *et al.* 2003; Cronin *et al.* 2006). Phanerogams convert nutrients from inorganic into particulate and dissolved organic forms, control phytoplankton growth, retain suspended matter, stabilise surface sediments and provide nursery areas for invertebrates and fish (Phillips & Menez 1988; Newman 1991; Caffrey & Kemp 1992; Blanchet *et al.* 2004; Hasegawa *et al.* 2008). Addition-

ally, the radial oxygen loss from roots provides oxygen into anoxic sediments, which supports the reoxidation of reduced compounds (i.e., ammonium, methane and sulphides), the precipitation of soluble reactive phosphorus with ferric iron (Deborde *et al.* 2008) and the removal of nitrogen via nitrification coupled denitrification (Reddy *et al.* 1989; Caffrey & Kemp 1992; Ottosen *et al.* 1999). Due to their overall importance, seagrasses have been studied in transitional brackish zones and coastal environments all around the world (Bortone 2000; Hemminga & Duarte 2000; Nielsen *et al.* 2004). Less attention has been dedicated to the submerged vegetation of freshwater environments, particularly in river ecosystems (Hilton *et al.* 2006).

In most of developed countries, rivers are heavily impacted by anthropogenic activities through damming, water withdrawal for irrigation, trans-basin diversions, disturbance of lateral and longitudinal connectivity (Décamps *et al.* 1995; Naiman *et al.* 1995; Michener & Haeuber 1998; Sommer *et al.* 2001; Wissmar & Bisson 2003; Adam *et al.* 2007). In regulated rivers, the altered hydrology affects solid transport and sedimentation rates and can amplify the effects of nutrient loadings, inducing eutrophication processes (Dodds 2006; Hilton *et al.* 2006; Rossetti *et al.* 2008). Current velocity and water turbulence have also been recognized as prime factors regulating growth and distribution of submerged

macrophytes (Madsen *et al.* 2001; Sand-Jensen 2008). River eutrophication is a relatively old concept (Ohle 1955), but its development and related effects are not yet fully understood, due to the complex interactions establishing between biotic and physical factors under altered hydrological conditions (Dodds 2006; Hilton *et al.* 2006). Among others, studies on effects of either increasing nutrient loadings or changing hydrology have mainly addressed macrophyte biodiversity, species succession, and interactions between epiphytes and macrophytes in rivers (Wilby *et al.* 2001; Wade *et al.* 2002). Whilst, to our knowledge, only a few studies have focused on benthic metabolism and sedimentary buffering capacity (Madsen *et al.* 2001; Caraco & Cole 2002).

This paper aims at comparing benthic metabolism, nutrient exchanges and denitrification rates in vegetated (*Vallisneria spiralis* L.) and unvegetated areas within a reach of the Mincio River (Northern Italy), which is nutrient enriched from surrounding farmlands and wastewater treatment plants and where macrophyte meadows are expanding. Biogeochemical processes, in particular nitrogen pathways and fate, are discussed with respect to the seasonal evolution of the *V. spiralis* meadows and the benthic buffering capacity against nitrogen.

## 2. METHODS

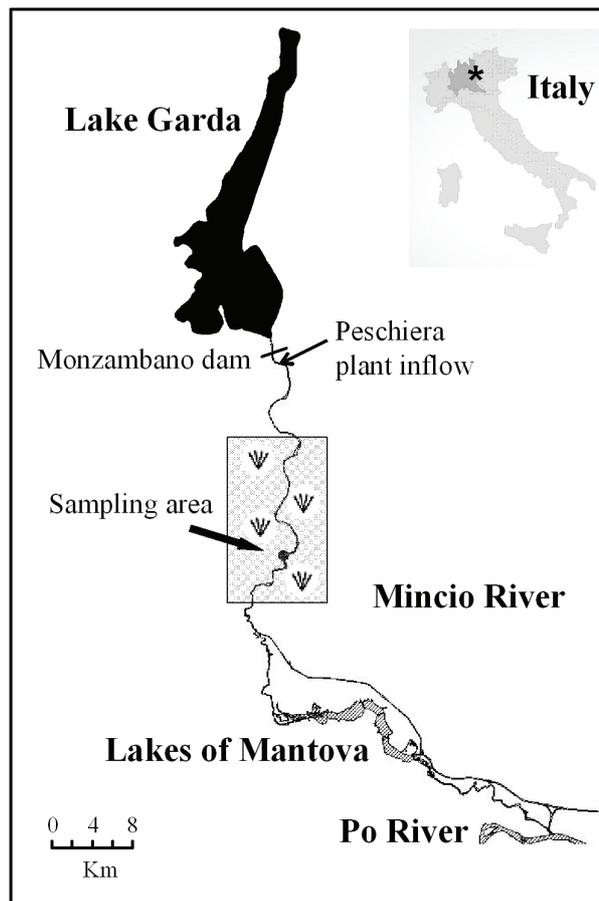
### 2.1. Study site

The Mincio River, a tributary of the Po River, originates from the Lake Garda, the largest Italian Alpine lake (Fig. 1). The river is regulated upstream by a dam which controls the water discharge from the lake. In winter, the water is stored in the lake causing a reduction of river flow to 10–12 m<sup>3</sup> s<sup>-1</sup>. In summer, the water discharge from the lake is 50–70 m<sup>3</sup> s<sup>-1</sup>, but up to 75% is immediately diverted into irrigation canals. The upper river reach, which is 30 km long, is colonised by vegetation with a major dominance of *V. spiralis*. The stretch considered in this study is 8 km long and ~1 m deep, with a current velocity of 0.5–1.0 m s<sup>-1</sup>. In the last decade, the average annual flow was 15 m<sup>3</sup> s<sup>-1</sup> of which 2–3 m<sup>3</sup> s<sup>-1</sup> originated from groundwater inputs.

Nutrients are delivered from the surrounding farmland and from the wastewater treatment plant of the town of Peschiera (up to 600,000 population equivalents in summer).

The lateral system composes of vegetated natural banks, whilst the river bed is made by coarse material and surrounded by marginal areas with meadows of submerged macrophytes (*V. spiralis*, *Potamogeton crispus*, *P. trichoides*, *P. perfoliatus*, *P. pectinatus*, *Myriophyllum spicatum*, *M. verticillatum*, *Ranunculus trichophyllus*, *Elodea canadensis*). Marginal areas of the river bottom, previously covered by coarse material, are actually covered by fine, muddy particles which allow the macrophyte meadows to expand. Here, the dominant species is *V. spiralis*, a stoloniferous species, capable of

rapid clonal extension which often forms expansive meadows (Hauxwell *et al.* 2007). Thirty years ago, this macrophyte was mostly confined to southern river stretches, characterized by slow current velocity.



**Fig. 1.** Overview of the Mincio River. The dam regulating the water flow, the approximate location of the main wastewater input and the sampling area are evidenced.

### 2.2. Sampling program

Samplings of water, bare sediments and sediments colonised by *V. spiralis* were conducted in February, April, June, August, October, and November 2007. Bare sediments were sampled in confined areas devoid of vegetation adjacent to large macrophyte stands. Sediment samples were collected with cores of three different dimensions (diameter × length), according to the standard for measuring biogeochemical processes in benthic systems (Dalsgaard *et al.* 2000): sediment characterisation (5×30 cm, n=3); flux measurements in bare sediments (8×40 cm, n=4); flux measurements in *V. spiralis* vegetated sediments (20×40 cm, n=4). Cores used for flux measurements in vegetated and unvegetated areas were previously tested on the same substrate to ensure not significant differences between measured fluxes.

All cores were cooled and brought to the laboratory within a few hours from sampling for further processing

and incubation procedures. In parallel, growth rates of *V. spiralis* were measured with the leaf marking technique. During each sampling event, current velocity (Flow probe, mod. FP101, Global Water, Gold River, CA, USA), temperature and dissolved oxygen (YSI multiple probe, mod. 556, Yellow Springs, OH, USA) were measured in situ and water samples were collected and filtered (GF/F Whatman) for inorganic nitrogen and soluble reactive phosphorus determination. On each sampling date, approximately 200 L of water were collected and brought to the laboratory for performing incubations. Cores and water were collected generally between 11:00 a.m. and 2:00 p.m.; concurrently, photosynthetically active radiation (PAR) was measured with a quantum sensor (Delta OHM, mod. HD 9021, Padua, Italy).

### 2.3. Sediment characterisation

Benthic microalgal biomass was measured as chlorophyll-*a* (Chl-*a*) concentration in the top 0.5 cm sediment layer of bare sediment sites and determined spectrophotometrically after extraction with 90% acetone (Lorenzen 1967). Sediment porosity, organic carbon, nitrogen and phosphorus contents were analysed in the 0-5 cm sediment horizon. Porosity was determined from the dry (70 °C) and wet weights of known sediment volumes according to equation (1):

$$\phi = \frac{V_v}{V_t} \quad (1)$$

where  $V_v$  is the volume of the void space between sediment particles, calculated as difference between the wet and dry weight of a known sediment volume ( $V_t$ ), typically 5 mL.

Dried sediments were also analyzed for organic C and N content (CHNS-O EA 1108 Carlo Erba Elemental Analyzer) and organic P content according to Aspila *et al.* (1976).

### 2.4. Dissolved oxygen, inorganic carbon and nutrient flux measurements

In the laboratory, cores were submerged with the top open in a tank containing river water which was aerated and kept at field temperature with a light/dark cycle similar to that of the sampling period. During the pre-incubation phase, water stirring inside cores and head-water exchange with the water in the incubation tank was ensured by small aquarium pumps (flow  $\sim 2.5$  L  $\text{min}^{-1}$ ) in the cores containing *V. spiralis* and by magnetic bars driven by an external motor at 60 rpm in the cores with bare sediments. Water stirring inside cores was on also during incubations to prevent stagnation and establishment of concentration gradients within the cores. Magnetic bars, suspended 5 cm above the sediment-water interface and aquarium pumps ensured

homogeneous stirring of the water overlying sediments without resuspension of the sediment particles.

The day after the sampling, the water in the tank was renewed and cores were incubated under light and dark conditions for dissolved oxygen ( $\text{O}_2$ ), dissolved inorganic carbon (DIC), soluble reactive phosphorus (SRP),  $\text{NH}_4^+$  and  $\text{NO}_3^-$  fluxes measurements. Light conditions were set as the average irradiance of the sampling period with a 1000-W halogen lamp. Incubations started by lowering the water in the tank just below that of the core top and by sealing the cores with floating lids provided with a sampling port. Incubations were performed according to Dalsgaard *et al.* (2000). Incubation time (from 1.5 to 5 hours) decreased with increasing water temperatures and was set in order to keep oxygen concentration at the end of the incubation within 20% of the initial value. Water samples were collected at regular time intervals with plastic syringes. Samples for  $\text{O}_2$  determinations were transferred to glass vials (Exetainers, Labco, High Wycombe, UK) and Winkler reagents were added immediately (Strickland & Parsons 1972). Samples for DIC were also transferred to glass vials and immediately titrated with 0.1 N HCl (Anderson *et al.* 1986). Samples for inorganic nutrients determination were filtered through Whatman GF/F glass fibre filters, transferred to glass (SRP) and polyethylene ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) vials and then frozen. Standard spectrophotometric techniques were used for SRP (Valderrama 1977),  $\text{NH}_4^+$  (Bower & Holm-Hansen 1980) and  $\text{NO}_3^-$  (Golterman *et al.* 1978).

Hourly flux rates of  $\text{O}_2$ , DIC, SRP,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were calculated with a linear regression of concentrations *versus* time and expressed as rate per square meter. Solute evolution in the light and dark periods was calculated by multiplying hourly rates by the hours of light and dark of the corresponding period and summed up to obtain daily rates. Annual rates were obtained by multiplying daily rates by the number of days in each sampling period and summing up to one year.

Oxygen and inorganic carbon fluxes were used to calculate the net benthic production (NBP=light flux), benthic respiration (BR=dark flux) and gross benthic production (GBP= NBP-BR).

Nitrogen and phosphorus assimilation by benthic microalgae was estimated from NBP and GBP rates assuming constant molar ratio C:N:P=106:16:1 (Sundbäck *et al.* 2000). Retention of N and P in microphytobenthos biomass was calculated assuming C:Chl-*a*=30 (de Jonge 1980). Nitrogen and phosphorus assimilation by *V. spiralis* was estimated from NBP and GBP using C:N:P values measured on each sampling date.

### 2.5. Denitrification measurements

Following measurements of dissolved gas and nutrient fluxes, sedimentary dark denitrification rates were measured in the same set of cores with the isotope-pairing technique (Nielsen 1992). This method quanti-

fies total denitrification ( $D_t$ ) in 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 ( $D_n$ ). At the beginning of the experiment,  $^{15}\text{NO}_3^-$  was added to the water column to increase the nitrate pool by ~30%. The  $\text{NO}_3^-$  concentration was measured prior to the addition of  $^{15}\text{NO}_3^-$  and at the time the cores were closed (within 5 minutes from the addition of  $^{15}\text{NO}_3^-$ ) in order to calculate the  $^{14}\text{N}/^{15}\text{N}$  ratio in the  $\text{NO}_3^-$  pool. Incubations lasted variable periods, ranging from 1 (summertime) to 4 (winter-time) hours. At the end of the incubation 5 to 10 mL of 7M  $\text{ZnCl}_2$  was added to the water phase and then sediment and water were mixed. Part of the slurry was then transferred into 12.5 ml gas-tight vials;  $^{14}\text{N}^{15}\text{N}$  and  $^{15}\text{N}^{15}\text{N}$  abundance in  $\text{N}_2$  was analysed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark. The rates of denitrification were calculated according to the equations and assumptions of Nielsen (1992):

$D_{15} = p(^{15}\text{N}^{14}\text{N}) + 2p(^{15}\text{N}^{15}\text{N})$  and  $D_{14} = p(^{15}\text{N}^{14}\text{N}) + 2p(^{14}\text{N}^{14}\text{N})$ , where  $D_{15}$  and  $D_{14}$  = rates of denitrification based on  $^{15}\text{NO}_3^-$  and  $^{14}\text{NO}_3^-$ , respectively; and  $p(^{14}\text{N}^{14}\text{N})$ ,  $p(^{15}\text{N}^{14}\text{N})$  and  $p(^{15}\text{N}^{15}\text{N})$  = rates of production of labelled and unlabelled  $\text{N}_2$  species. Because the  $p(^{14}\text{N}^{14}\text{N})$  cannot be readily measured, estimation of  $D_{14}$  was obtained from:  $D_{14} = D_{15} \times p(^{15}\text{N}^{14}\text{N})/2p(^{15}\text{N}^{15}\text{N})$ . The proportion of  $D_{14}$  supported by unlabelled  $\text{NO}_3^-$  from the water column ( $D_w$ ) was calculated from:  $D_w = D_{15} \times f/(1-f)$ , where  $f$  = mole fraction of  $^{14}\text{NO}_3^-$  in the water column. The coupled nitrification-denitrification ( $D_n$ ) was calculated by difference as:  $D_n = D_{14} - D_w$ .

Theoretical denitrification rates of nitrate diffusing into anoxic sediments from the water column were estimated according to equation (2), proposed by Christensen *et al.* (1990) for unvegetated sediments:

$$D_w = F_{O_2} \times 0.8 \times \left[ \sqrt{1 + 0.82 \times \frac{C_{\text{NO}_3^-}}{C_{O_2}} \times \frac{1}{0.8}} - 1 \right] \quad (2)$$

where  $F_{O_2}$  is the dark sediment oxygen demand and  $C_{\text{NO}_3^-}$  and  $C_{O_2}$  are the water column nitrate and oxygen concentrations, respectively.

## 2.6. Macrophyte biomass, elemental composition and leaf growth rates

At the end of incubations for flux and denitrification measurements macrophytes were collected from each core by sediment sieving. *V. spiralis* specimen were sorted, separated into above (leaves) and below ground (roots+rhizomes) biomass, rinsed to remove epiphytes and desiccated at 70 °C until constant weight was reached. The dried biomass was powdered and analyzed for organic C and total N and P content as previously described for sediments.

From February to December 2007, growth rates of the above ground biomass of *V. spiralis* were measured monthly with the leaf marking technique (Sand-Jensen 1975). Scuba divers tagged 20 to 30 randomly selected shoots monthly from an area immediately adjacent to that sampled with cores. The benchmark was made by carefully punching two needle holes in each leaf, at a common reference distance (approximately 1 cm) from the sediment-water interface. Depending on the season, after 10 to 25 days the tagged shoots were retrieved, gently washed, and frozen at -20 °C. In the meantime a new set of shoots was marked for further measurements.

The leaf growth was determined as the distance from the reference point to the needle scars of each leaf. New leaves (leaves without any mark) above the reference point were then counted and measured. In broken leaves, the missing part was not included in calculations as we considered impossible to establish whether the leaf apex was broken before or after the marking. According to these assumptions, the leaf net growth rate (NGR) was calculated with the exponential growth model after measurements of single leaf length and width. In order to convert increase in biomass from surface measurements, we determined for each incubation period the surface areas and dry weight at 70 °C on a set of leaves (n=30). We then used a conversion factor of weight per leaf surface area ( $\mu\text{gD}_w \text{ mm}^{-2}$ ) to calculate the total above-ground biomass. Further, we converted the biomass increase in carbon units using the above-ground biomass and the carbon content in leaves, thus obtaining the DIC uptake ( $\text{mmol m}^{-2} \text{ d}^{-1}$ ). The plastochrone interval (PI) was calculated according to the formula proposed by Jacobs (1979), as the time (days) necessary to produce a new leaf per shoot.

## 2.7. Data analyses

Statistical analyses were performed using the SPSS statistical package (SPSS Inc. USA, ver. 15.0). Differences between vegetated and unvegetated sediments were tested using one or two-way ANOVA with sites and sampling dates as factors; differences were accepted as significant at  $p < 0.05$ . Pearson correlation analyses were used to study correlation between variables.

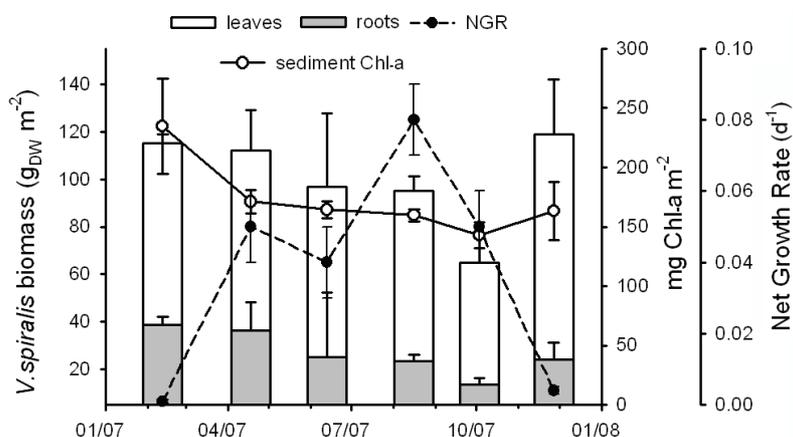
## 3. RESULTS

### 3.1. Water and sediment characteristics

Irradiance and water temperature exhibited a typical seasonal pattern, water flow decreased from April to August due to water withdrawal for irrigation (Tab. 1). Dissolved inorganic nitrogen (DIN) was mostly accounted by nitrate. Average DIN to SRP molar ratio ranged from 50.0 to 88.8. Over the whole sampling period dissolved oxygen ( $89.4 \pm 14.4\%$ ) was slightly below saturation, whilst carbon dioxide ( $377.2 \pm 182.6\%$ ) was always oversaturated (data not shown).

**Tab. 1.** Main features of the sampling site. Light fluxes are reported as the average irradiance of the sampling date, which were used for light incubation. Temperature, water flow and inorganic nutrient concentrations are daily averages±standard deviations (n=7).

Date	Flow (m <sup>3</sup> s <sup>-1</sup> )	Temperature (°C)	Irradiance (μE m <sup>-2</sup> s <sup>-1</sup> )	[NH <sub>4</sub> <sup>+</sup> ] (μM)	[NO <sub>2</sub> ] (μM)	[NO <sub>3</sub> <sup>-</sup> ] (μM)	[SRP] (μM)
08/02/07	13.2±1.3	9.4±1.1	180	16.0±1.1	3.6±0.2	65.7±4.7	1.7±0.1
18/04/07	6.4±2.6	15.2±2.2	320	5.1±0.7	2.7±0.1	67.0±3.1	1.3±0.1
13/06/07	10.8±1.3	22.4±1.8	480	3.4±0.9	2.1±0.5	122.5±4.2	1.6±0.2
16/08/07	8.1±1.3	25.1±2.1	430	4.3±0.3	1.2±0.1	122.4±19.7	1.5±0.1
03/10/07	13.3±0.8	21.3±2.8	290	6.7±0.5	1.6±0.4	114.4±10.1	4.1±0.1
27/11/07	11.3±2.4	11.3±1.0	210	5.2±0.5	4.6±0.3	314.3±5.7	3.6±0.2



**Fig. 2.** Variations of *Vallisneria spiralis* biomass, benthic chlorophyll-*a* (Chl-*a*) (0-5 cm sediment horizon) and net growth rates of *V. spiralis* (NGR) from February to November 2007.

Chl-*a* extracted from the upper 0.5 cm sediment layer, a proxy for microphytobenthos biomass, peaked in February ( $234\pm 40$  mg m<sup>-2</sup>) and declined to 142 mg m<sup>-2</sup> in October (Fig. 2).

The surface sediment (0-5 cm horizon) was muddy, with an average porosity of  $0.77\pm 0.11$ . The sedimentary organic C ( $9.93\pm 0.93\%$ ) and N ( $0.56\pm 0.12\%$ ), and total P ( $0.06\pm 0.02\%$ ) contents were rather constant during the whole study period.

### 3.2. *Vallisneria spiralis* biomass, elemental composition and growth rates

Total biomass of *V. spiralis* averaged  $99.8\pm 18.3$  g m<sup>-2</sup> as dry matter, with a slight decay in summer (Fig. 2). On average, the above to below ground biomass ratio was  $2.8\pm 0.8$ , spanning from 2.0 in February to 3.9 in November.

The elemental composition of the whole biomass of *V. spiralis* (pooled data of leaves and roots) was rather constant for organic C ( $31.35\pm 2.06$ ) and N ( $3.07\pm 0.35$ ), whilst total P increased in late autumn, especially in the below ground tissues, coinciding with high SRP concentrations in the water column (Tabs 1 and 2). Accordingly, the C:N molar ratio ( $12.0\pm 1.1$ ) was rather constant, while the C:P molar ratio decreased mainly in the root tissues (Tab. 2).

The leaf NGR followed a typical seasonal trend with a summer peak ( $0.080\pm 0.010$  d<sup>-1</sup>) and a minimum in December ( $0.0011\pm 0.0003$  d<sup>-1</sup>). A significant positive correlation was found between NGR and water temperature ( $r=0.48$ ,  $p<0.01$ ,  $n=90$ ), while NGR and mean irradiance were not correlated.

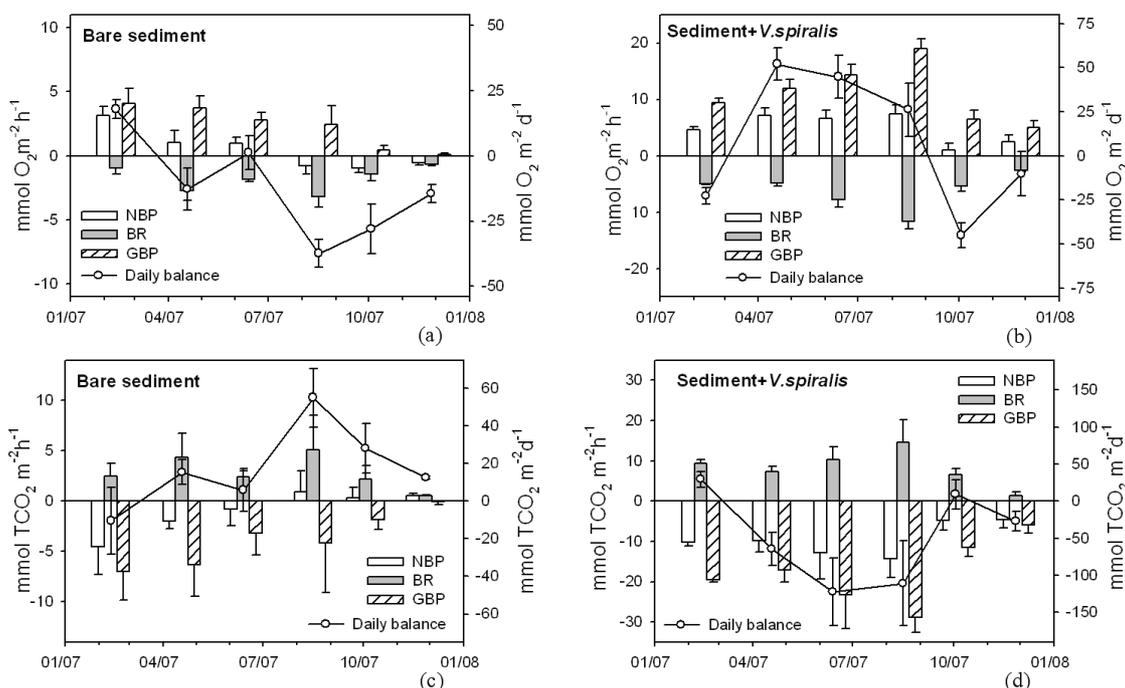
The leaf dry biomass to leaf area ratio ranged from  $10.2\pm 0.5$  to  $16.8\pm 0.7$  μg mm<sup>-2</sup>, through the growth period. Mean production of new leaves per shoot varied from 0 (November) to  $7.4\pm 0.5$  (May) and was correlated with NGR ( $r=0.58$ ,  $p<0.05$ ,  $n=10$ ).

The number of leaves per shoot differed greatly among plants and were comprised between 2 and 18 ( $n=90$ ). Shoot height peaked in early August at which time plants were about 60 cm tall. Dry weight of single shoots presented great variability, with a maximum in October ( $336.3\pm 60.3$  mg shoot<sup>-1</sup>) and a minimum in May ( $138.8\pm 13.3$  mg shoot<sup>-1</sup>).

The plastochrone interval (PI) was inversely correlated with NGR ( $r=-0.83$ ,  $p<0.01$ ,  $n=10$ ). Higher NGRs of *V. spiralis* in spring and late summer resulted in rapid renewal of leaves with PI close to 3.3 days. In late autumn and winter, much lower NGRs were coupled to extremely elevated PI, and no new leaves grew during the last sampling in December. From NGR, above-ground biomass and its elemental composition we calculated the total inorganic carbon fixed by the

**Tab. 2.** Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and C:N and C:P molar ratios in *Vallisneria spiralis* leaves and roots.

	TOC (%)	TN (%)	TP (%)	C:N (mol:mol)	C:P (mol:mol)
<b>Leaves</b>					
08/02/2007	32.50	3.14	0.83	12.1	100.9
18/04/2007	32.09	3.61	0.79	10.4	105.3
13/06/2007	33.70	3.35	0.59	11.7	147.6
16/08/2007	33.15	3.29	0.57	11.8	149.2
03/10/2007	34.30	3.34	0.85	12.0	103.8
26/11/2007	31.76	3.03	0.90	12.2	91.2
<b>Roots</b>					
08/02/2007	32.81	3.11	0.37	12.3	231.1
18/04/2007	29.22	3.16	0.50	10.8	149.8
13/06/2007	29.49	2.38	0.45	14.5	168.7
16/08/2007	28.83	2.52	0.58	13.3	129.3
03/10/2007	28.43	2.93	1.29	11.3	57.1
26/11/2007	29.91	2.96	1.17	11.8	66.3

**Fig. 3.** Hourly net benthic production (NBP), benthic respiration (BR) and gross benthic production (GBP) and daily production in bare and *Vallisneria spiralis* vegetated sediments of the Mincio River. Measures were made as dissolved oxygen ( $O_2$  - a, b) and dissolved inorganic carbon (DIC - c, d) fluxes with light and dark incubation of intact cores. Note that scales are different. Averages $\pm$ standard deviations ( $n = 4$ ) are reported.

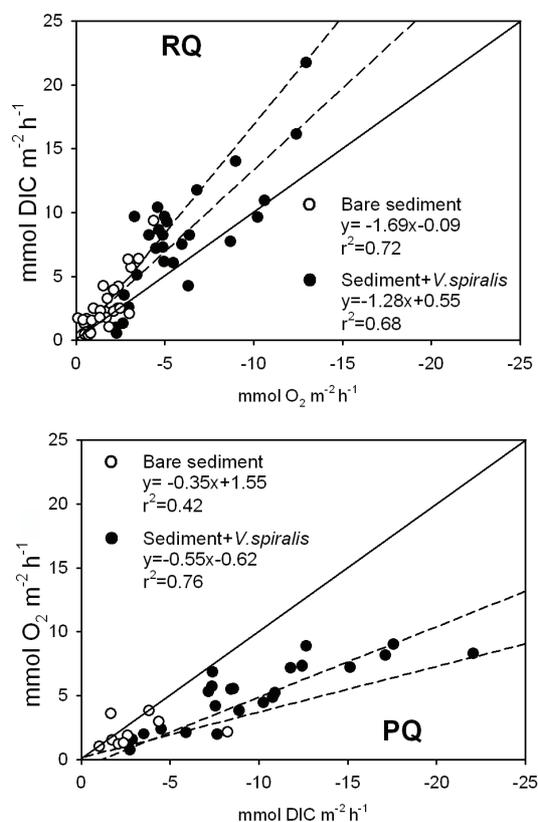
aboveground tissues, which ranged between 2.2 and 157.4 mmol C m<sup>-2</sup> d<sup>-1</sup>.

### 3.3. Benthic metabolism and fluxes

In the *V. spiralis* stands, net benthic production (NBP), respiration (BR) and the resulting gross benthic production (GBP) rates underwent a clear seasonal trend (Fig. 3). Daily NBP and BR rates of vegetated sediments were up to five times higher than in bare sediments, as a result of the macrophyte activity. Accordingly, GBP increased with water temperature and peaked in August. In the unvegetated sediments, NBP

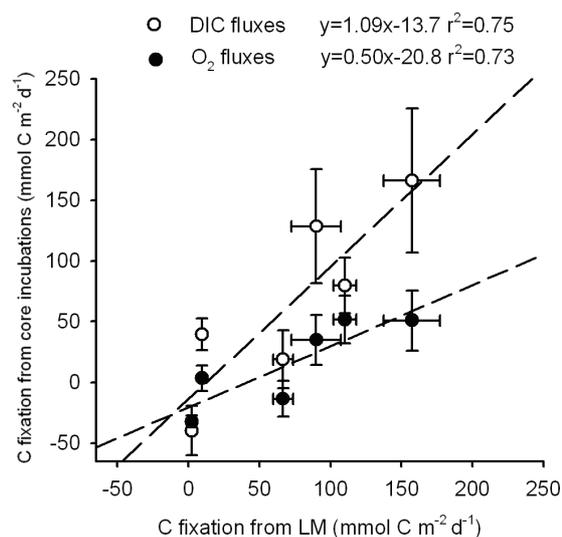
and GBP maximum values were recorded in spring, whilst the highest BR were measured in August, coinciding with the temperature peak.

At the annual scale, the bare sediment was a net  $O_2$  sink ( $-4.40 \pm 0.95$  mol m<sup>-2</sup> y<sup>-1</sup>) and a DIC source ( $6.41 \pm 1.80$  mol m<sup>-2</sup> y<sup>-1</sup>). Overall, the *V. spiralis* meadow was net autotrophic, with an annual NBP of  $2.73 \pm 1.56$  mol m<sup>-2</sup> y<sup>-1</sup> and  $-17.2 \pm 4.8$  mol DIC m<sup>-2</sup> y<sup>-1</sup>. Benthic vegetation also accounted from 44 to 83% of BR. Respiratory quotients  $RQ > 1$  coupled with low photosynthetic quotients  $PQ < 1$  evidenced a strong imbalance between the DIC and  $O_2$  dynamics (Fig. 4).



**Fig. 4.** Respiratory (RQ) and photosynthetic (PQ) quotients calculated with data from the six sampling periods for bare and *Vallisneria spiralis* vegetated sediments. The continuous line indicates identical fluxes of the two gases. Details in the text.

The benthic metabolism was also analysed estimating carbon fixation rates with different independent methods. First, we calculated the carbon fixation with the leaf marking growth data. Leaf NGR was multiplied by the above-ground biomass of *V. spiralis* and its C content measured on each sampling date. This technique underestimated the real carbon fixation as we did not consider the growth of the below-ground quota, which on average accounted for the 27% of the total biomass, as well as we did not take into account grazing and mechanical leaf losses. Carbon fixation was also estimated from NBP, BR and GBP rates. For this purpose, data of vegetated sediments were corrected with the corresponding data of bare sediments. The assumptions we made were that sedimentary process rates were not different in bare and vegetated sediments and that DIC was taken up by *V. spiralis* from water. Inorganic carbon fixation was also calculated from oxygen fluxes using  $PQ = 1.2$  (Kemp *et al.* 1986; Schramm *et al.* 1984; Wetzel & Likens 1991). We found a good agreement between fixation rates estimated with the leaf marking and with DIC fluxes, while oxygen fluxes underestimated carbon fixation rates, which were on average 50% lower (Fig. 5).

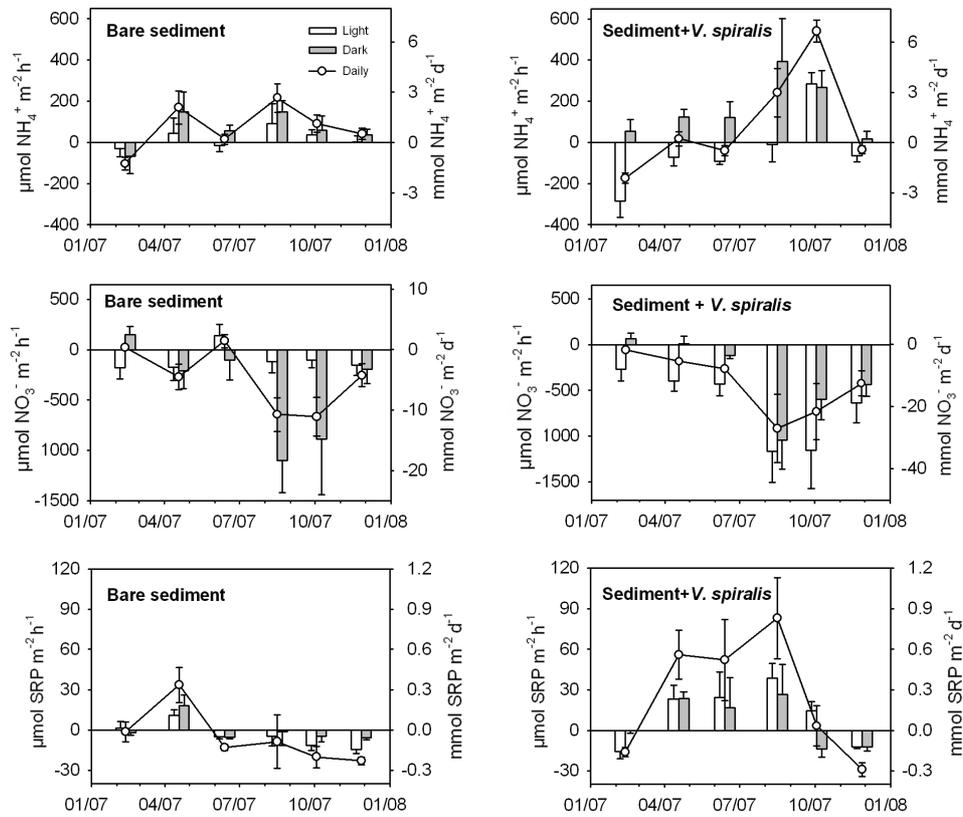


**Fig. 5.** Dissolved inorganic carbon (DIC) uptake calculated from growth rates obtained via the leaf marking techniques (LM) is plotted together with inorganic carbon uptake calculated from core incubations, in particular from DIC (white dots) and  $O_2$  fluxes (black dots). For the latter we applied a photosynthetic quotient of 1.2. See the text for major details.

Ammonium fluxes were greatly affected by benthic vegetation (Fig. 6). Hourly dark rates correlated with water temperature ( $r = 0.74$ ;  $p < 0.01$ ,  $n = 24$ ), with a net sediment release in August, especially in the macrophyte stand. Daily  $NH_4^+$  fluxes showed how bare sediments were mainly a net source of  $NH_4^+$  to the water column, whilst the *V. spiralis* meadow was a net sink during the active growth and a net source during the senescence phase. Overall, the annual budget evidenced that both bare ( $321 \pm 91$  mmol  $m^{-2} y^{-1}$ ) and vegetated sediments ( $409 \pm 102$  mmol  $m^{-2} y^{-1}$ ) were net sources of  $NH_4^+$  to the water column.

The large availability of  $NO_3^-$  in the water column was associated with high  $NO_3^-$  uptake rates at both vegetated and bare sediment sites (Fig. 6). In the bare sediment,  $NO_3^-$  uptake was usually greater in the dark than in the light, whilst in the *V. spiralis* stand  $NO_3^-$  consumption prevailed in the light. Based on these findings we assumed that nitrate fluxes were controlled by both microbial denitrification and benthic vegetation in the meadow, while microbial processes prevailed in the bare sediment. Overall, the macrophyte meadow ( $-4581 \pm 1378$  mmol  $m^{-2} y^{-1}$ ) was a significantly higher nitrate sink than unvegetated sediment ( $-1727 \pm 854$  mmol  $m^{-2} y^{-1}$ ).

In the *V. spiralis* meadow, SRP fluxes were positively correlated with temperature ( $r = 0.65$ ,  $p < 0.01$ ,  $n = 24$ ) (Fig. 6). A significant interaction term (two-way ANOVA, site  $\times$  date,  $p < 0.05$ ) indicated that differences between SRP fluxes depended on season. Only slight SRP uptake rates were determined in the bare sediment, where no significant differences were found between



**Fig. 6.** Light, Dark and Daily fluxes of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and SRP in bare and *Vallisneria spiralis* vegetated sediments of the Mincio River. Averages  $\pm$  s.d. ( $n = 4$ ) are reported.

light and dark conditions and among seasons (Fig. 6). On an annual basis, bare sediment was a net SRP sink ( $-19.4 \pm 13.4 \text{ mmol m}^{-2} \text{ y}^{-1}$ ), while the *V. spiralis* meadow was a net SRP source to the water column ( $89.3 \pm 31.8 \text{ mmol m}^{-2} \text{ y}^{-1}$ ).

### 3.4. Denitrification rates

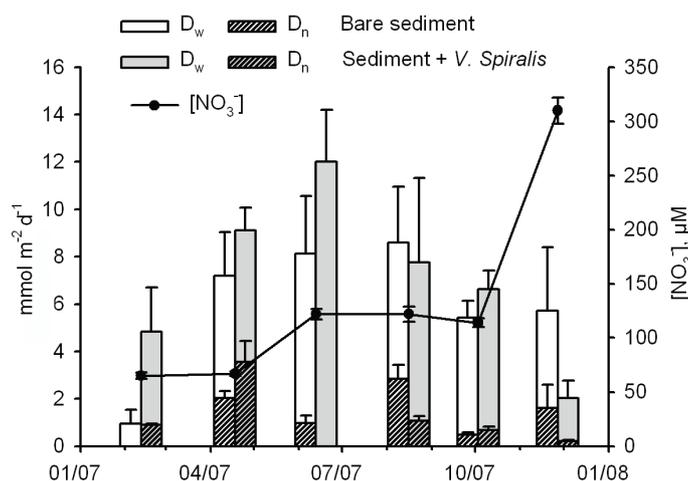
Total denitrification measured in the dark was mostly supported by the reduction of  $\text{NO}_3^-$  diffusing to anoxic sediments from the water column. The annual  $D_w$  accounted for more than 80% of  $D_t$  in both bare sediments and *V. spiralis* stands (Fig. 7).  $D_t$  rates were positively correlated with water temperature ( $r=0.81$ ,  $p < 0.01$ ,  $n=48$ ) and followed a clear seasonal pattern with a summer peak. Differences in denitrification rates were significant between sampling dates (ANOVA,  $p < 0.01$ ), but not between sites. Overall,  $\text{NO}_3^-$  removal by denitrification was  $2173 \pm 277 \text{ mmol m}^{-2} \text{ y}^{-1}$  in the bare sediment and  $2782 \pm 634 \text{ mmol m}^{-2} \text{ y}^{-1}$  in the macrophyte meadows.

## 4. DISCUSSION

### 4.1. Benthic vegetation: biomass, growth and feedbacks

In the upper reach of the Mincio River, *V. spiralis* formed homogeneous stands but with biomass lower

than that reported for the same species and comparable to that reported for *V. americana* in running waters (Briggs & Maher 1985; Donnermeyer & Smart 1985). To our knowledge this is the first study reporting growth rates of *V. spiralis* measured with the leaf-marking technique, which instead has been extensively used mainly with seagrass species (Hauxwell *et al.* 2007). Unexpectedly, in our study the biomass minimum was attained in summer when leaf NGR peaked. Likely, the *V. spiralis* meadow underwent consistent leaf loss, as evidenced by the macrophyte wrack which accumulated downstream (data not shown). Nonetheless, the meadow was spreading and covering the whole river bed along the considered river reach. Among other factors, we assumed that the meadow formation and persistence was supported by plant propagules delivered from the Lake Garda and from the meadow itself and was facilitated by low water depth and slow water motion (Riis & Sand-Jensen 2006; Riis 2008). Likewise, the rhizosphere development gave further advantages, allowing macrophyte to resist against eradication (Stevenson 1988; Sand-Jensen 2008). The annual average root to shoot ratio (RSR) estimated from the below to above ground biomass of *V. spiralis* was  $0.37 \pm 0.10$ , which was among the highest RSR reported for submerged freshwater aquatic plants (Stevenson 1988).



**Fig. 7.** Total denitrification rates, splitted in the contributions of  $D_w$  (denitrification of nitrate from the water column) and  $D_n$  (denitrification coupled to nitrification) in bare and *Vallisneria spiralis* vegetated sediments and water  $NO_3^-$  concentration at the sampling site. Averages  $\pm$  s.d. ( $n = 4$ ) are reported.

Nutrient availability was assumed not to limit macrophyte growth, the minimum N (3.03%) and P (0.57%) content in plant tissues being far above the lower limit for *V. spiralis* to actively grow (N=1.3% and P=0.13%, Gerloff & Krowbholz 1966).

#### 4.2. Benthic vegetation and ecosystem processes

Oxygen and dissolved inorganic carbon fluxes resulted stoichiometrically unbalanced in both bare and vegetated sediments. High oxygen consumption rates were mainly found in bare sediments and during the senescence of *V. spiralis*. Bare sediments were net heterotrophic with an average respiratory quotients  $RQ > 1$ , evidencing that organic matter was metabolized also anaerobically and/or reduced carbon metabolites (e.g.,  $CH_4$ ) were re-oxidised to  $CO_2$  (Andersen & Kristensen 1988).

The *V. spiralis* meadow was net autotrophic, but with a photosynthetic quotient  $PQ < 1$ , which was on average low in comparison with  $PQ$ s of other freshwater submerged macrophytes (Kemp *et al.* 1986; Sand-Jensen *et al.* 2007). Within the meadow, the oxygen release to inorganic carbon uptake ratio was thus unbalanced, with a missing  $O_2$  quota. Since  $RQ$  was close to 1, we assumed that aerobic and anaerobic microbial processes were balanced and that missing  $O_2$  quota was not due to respiratory processes but was rather transported into the sediments. Oxygen transport and an active radial oxygen loss by *V. spiralis* was further demonstrated with ongoing *in situ* experiments (Bartoli, unpubl. data).

The missing quota of  $O_2$  from GBP in the *V. spiralis* meadow can be proved also with the comparison of carbon fixation data which were independently estimated from NGR, DIC and  $O_2$  fluxes (Fig. 5). We can argue that carbon fixation assessed by  $O_2$  fluxes underestimated the actual rates by some 50%, which can be

accounted by the  $O_2$  not released into the water column but transferred to roots (see also Kemp *et al.* 1986).

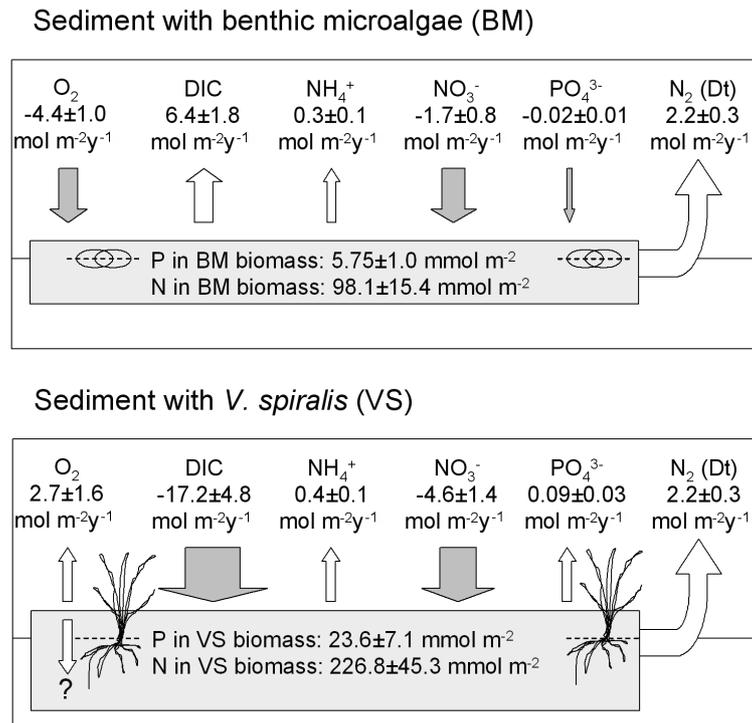
Overall, the carbon fixation rates in the range  $0.02$ – $0.16$  mol  $m^{-2} d^{-1}$  were consistent with highly productive systems, e.g. lake littorals and freshwater wetlands (Titus & Stephens 1983; Briggs & Maher 1985). Finally, the comparison of bare and vegetated sites indicated that the development of *V. spiralis* stands turned the riverbed from heterotrophic (*sensu* Vannote *et al.* 1980) to autotrophic and from a carbon source to a carbon sink.

#### 4.3. Biogeochemical implications for nutrients losses

We studied relationships between benthic vegetation and biogeochemical processes, addressing uptake and retention within biomass, water-sediment exchanges and denitrification. The water in the northern reach of the Mincio River was polluted by  $NO_3^-$ ; the river was less impacted by phosphates, although SRP concentrations were higher than in the Lake Garda outflow.

From Chl-*a* and *V. spiralis* biomass data we calculated N and P average pools in standing stocks. Theoretical N and P uptake were also assessed from C:N:P ratios and NBP estimated from DIC fluxes.

Benthic microalgae immobilised  $5.75 \pm 0.96$  mmol P  $m^{-2}$  (annual average), which was equivalent to the P amount retained by *V. spiralis* roots. The above ground biomass of *V. spiralis* was a major P storage with  $18.02 \pm 5.48$  mmol P  $m^{-2}$ . Theoretical SRP uptake from microphytobenthos averaged  $0.04 \pm 0.01$  mol  $m^{-2} y^{-1}$  meaning that about 50% of SRP requirements came from the porewaters. The theoretical P uptake by *V. spiralis* was  $0.35 \pm 0.13$  mol  $m^{-2} y^{-1}$ , but the meadow was a net SRP source to the water column, indicating that the SRP assimilation by *V. spiralis* was not sufficient to compensate for benthic regeneration. Differences between vegetated and unvegetated areas were likely



**Fig. 8.** N and P pools in the standing stock of benthic microalgae and *Vallisneria spiralis* (annual averages ± s.d.) and annual fluxes of O<sub>2</sub>, DIC, inorganic nutrient fluxes and denitrification rates in bare and vegetated sediments.

due to the accumulation of macrophyte debris and riverine particulate matter which fuelled microbial decomposition of the accumulated detritus (Stevenson 1988; Madsen *et al.* 2001) (Fig. 8).

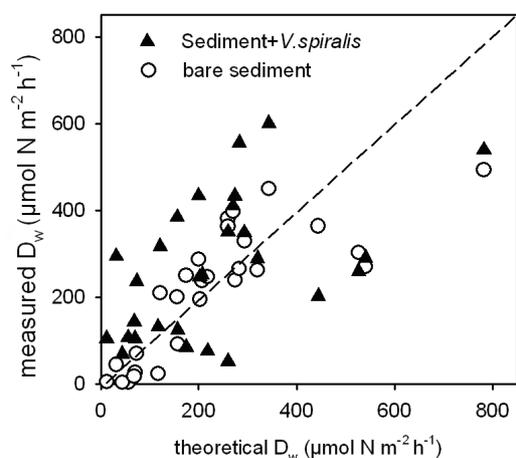
The N storage by microphytobenthos averaged 92.08±15.36 mmol N m<sup>-2</sup> and the theoretical N requirements to sustain microalgal production, estimated in 0.48±0.26 mol m<sup>-2</sup> y<sup>-1</sup>, was mainly supplied by sediment regeneration as the sediment itself was a net ammonium source to the water column. *V. spiralis* retained 226.8±45.3 mmol N m<sup>-2</sup> of which 75% was in the above ground portion. N requirements by the macrophyte (3.46±1.49 mol m<sup>-2</sup> y<sup>-1</sup>) were in part sustained by pore-water (Lodge *et al.* 1989; Schramm 1999; Nielsen *et al.* 2004) and hyporheic flow (Carignan 1982; Hilton *et al.* 2006) and in part (about 60%) by water column nitrate.

In this study we measured only dark denitrification as we assumed that this river stretch was not nitrogen limited and with little to no competition between primary producers, nitrifiers and denitrifiers, according to Rysgaard *et al.* (1995). Denitrification rates were among the highest reported in the literature (Seitzinger 1988; Piña-Ochoa & Álvarez-Cobelas 2006), although we believe that they were underestimated, mostly due to a methodological limitation (see later). On average, denitrification of dissolved nitrate from the water column accounted for more than 80% of the total denitrification. Light incubations would have probably resulted in D<sub>n</sub> rates higher than those measured in the dark incubations because benthic microalgal activity might potentially

increase oxygen availability at the interface and oxygen transport into the uppermost sediment horizon, thus stimulating the nitrification coupled denitrification. This is a controversial issue, since Rysgaard *et al.* (1995) found that D<sub>n</sub> was stimulated by microphytobenthos in absence of competition with bacteria, when NO<sub>3</sub><sup>-</sup> concentration in the water column was >20 μM, whilst studies in oligotrophic shallow waters demonstrated that light stimulated uptake by microphytobenthos and inhibited denitrification due to strong competition between algae and bacteria (Sunbäck *et al.* 2000). Caffrey & Kemp (1992) reported that nitrification activity was enhanced in macrophyte beds, as a consequence of radial oxygen loss which supported nitrification coupled denitrification well below the upper oxic sediment layer. In our site competition between denitrifiers and macrophytes was likely avoided by the very high NO<sub>3</sub><sup>-</sup> concentration (65 to 315 μM) and water renewal. The denitrification process was also fuelled by the availability of labile organic matter, deriving from particles sedimentation, vegetation debris and roots exudates.

We also calculated D<sub>w</sub> rates with the model of Christensen *et al.* (1990), which were consistent with measured D<sub>w</sub> data mainly in bare sediments (Fig. 9). However, a greater variability was found in vegetated than in bare sediment, with values both above and below those estimated with the model. Benthic vegetation can influence microbial denitrification in different ways. Processes can be stimulated by leaves, that can

support microbial communities, while exudates and organic debris can provide energy to heterotrophic microbial metabolism, namely  $D_w$ .



**Fig. 9.** Measured and modelled denitrification of nitrate diffusing to anoxic sediment from the water column ( $D_w$ ); see the text for major details.

Nitrification coupled denitrification is often occurring in the rhizosphere, thanks to radial oxygen loss and redox conditions which establish in microlayers around root hairs (Caffrey & Kemp 1992). Methodological constraints have also to be considered. The isotope pairing technique is a reliable method for measuring denitrification in unvegetated and not bioturbated sediments, whilst it can underestimate denitrification either in the deeper sediments horizons or in the rhizosphere. Reddy *et al.* (1989) found a conspicuous transport of oxygen in the rhizosphere of three wetland plants which was coupled to very high coupled nitrification-denitrification rates, up to 300–400  $\mu\text{mol N m}^{-2} \text{h}^{-1}$ . Other examples of submerged macrophytes with elevated radial oxygen loss which stimulate porewater N removal are *Littorella uniflora* and *Lobelia dortmanna* (Sand-Jensen *et al.* 1982; Risgaard-Petersen & Jensen 1997). For seagrasses that are morphologically similar to *V. spiralis* (e.g., *Zostera marina*) and other stream macrophytes as *Potamogeton perfoliatus*, radial oxygen loss is a minor fraction of photosynthetic oxygen production which keeps very low nitrification coupled denitrification rates (Sand-Jensen *et al.* 1982; Kemp *et al.* 1986; Risgaard-Petersen *et al.* 1998; Ottosen *et al.* 1999). To our knowledge no previous work addressed denitrification processes in benthic systems with *V. spiralis*, and further studies will be required to highlight also the rhizosphere role, e.g. labelling the porewater with  $^{15}\text{NH}_4^+$  (Risgaard-Petersen & Jensen 1997).

## 5. CONCLUSIONS

The difference between benthic exchanges occurring in *V. spiralis* meadow and bare sediments in the Mincio

River can be highlighted analysing the annual fluxes of  $\text{O}_2$ , DIC and nutrients (Fig. 8). The most significant differences deal with nutrient pools in primary producers standing stocks, with higher N and P immobilisation and slower turnover in macrophyte biomass. But the main effect of the riverbed colonisation by *V. spiralis* was to switch benthic metabolism from heterotrophic to autotrophic. The macrophyte meadow had a relatively less important role as oxygen source to the water column compared to its capacity to sequester inorganic carbon. Inorganic carbon uptake by macrophytes resulted in downward transport of fixed organic carbon as leaves or plant debris and in an attenuation of  $\text{CO}_2$  supersaturation values in the water column, which is typical of running waters.

Benthic vegetation regulated key biogeochemical processes, as nitrate uptake and denitrification which are of paramount importance due to river pollution by nitrates. Benthic vegetation, which established probably due to hydromorphological changes, fixed annually an amount of nitrogen comparable to that lost via denitrification, although plant uptake represents only a temporary N storage. The denitrification and macrophyte uptake rates estimated in this study are relevant compared to those reported in other studies, although they are some orders of magnitude lower than  $\text{NO}_3^-$  loadings.

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