Mitigation of nitrogen pollution in vegetated ditches fed by nitrate-rich spring waters

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Abstract

In permeable soils, excess nitrate from agriculture is transported vertically and accumulates in aquifers. However, it can come back to the surface via groundwater movement and pollute watercourses. We hypothesized that vegetated ditches may dissipate significant amounts of nitrate from spring waters, and represent a buffer system to protect downstream water bodies from eutrophication. To test this hypothesis, nitrate removal was measured in ditches fed by nitrate-rich groundwater in presence and absence of emergent vegetation. Reach-scale methods (N_2 open-channel, N budgets) were coupled with laboratory incubations of sediment cores (benthic N fluxes, isotope pairing) and plant N uptake estimation. Studied ditches are representative of a wide hydrological network in Northern Italy, within the so-called "spring-belt" (Po River plain), a NO_3^--vulnerable area with high density of contaminated springs.
Results indicated a greater reach-scale N removal in vegetated (38-84 mmol N m^{-2} d^{-1}) as compared to unvegetated condition (12-45 mmol N m^{-2} d^{-1}). Denitrification was the dominant N-removal pathway, while plant uptake represented a minor fraction of the net N abatement. Large development of interfaces for microbial growth provided by aquatic vegetation and more opportunities for biotic interactions are features that promote nitrate reduction in the ditch network. Despite the vegetated ditches were significant N-reactors, denitrification provided a little N-removal to in-stream high nitrate loads, with the exception of periods when plant coverage and water retention time peaked. Management of N-saturated ditches may consist in the enlargement of stretches to increase water retention and amplify the interfaces where biofilms develop, though preserving hydraulic efficiency. The maintenance of vegetation in the ditch networks would result in a significant N-abatement on a larger scale.

Keywords

Ditch network; nitrate pollution; springs; vegetation; denitrification; N_2 open-channel method

1. Introduction

In the last century, the intensification of agricultural activities and soil loss due to urbanization have deeply simplified the landscape in lowland areas, by removing natural habitats as wetlands and riparian vegetated zones (Groffman et al., 2003; Hefting et al., 2014). These human-impacted watersheds have lost their capacity to buffer excess nutrient loads, whose delivery to coastal waters is further accelerated by river morphological alterations such as channelization, burial, and water withdrawal, and by decreased connectivity between riverbeds and floodplains (Roley et al., 2012; Beaulieu et al., 2015). Modern agriculture has further modified the landscape through the implementation of extensive artificial canal networks, dug ex novo for wetland reclamation or resectioned in former natural river networks. In both cases, they often constitute a capillary network arranged to maximize multiple water uses, as drainage and irrigation, and have become integral components and ubiquitous features of many productive agro-ecosystems (Pierce et al., 2012; Dollinger et al., 2015).

Small-size watercourses, no matter if natural or artificial, are the interfaces between agricultural lands and downstream aquatic ecosystems, such as rivers, estuaries or lagoons and coastal waters. Such canals are characterized by multiple interfaces between water, sediment and aquatic vegetation (Marion et al., 2014; Pinay et al., 2015). Here, nitrogen (N) removal takes place as a result of several plant and microbially-mediated N transformations, among which the dominant are assimilation and denitrification, the reduction of nitrate (NO_3^-) to N gases under anaerobic conditions (Schaller et al., 2004; Bernot and Dodds, 2005, Mulholland et al., 2008). Small canals and ditches can contribute significantly to watershed N dynamics because of their capillary distribution and high metabolic capacity. The latter is sustained by the high ratio between bio-reactive surfaces, directly or indirectly ascribable to aquatic macrophytes, and water volumes (Marion et al., 2014; Srivastava et al., 2016).

Recent studies have suggested a new perspective on the role of canal networks in watershed N dynamics by proving that agricultural basins can generate large N excess but little export (Bartoli et

al., 2012; Castaldelli et al., 2013; Romero et al., 2016). Interest has grown on the identification and parametrization of landscape elements that provide high rates of N removal. However, the debate is still open if intensively cultivated systems may maintain or not these relevant ecosystem functions, since aquatic vegetation is generally considered an impediment for water circulation and mechanically removed during routine management practices of ditches (Duncan et al., 2013; Pinay et al., 2015).

While many studies have investigated N removal in wetlands and afforested riparian zones (e.g. Balestrini et al., 2008; Tournebize et al., 2017), the process is understudied in ditches and canals (Pierobon et al., 2013; Taylor et al., 2015; Balestrini et al., 2016; Iseyemi et al., 2016). Although there are profound implications for the re-establishment of beneficial ecosystem services in agricultural basins with extensive water networks, only a few studies provided field experimental data on N dynamics in ditches suitable to upscaling at the watershed level (e.g. Birgand et al., 2007; Castaldelli et al., 2015). Conventional methods applied on intact sediment cores (i.e. isotope pairing technique; Nielsen 1992) give precise estimates of denitrification rates but cannot be used to infer about processes in lotic environments where multiple riverine habitats and interfaces exist (e.g. sediments with irregular associations of submerged and/or emergent macrophytes). The N_2 open-channel method provides direct whole-system estimates of denitrification in running waters derived from accurate measurements of N_2 concentrations in a conceptual moving parcel of water while accounting for gas exchanges with the atmosphere. This methodology integrates small-scale spatial and temporal variability in processes and overcomes the limitations inherent in the upscaling of results from the laboratory to the field (e.g. measurements performed over small surfaces, incubation artifacts, etc.). Further, this method quantifies denitrification under natural conditions at spatial and temporal scales appropriate to assess its relevance to watershed N fluxes modelling and management (Gardner et al., 2016; Reisinger et al., 2016). Eventually, the net effect of vegetation on in-stream N metabolism can be discriminated if two conditions (vegetated and unvegetated) are compared, and the multiple N pathways can be disentangled if several methods are concomitantly applied (Castaldelli et al., 2015).

The aim of the present study was to quantify N removal in ditches fed by spring water contaminated by NO_3^-, in the presence and absence of emergent vegetation and along its growth cycle. Studied ditches belong to the "spring-belt", an area of the Po valley (Northern Italy) where groundwater interacts with surface waters due to many permanent man-modified resurgences, locally known as "*fontanili*", originating from changes in slope profile and soil permeability. Here, NO_3^--contaminated groundwater may pollute rivers, canals, ditches and downstream water bodies, if NO_3^- is not intercepted (Laini et al., 2011; Sacchi et al., 2013; Viaroli et al., 2015). In this study, denitrification rates estimated in ditches by the N_2 open-channel method were compared to reach-scale budgets of inorganic N species and to benthic N fluxes measured by sediment core incubations, according to an experimental protocol previously validated for similar watercourses in the lower portion of the Po River basin (Castaldelli et al., 2015). As NO_3^- availability from the groundwater feeding the ditch network is always not limiting and almost constant during the year, we hypothesize high denitrification rates and emergent vegetation as the primary control on in-stream N dynamics, by sustaining quantitatively relevant microbial processes responsible for N removal. In fact, under N excess the competition between uptake by primary producers and microbial denitrification is smoothed and high rates of both processes can simultaneously occur (Soana et al., 2015; Racchetti et al., in press).

Results from this and other similar studies may define appropriate management practices of ditches and canals, targeting NO_3^- removal. In the development of the hydrological network, from small waterways to large and deep canals, shallow ditches represent the level at which it is possible to operate effective management practices, avoiding hydrological risks and economically unsustainable investments. The hypothesis of intervention implies the recovery of dense vegetation stands in suitable stretches, to enhance NO_3^- removal.

2. Material and Methods

113 *2.1 Study area and experimental approach*

114 The study was carried out in two ditches, V (with in-stream vegetation) and U (without in-stream
115 vegetation), located in the eastern part of the Metropolitan City of Milan (Lombardy Region, Northern
116 Italy). The two sites belong to the central, flat, agricultural plain of the Po River watershed, within
117 the Lambro River sub-basin (Fig. 1). This territory is highly urbanized and industrialized, but large
118 portions still host agricultural lands, crossed by dense networks of artificial canals and ditches, built
119 over the course of centuries for drainage and irrigation purposes. The two ditches (V: 45°27'25.78"N,
120 9°24'59.23"E; and U: 45°27'25.21"N, 9°24'47.91"E) are adjacent, similar in length, uniform in
121 morphology and without any lateral surface water input or output along the studied stretches (Fig.1,
122 Table 1). They are fed by NO_3^--rich groundwater, originating from the same spring named "Quattro
123 Ponti" (Fig.1; Table 1) and are representative of this territory, where many springs feed steadily a
124 dense hydrological network of ditches and canals although with seasonal variations of discharge
125 (Laini et al., 2011). The fields surrounding the two investigated sites, cultivated with maize, affected
126 ditch flow only during extremely heavy rain events, which anyway did not take place during sampling
127 periods.

128 Macrophyte coverage at V was persistent over the three sampling dates and included the emergent
129 reed canary-grass *Typhoides arundinacea* L. Moench (syn *Phalaris arundinacea* L.) and some
130 submerged species, among which *Elodea canadensis* was the most widespread. As described
131 afterwards in section 2.3, species-specific coverage was measured only for the dominant species, *T.*
132 *arundinacea*, whose biomass accounted on average >90% of the total plant biomass along the studied
133 stretch in all samplings. At the end of the growing season, after the crop harvest on the nearby fields,
134 the local water authority makes a vegetation mowing, usually in October. U is bordered by a narrow
135 riparian strip (< 4 m) on both sides consisting of hardwoods, mostly oaks (*Quercus robur*) and elms
136 (*Ulmus* sp.). The presence of the canopy naturally hampers the development of in-stream aquatic
137 vegetation. Coarse particulate organic matter and woody debris are randomly present in the ditch bed.
138 At both sites, phytoplankton is not a relevant primary producer (chlorophyll-*a* <0.5 µg L^{-1}).

Three sampling campaigns were carried out in May, July, and September 2014, during stable hydrological and meteorological conditions and with no effect of rainfalls. Samplings were performed in the dark and in the light, from 02:30 a.m. to 05:30 a.m. and from 01:30 p.m. to 04:30 p.m., respectively. The experimental programme was planned to cover three key stages of the plant growth cycle, namely the spring growth phase, the summer areal coverage peak, and the maturity phase at the end of summer. Two sampling stations (upstream and downstream) were selected on each ditch, and three experimental approaches were applied: 1) N_2 open-channel method; 2) reach-scale balance of inorganic N species and, 3) incubation of bare sediment cores. Methods 1) and 2) were applied both in the dark and light phase to discriminate the effect of photosynthetic processes on N metabolism. Finally, for V, N uptake by in-stream vegetation was calculated by areal coverage, growth rates and biomass elemental composition, and compared to the reach-scale balance of N species.

2.2 N_2 open-channel method

Reach-scale denitrification was modelled according to the method proposed by Laursen and Seitzinger (2002). The approach provides the direct estimate of the process by measuring the variation of the end-product N_2 in a parcel of water as it moves downstream, from the N_2:Ar ratio analysed by Membrane Inlet Mass Spectrometry (MIMS). A model-based approach is used to solve for denitrification rates, correcting the variations of N_2 for re-aeration during downstream transport. Both N_2 and Ar concentrations are assumed to change during downstream transport as a function of re-equilibration with the atmosphere, and for N_2 also as a function of microbial activity. Denitrification is estimated based on the production rate required to explain the excess N_2, namely the measured change not accounted for by atmospheric exchanges. This approach allows for the calculation of *in situ* net N_2 fluxes (i.e. total denitrification, including coupled nitrification/denitrification, minus N_2 fixation) at a reach-scale, where accurate NO_3^- mass balances can be also performed.

164 The selected ditches were sampled from access points using a Lagrangian two-stations (upstream and
165 downstream) approach. Water samples for dissolved N_2 analyses were collected from the same water
166 parcel as it moved downstream. Mean velocity and travel time of the water parcel between the two
167 sampling locations were calculated from the wet section area and discharge. Depth was measured at
168 equally spaced vertical transects, from a minimum of 6 to a maximum of 10 depending upon the ditch
169 width. Water velocity was measured in a grid of points along the ditch sections, typically every 0.5
170 and 0.1 m along the horizontal and vertical axes, respectively, by means of a current meter (Open
171 Stream CurrentMeter 2100) mounted on a measuring pole with centimetric resolution, equipped with
172 a modified propeller for low flow conditions. Data were then integrated to calculate ditch discharge
173 by means of the software Surfer® 11 (Golden Software, LLC). This procedure was replicated at
174 multiple cross sections, three at least, for each ditch and average discharge was obtained.
175 Water temperature, oxygen and conductivity were measured with a multiparametric probe (Ocean
176 Seven, 316, Idronaut, Italy). Samples for N_2:Ar were withdrawn in triplicate directly from the ditches
177 using a glass syringe and transferred into 12-mL glass-tight vials (Exetainer, Labco, High Wycombe,
178 UK), flushing at least 3 times the vial volume and preserved by adding 100 μL of $ZnCl_2$ saturated
179 solution. The N_2:Ar ratio in water samples was measured using a MIMS (Bay Instruments, Easton,
180 MD; Kana et al., 1994) at the laboratory of Aquatic Ecology, University of Ferrara. The coefficient
181 of variation calculated from replicated N_2:Ar samples (n=10) was 10-fold lower (~0.04%) than N_2
182 measurements (~0.4%), in accordance to Laursen and Seitzinger (2002). N_2 concentration was
183 calculated from the measured N_2:Ar multiplied by the equilibrium Ar concentration at the *in situ*
184 water temperature, determined from the solubility equation (Weiss, 1970; Taylor et al., 2015).
185 Net N_2 fluxes at the reach scale were simulated at 1-min time steps by providing the following model
186 input parameters: measured N_2 concentrations and water temperature at upstream and downstream
187 stations, average depth and width, gas transfer velocity (k600), Schmidt number coefficient (2/3 for
188 surfaces without waves; Jähne et al., 1987), and travel time of the water parcel from upstream to
189 downstream, calculated from average current velocity (Laursen and Seitzinger, 2002). If the ratio of

190 stream velocity to stream depth is higher than 0.03 s^{-1}, benthic turbulence is considered the primary
191 driver of gas exchanges (Schwarzenbach et al., 1993). As this criterion was fulfilled for the
192 investigated reaches, the oxygen reaeration coefficient (KO_2, 20°C, d^{-1}) was calculated by means of
193 a set of empirical equations (Haider et al., 2013), which use average current velocity and water depth
194 as the only variables affecting reaeration. The transfer velocity for oxygen (kO_2, 20°C, cm h^{-1}) was
195 obtained by multiplying each reaeration coefficient by the correspondent water depth, assuming a
196 well-mixed water column, and finally normalized to a Schmidt number of 600 (k600, for CO_2 at 20
197 °C, cm h^{-1}) (Jähne et al., 1987; Wanninkhof, 1992). A conservative approach was adopted in this
198 study, as a set of depth–velocity equations was applied with the aim of providing a range of k600
199 values, likely including the true value of each investigated ditch (Laini et al., 2011; Castaldelli et al.,
200 2015). Simulations were run by varying the gas transfer parameterizations and a range of N_2
201 production rates was obtained for each reach in each sampling period. The upstream–downstream
202 balance of inorganic N species sets a limit to the minimum in-stream N removal and the comparison
203 to the N_2 open-channel method helped to identify the more realistic reaeration coefficient for each
204 system. Hourly rates (mmol N m^{-2} h^{-1}) were multiplied by the correspondent number of light and dark
205 hours in each of the three investigated months (15, 16, 13 hours of light in May, July, and September,
206 respectively) and summed to obtain daily values (mmol N m^{-2} d^{-1}).
207
208 *2.3 Reach-scale inorganic N mass balance and N uptake by vegetation*
209 Samples for dissolved inorganic N (DIN) as sum of NO_3^-, nitrite (NO_2^-) and ammonium (NH_4^+) were
210 collected in triplicate simultaneously to dissolved N_2:Ar, filtered through Whatman GF/F glass fiber
211 filters, transferred to polyethylene vials and frozen for later analysis. NH_4^+ was determined on a
212 double beam Jasco V-550 spectrophotometer using salicylate and hypochlorite in the presence of
213 sodium nitroprusside (Bower and Holm-Hansen, 1980). NO_2^- and NO_3^- were measured on a
214 Technicon AutoAnalyser II (Armstrong et al., 1967). Detection limits were 0.5 µM, 0.1 µM, and 0.4
215 µM for NH_4^+, NO_2^-, and NO_3^-, respectively. Precision ranged between ±3% and ±5% for the three

nutrient analyses. DIN mass balance (mol N h^{-1}) was determined as the difference in DIN load (calculated multiplying concentrations by water flow) between upstream and downstream sampling stations. Uncertainty in reach-scale mass balances was estimated by considering the variability in replicates for NH_4^+, NO_2^-, and NO_3^- determination and the potential errors in flow measurements (Bukaveckas and Isenberg, 2013). Net DIN loss along each selected ditch was divided by the corresponding riverbed surface to obtain areal rates (mmol N m^{-2} h^{-1}). Daily rates (mmol N m^{-2} d^{-1}) were calculated from hourly dark and light rates as described in section 2.2 for reach-scale N_2 fluxes. Since NO_3^- was the dominant form of DIN (NO_3^-:DIN > 99% in all samples), and NH_4^+ and NO_2^- concentration were close or below detection limits, hereafter we used the term NO_3^- removal instead of DIN removal.

At V, vegetation sampling was performed for the dominant species (*T. arundinacea*) according to Pierobon et al. (2013). Briefly, macrophyte % coverage (areal cover of the stream bed) and plant biomass were estimated in five replicates on each sampling date. Biomass was harvested with a 50x50 cm frame randomly positioned and the dry weight (DW) measured after 36 h at 60°C. Average values of relative growth rates (RGR, % day^{-1}) representative of the three key stages of the plant growth cycle (spring, summer, and autumn) were calculated from biomass dry weight between two subsequent sampling campaigns. Uptake by in-stream vegetation was then obtained from the measured relative growth rates and areal coverages, and using an average N content in *T. arundinacea* biomass (Borin and Salvato, 2012). The biomass loss via herbivory was neglected due to the refractory nature of the macrophyte biomass.

2.4 Fluxes of O_2, N_2, and DIN and denitrification rates in sediment cores

In May, twelve intact sediment cores (Plexiglas liners, i.d. 4.5 cm, height 20 cm) were collected manually from each ditch, eight for measurements of benthic fluxes and four for sediment characterization. At V, the sediments were sampled within the *T. arundinacea* stands but avoiding

241 the inclusion of plants into the cores. Sampling, pre-incubation and incubation procedures were
242 performed according to a standard protocol (Dalsgaard et al., 2000). Briefly, once in the laboratory,
243 eight cores from each ditch were submersed into incubation tanks with *in situ* water and left overnight.
244 Homogeneous mixing of the water column without sediment resuspension was ensured by a rotating
245 teflon-coated magnetic bar fixed to the inner wall of each liner a few centimetres above the sediment
246 surface and driven by an external motor (40 rpm). Water was stirred during the whole pre-incubation
247 and incubation periods. Dark gas (O_2, N_2) and dissolved inorganic N (NO_3^-, NO_2^-, NH_4^+) exchange
248 rates between water and sediment were measured by start-end batch incubations performed in ditch
249 water at ~16°C, the average temperature typically maintained by the lowland springs water all year
250 round (Laini et al., 2011). Incubation time (~2 h) was set in order to keep the variation of dissolved
251 oxygen within ~20% of the initial value. The day after sampling, the water inside the cores was
252 replaced with fresh ditch water and the incubations started when each liner was sealed with a floating
253 Plexiglas lid. Oxygen was measured with a microsensor (OX-500, Unisense, Science Park Aarhus,
254 Denmark) directly inside the cores at the beginning and end of the incubation, and simultaneously,
255 water samples were collected from each core with a glass 100-mL syringe. Water samples for N_2:Ar
256 ratio and DIN measurements were collected and analyzed as previously described. Hourly dark fluxes
257 of O_2, N_2, NH_4^+, NO_3^- and NO_2^- were calculated from the rate of change in concentrations with time
258 and expressed as rate per square meter, according to the equation:

259 $$F = \frac{(C_f - C_0) \cdot V}{A \cdot t},$$

260 where F (μmol m^{-2} h^{-1}) is the flux, C_0 and C_f (μM) are the concentrations at the beginning and at the
261 end of incubation, respectively, V (L) is the water volume in the core, A (m^2) is the surface of the
262 sediment core, and t (h) is the incubation time. Negative values indicate a flux from the water column
263 to the sediment (net consumption), while positive values indicate a flux from the sediment to the
264 water column (net release). Daily fluxes were calculated by multiplying hourly rates by 24, since in

groundwater feeding lowland springs chlorophyll-*a* concentration is very low and photosynthetic activity considered irrelevant.

On the same set of cores used for benthic flux determinations, dark denitrification rates were measured by the isotope pairing technique (IPT, Nielsen, 1992; Dalsgaard et al., 2000), which allows for partitioning the total denitrification rates (D_{tot}) into denitrification of NO_3^- diffusing to the anoxic sediment from the water column (D_W), and denitrification of NO_3^- produced by nitrification within the oxic sediment (D_N). At the beginning of the incubation, labelled NO_3^- (15 mM $Na^{15}NO_3$ solution, 98 atom% enrichment) was added to the water column of each core to have a final ^{15}N atom% enrichment of ~30%. The NO_3^- concentration was measured prior and after the addition of $^{15}NO_3^-$ at the time the cores were closed in order to calculate the ^{14}N:^{15}N ratio in the NO_3^- pool. Cores were incubated in dark conditions as previously described for benthic flux measurements. At the end of incubation, the whole sediment and water phases of each core were gently mixed and an aliquot of the slurry was transferred into 12-mL glass-tight vials and preserved by adding 200 μL of $ZnCl_2$ saturated solution. The IPT samples were analysed for $^{29}N_2$ and $^{30}N_2$ by MIMS (Lunstrum and Aoki, 2016). Denitrification rates were calculated according to the equations and assumptions of Nielsen (1992).

Finally, the top 0-1 cm sediment layer of four cores from each ditch was analysed for bulk density (measured as the weight of a known volume of fresh material) and porosity after oven drying at 70 °C. Organic matter content (OM, %) was quantified as loss on ignition (LOI) in a muffle furnace at 350°C for 3 hours on dry powdered sediment aliquots.

2.5 Statistical analyses

The effect of factors *site* (V, U), *months* (May, July, September), and *light condition* (light, dark) on reach-scale net N_2 fluxes was tested by means of a three-way ANOVA. Differences between the two ditches in N_2 concentrations, core benthic fluxes and sediment features were tested via one-way

ANOVA. Normality (Shapiro–Wilk test) and homoscedasticity (Levene's test) were previously examined. Log-transformed data satisfied assumptions of normality. Statistical significance was set at $p \leq 0.05$. Statistical analyses were performed with SigmaPlot 11.0 (Systat Software, Inc., CA, USA).

3. Results

3.1 General features of the investigated reaches

Morphometric characteristics and water and sediment features of the two investigated ditches are reported in Table 1. Discharge showed a wide variation in the study period in both ditches, ranging from 10 to >140 L s^{-1}, with the lower values detected in July since water flow from the spring decreased due to management for crop irrigation. At V, this period overlapped with the macrophyte coverage peak and the lowest values of water velocity. k600 values predicted as a function of current velocity and water depth were in the range 1.2-4.6 and 0.91-6.38 cm h^{-1}, at V and U, respectively. Chemico-physical features of the inflowing water reflected the typical quality of emerging groundwater feeding the canal network in the study area. Temperature remained almost constant throughout the study period (15-17°C), oxygen was generally undersaturated (74-94%), conductivity was always higher than 566 µS cm^{-1}, and NO_3^- concentrations were stably high and in the range 355-395 µM (Table 1). Dissolved N_2 concentrations at upstream sampling stations were in the range of 573-612 µM and not significantly different between V and U ($p>0.05$), but were significantly higher than theoretical water-atmosphere equilibrium concentrations ($p<0.01$) which correspond to a constant oversaturation in the range 101-105% (Table 1).

3.2 Reach-scale fluxes of N_2 and NO_3^- and N uptake by vegetation

N_2 concentrations measured at downstream sampling stations always exceeded concentrations predicted by de-gassing with the atmosphere and the excess was ascribed to in-stream N_2 production. The only exception was measured at U, in May, in light conditions, when a null N_2 production was

315 obtained. N_2 production was significant different between ditches ($p<0.01$) varying in the ranges 1.2-
316 5.5 and 0-2.1 mmol N m^{-2} h^{-1} in presence (V) and absence (U) of vegetation, respectively (Fig. 2). N_2
317 fluxes were systematically higher in dark than in light conditions ($p<0.01$) at V and varied among
318 seasons ($p<0.01$), with the highest N_2 production rates measured in July for both ditches. No
319 significant interactions among the three factors were found.
320 Despite the constancy of NO_3^- concentrations (NO_3^-:DIN always >99%), inflow NO_3^- loads varied
321 between 13 and ~201 mol N h^{-1}, according to discharges variation of the two ditches throughout the
322 study period (Table 2), with the lower loads detected in July at V and the highest ones in September
323 at U. In all sampling conditions, NO_3^- load measured downstream was lower than upstream in the
324 range 0.3-20%. The highest NO_3^- removal rates expressed on an areal basis (2.5 mmol N m^{-2} h^{-1}) were
325 detected at V in July (dark condition) when discharge was the lowest and retention times the highest,
326 while the lowest one (0.4 mmol N m^{-2} h^{-1}) was measured at U in September (dark condition) (Fig. 2).
327 Throughout the study period, NO_3^- abatement ranged between 0.5 and 2.5 mmol N m^{-2} h^{-1} in the
328 vegetated ditch and between 0.4 and 1.6 mmol N m^{-2} h^{-1} in the unvegetated one (Fig. 2). N_2 production
329 rates were positively correlated with NO_3^- removal rates ($r=0.77$, $p<0.01$, $n=12$). For each sampling
330 date, the highest rates (N_2 production or NO_3^- consumption) were measured in the vegetated ditch
331 during the dark phase.
332 The portion of ditch bed covered by *T. arundinacea* stands was 15%, 56%, and 42% in May, July and
333 September respectively, with minimum average biomass in spring (100-300 gDW m^{-2}) and maximum
334 values in autumn (550-650 gDW m^{-2}). The maximum values of RGR were obtained in May (4.0-7.0
335 % day^{-1}), intermediate rates in July (2.0-3.5% day^{-1}) and the minimum values in September (0.1-0.2
336 % d^{-1}), resulting in a net daily biomass accumulation of 8-14, 10-18, and 1-2 g DW m^{-2} day^{-1} in the
337 three seasonal sampling campaigns, respectively. N uptake was maximum in July (11-19 mmol N m^{-2} d^{-1}), intermediate in May (9-15 mmol N m^{-2} d^{-1}) and minimum in September (1-2 mmol N m^{-2} d^{-1}).
339
340 *3.3 Fluxes of O_2, N_2, and DIN and denitrification rates in sediment cores*

341 Benthic fluxes of gas (O_2, N_2) and inorganic N forms (NH_4^+, NO_3^-) and denitrification rates measured
342 in sediment cores were significantly different between the two ditches ($p<0.01$). Sediment oxygen
343 consumption was 2 times higher at V (2.66±0.49 mmol O_2 m^{-2} h^{-1}) than U (1.35±0.42 mmol O_2 m^{-2}
344 h^{-1}) (Fig. 3). Sediments from V had significantly higher ($p<0.05$) OM content and porosity and
345 significantly lower ($p<0.05$) density than sediments from U (Table 1). NH_4^+ was always released
346 from the sediment to the water column, with a significantly higher flux at V (0.14±0.08 mmol N m^{-2}
347 h^{-1}) than at U (<0.01 mmol N m^{-2} h^{-1}). Benthic compartment was a sink for NO_3^-+NO_2^- with
348 consumption rates higher at V (-0.77±0.44 mmol N m^{-2} h^{-1}) than at U (-0.22±0.07 mmol N m^{-2} h^{-1}).
349 They were consistent with net N_2 fluxes which were on average more than 4 times higher at V
350 (0.59±0.25 mmol N m^{-2} h^{-1}) than at U (0.13±0.1 mmol N m^{-2} h^{-1}).
351 Total denitrification rates (Dtot) measured via IPT were 0.48±0.13 mmol N m^{-2} h^{-1} at V and 0.06±0.02
352 mmol N m^{-2} h^{-1} at U, and the fraction sustained by water column NO_3^- (Dw) on average accounted
353 for 86±12% of Dtot at U and for 68±21% at V (Fig. 3).
354
355 *3.4 Reach-scale vs core measurements*
356 Throughout the study period, at V reach-scale NO_3^- removal varied between 22 and 50 mmol N m^{-2}
357 d^{-1} and was consistent with the correspondent N_2 production rates (38-84 mmol N m^{-2} d^{-1}) (Fig. 4).
358 The maximum values detected for open-channel N_2 flux and NO_3^- abatement at U corresponded to
359 the lower extremes of the ranges measured at V. Net N daily accumulation in plant biomass accounted
360 for 5-8% of the daily reach-scale NO_3^- removal in May, 12-21% in July and 1-4% in September.
361 Reach-scale rates (N_2 production and NO_3^- consumption) were systematically higher throughout the
362 study period than those obtained in laboratory by incubations of sediment cores (Fig. 4) with the
363 maximum discrepancy found in summer for both V and U. For both ditches, there was no significant
364 difference ($p>0.05$) between rates of N_2 production obtained by N_2:Ar measurement and
365 denitrification rates determined via IPT (Fig. 3).
366

4. Discussion

4.1 Vegetation promotes denitrification in NO_3^--rich ditches

In the present study, both NO_3^- consumption (measured via reach-scale mass balance) and denitrification (measured via N_2 open-channel method) were 1.2-3.0 and 1.5-3.3-fold higher in the vegetated ditch compared to the unvegetated one, respectively. Aquatic vegetation can contribute to decreased N loads through two main pathways: directly via assimilation and incorporation into biomass, and indirectly, via the stimulation of microbially-mediated processes among which denitrification is the most important. Assimilation is only a temporary sequestration since N may be buried or recycled back to the aquatic system via leaching and mineralization of plant litter, while denitrification is a permanent N-removal (Schaller et al., 2004; Soana et al., 2015). Compared to daily NO_3^- removal, N assimilation by *T. arundinacea* stands was irrelevant in May and September, but not in July when plant coverage peaked and NO_3^- uptake from the water column could not be excluded. However, *T. arundinacea,* like other helophytes, has a well-developed root system and assimilates N preferentially from the sediments in the form of NH_4^+ (Brix et al., 1994).

Rooted macrophytes promote a number of "hidden dynamics", both in the sediment around roots and in the periphytic mats, where N processing (e.g. uptake, ammonification, nitrification and denitrification) occurs with rates higher than those ascribed to the sediment alone (Schaller et al., 2004; Soana and Bartoli, 2014). The presence of vegetation stimulates the accumulation of organic matter (e.g. trapping suspended particles, root exudate release, decaying plant litter), thus providing to the benthic compartment both labile organic carbon availability and anoxic niches required for NO_3^- dissimilation via denitrification (Li et al., 2016; Hang et al., 2016). Furthermore, submersed portions of plants, like stems and leaves, represent potentially available surfaces for hosting consortia of bacteria and microalgae. Biofilms can alter nutrient dynamics in shallow environments by being highly productive and promoting solute exchanges (Bastviken et al., 2003; Srivastava et al., 2016). Anyway, sediments without aquatic vegetation support denitrification too, even if with systematically lower rates. Anaerobic microsites around particulate organic matter and woody debris represent

393 suitable environments capable of promoting NO_3^- reduction by denitrifying bacteria (Schaller et al.,
394 2004; Stelzer et al., 2014).
395 For the vegetated ditch, reach-scale N_2 production and NO_3^- removal were systematically higher
396 during dark conditions on each sampling occasion. Diurnal patterns of denitrification generally
397 depend on oxygen dynamics resulting from a balance between photosynthesis and respiration
398 processes (Laursen and Seitzinger, 2004; Pellerin et al., 2009; Lupon et al., 2016). Plant or benthic
399 microalgal photosynthesis increases oxygen penetration, dislocating the denitrification zone deeper
400 from the sediment-water interface and reducing the diffusional rate of NO_3^- towards the oxic–anoxic
401 boundary where denitrification occurs. Conversely, in dark conditions, the oxic layer is thinner, the
402 mean diffusional path length is shorter, thus the supply of NO_3^- from the water column to the
403 denitrification zone is higher (Harrison et al., 2005; Nizzoli et al., 2014).
404 The general good agreement between reach-scale estimates of denitrification and NO_3^- consumption
405 suggests that denitrification of water column NO_3^- was the main process responsible for N removal
406 throughout the study period. Taking into account the associated variability by using a set of depth–
407 velocity equations providing a range of k600 values, N_2 production overlap NO_3^- removal in most
408 cases. Indeed, N_2 flux determinations suffer of the variability introduced by this conservative
409 approach, failing direct measurements of the reaeration coefficients. However, when N_2 fluxes were
410 on average greater than the corresponding NO_3^- fluxes, we can postulate two hypothesis: 1)
411 overestimation of N_2 fluxes due to overestimation of the gas transfer velocity; 2) contribution of
412 nitrification in producing NO_3^- subsequently denitrified to N_2. Determination of water-atmosphere
413 gas exchanges is usually a critical step in open-channel methods. The use of empirical relationships
414 for the calculation of gas transfer velocity carries a degree of uncertainty affecting the overall
415 estimation of metabolic rates. Extensive literature reviews have highlighted that mathematical
416 formulas including only the hydraulic parameters of water velocity and depth generally tend to predict
417 higher k600 values than the real ones, except for the datasets used to calibrate them (Cox et al., 2003).
418 As their performance is often uneven and contradictory, we cannot exclude that an overestimation of

419 reach-scale denitrification rates occurred for our investigated sites. Given that the identification of
420 the appropriate equation for each specific velocity-depth range is usually difficult, future applications
421 of the N_2 open-channel method should include the concomitant direct measurement of reaeration
422 coefficients in order to increase the accuracy of the denitrification estimates. Denitrification rates
423 measured by IPT in intact sediment cores demonstrated that the process was mostly supported by the
424 reduction of NO_3^- diffusing from the water column to anoxic sediments, but also nitrification in the
425 oxic layers was a source of NO_3^- for denitrification. This microbial path can be amplified in sediments
426 hosting living roots where the injection of oxygen, generally conspicuous for emergent wetland
427 plants, stimulates coupled nitrification/denitrification deeper in the rhizosphere (Borin and Salvato,
428 2012; Taylor et al., 2015). However, in NH_4^+-poor sites like ours, this pathway is likely to be of only
429 minor influence on the measured reach-scale N_2 fluxes.
430
431 *4.2 Main drivers of N removal*
432 Both proximal (biotic) and ecosystem-level (hydrologic) controls (Bernot and Dodds, 2005;
433 Seitzinger et al., 2006) regulated temporal variability of denitrification rates in the studied ditches.
434 On a daily basis, N-removal efficiency via denitrification was higher at night, likely due to anoxic
435 zones closer to the supply of NO_3^- from the water column. Otherwise, along the investigated period,
436 flow rate was the most variable parameter exerting a primary control on in-stream N dynamics and
437 denitrification. The highest rates of NO_3^- removal and N_2 production were detected in July, when
438 discharges were the lowest and retention times the highest. In fact, when the hydraulic residence time
439 is long, ditches and canals function as linear wetlands, where interactions between water volumes and
440 multiple biologically active interfaces are maximised, promoting N removal (Pinay et al., 2015;
441 Tournebize et al., 2017).
442 The N removal rates (1.8-3.3 kg N $km^{-1}d^{-1}$) measured in this study in the vegetated ditch overlap those
443 (1.5-5 kg N $km^{-1}d^{-1}$) previously measured with comparable methods in other vegetated ditches of the
444 Po River hydrological network, used primarily for drainage and characterised by lower NO_3^-

concentrations (30-150 µM) (Pierobon et al., 2013; Castaldelli et al., 2015). In the present study, the ditches were fed by NO_3^--rich groundwater with concentrations up to 390 µM almost constant during the year. Thus, similar denitrification rates, measured in ditches with 2.5-fold higher NO_3^- concentrations, may indicate either a saturation of the process or a limitation by other regulating factors (Bernot and Dodds, 2005, Mulholland et al., 2008). Temperature stably close to 15-17°C may have limited denitrification. In fact, in ditches studied by Castaldelli et al. (2015), not fed by springs and located near the Po delta, summer water temperature was up to 29 °C. Furthermore, denitrification, being an anaerobic respiration, is controlled by the presence of an electron donor, in most of the cases represented by labile organic carbon. In the studied ditches, biodegradable carbon was not phytoplanktonic, since phytoplankton was almost absent (chlorophyll-*a* <0.5 µg L^{-1}), but more likely was produced in form of root exudates or from other plant decaying materials. Concentrations of dissolved organic carbon < 2 mg L^{-1} were measured in the spring water feeding the studied ditches (Balestrini et al., 2016), indicating that organic carbon was unbalanced with respect to the large NO_3^- availability (4.9-5.5 mg N L^{-1} on average, along the investigated period), according to the theoretical ratio of 1.07 of denitrification stoichiometry (Picek et al., 2007). Moreover, we can speculate that, not only the amount of organic carbon but probably also its biodegradability could have further limited denitrification. OM derived from emergent plants and woody riparian strips is generally refractory and undergoes slow decomposition, while exudates from the roots of aquatic vegetation are more easily degradable and may fuel microbial metabolism (Karjalainen et al., 2001; Hang et al., 2016).

It is reported that conditions of OM limitation may also favour incomplete denitrification and N_2O production (Vilain et al., 2012; Zhao et al., 2014). This aspect was investigated in 15 ditches and canals, fed by spring water in the same geographical area, in early and late summer. Groundwater exhibited supersaturation of N_2O at the spring outlet, but in this specific case, no reach-scale N_2O production was evidenced (Laini et al., 2011). These outcomes suggest that N_2O concentrations in ditches are regulated by outgassing of spring water affected by groundwater dynamics, while the

contribution of in-stream microbial or plant activity appears to be negligible with respect to the emissions of this greenhouse gas.

4.3 Management practices of the ditch network aimed at maximizing the depuration capacity

The actual efficiency of small lotic ecosystems, such as ditches and canals, to mitigate N pollution can be really appreciated if they are considered as a whole, i.e. sediment, vegetation, biofilms and their multiple interactions. Reach-scale methods, that integrate water column and benthic compartments, allow the quantification of processes and dynamics not detectable through the laboratory incubations of sediment cores (Gardner et al., 2016; Reisinger et al., 2016). Therefore, they are more suitable for a better understanding of large-scale N processing with potential implications for management strategies of aquatic ecosystems in human-impacted catchments. Our results suggested that small size watercourses in agricultural landscapes are important hot spots of N removal especially in presence of vegetation. Even though high denitrification rates supported by agricultural ditches, NO_3^- loads transported by spring-fed waterways in the Lombardy Plain are very elevated (Laini et al., 2011; Sacchi et al., 2013), thus the in-stream NO_3^- removal measured in this study was actually relatively low. Downstream NO_3^- loads were generally lower than upstream ones by 0.2-3.9%, while during period of low discharge and maximum vegetation development, the percentage of in-stream reduction peaked at ~20%. These outcomes evidence one of the most valuable ecosystem services provided by aquatic vegetation, i.e. the mitigation of NO_3^- pollution, and sustain the need of increasing macrophytes biomass to favour denitrification. In routine management practices aimed at preserving the hydraulic performance of canals and ditches, aquatic vegetation is considered only as an impediment for water circulation and usually removed, sometimes together with sediments. A sustainable ditch maintenance able to harmonize the hydraulic functionality with some ecological issues is reliable and relatively costless. For example, the frequency and the extensions of the vegetation cutting can be regulated avoiding the complete and simultaneous removal in many ditches, while opting for spatially and temporally differentiated models of maintenance.

Also, a hydrological management of the ditch network able to maintain an appropriate water head, that means leaving enough water in the irrigation period and reducing the inflows during abundant rainy events, can support the removal of NO_3^-. An increase of the water residence time, a crucial factor for N retention, could be achieved by actions addressed at increasing the channel sinuosity and diversifying the flows, e.g. by inserting coarse substrates and large woody debris in the bed or by creating lentic units. On the other hand, hardwood riparian strips could act as a buffer for NO_3^- coming from the cultivated field through the subsurface flow paths. In the light of these outcomes, it is evident that a sustainable management of the agricultural catchments able to exploit the several ecosystems services provided by the natural systems (e.g. macrophytes and riparian zones) could contribute to counteract one of most widespread, costly and challenging environmental problems, i.e. NO_3^- pollution.

5. Conclusions

The results from the present study show that: 1) ditches with in-stream vegetation provide a significantly higher NO_3^- removal than unvegetated ones; 2) plant-mediated denitrification is the dominant N removal pathway; 3) a longer water residence and larger development of interfaces on aquatic vegetation promote N abatement; and 4) addition of labile organic carbon by in-stream macrophyte helps to overcome the OM limitation typical of ditches fed only by spring water. In the light of these evidences, the ditch management should turn to practices which, though guaranteeing the hydraulic efficiency, may at the same time favour the above cited processes, during the plant growth cycle. These actions may significantly enhance N remediation capacity and other ecosystem services supported by macrophytes in hydraulic networks fed by NO_3^--rich spring-water, with positive effects at the watershed level and on the terminal water bodies as, in the case of the Po basin, the coastal lagoon of the delta and north-western Adriatic Sea.

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References

Armstrong, F.A.J., Sterus, C.R., Strickland, J.D.H., 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. Deep-Sea Res. 14, 381–389.

Balestrini, R., Arese, C., Delconte, C., 2008. Lacustrine wetland in an agricultural catchment: Nitrogen removal and related biogeochemical processes. Hydrol. Earth Syst. Sci. 12 (2), 539–550.

Balestrini, R., Sacchi, E., Tidili, D., Delconte, C. A., Buffagni, A., 2016. Factors affecting agricultural nitrogen removal in riparian strips: examples from groundwater-dependent ecosystems of the Po Valley (Northern Italy). Agric. Ecosyst. Environ. 221, 132–144.

Bartoli, M., Racchetti, E., Delconte, C. A., Sacchi, E., Soana, E., Laini, A., Longhi, D., Viaroli, P., 2012. Nitrogen balance and fate in a heavily impacted watershed (Oglio River, Northern Italy): in quest of the missing sources and sinks. Biogeosciences 9(1), 361–373.

Bastviken, S.K., Eriksson, P.G., Martins, I., Neto, J.M., Leonardson, L., Tonderski, K., 2003. Potential nitrification and denitrification on different surfaces in a constructed treatment wetland. J. Environ. Qual. 32(6), 2414–2420.

Beaulieu, J.J., Golden, H.E., Knightes, C.D., Mayer, P.M., Kaushal, S.S., Pennino, M.J., Arango, C.P., Balz, D.A., Elonen, C.M., Fritz, K.M., Hill, B.H., 2015. Urban stream burial increases watershed-scale nitrate export. PloS one 10(7), e0132256.

Bernot, M.J., Dodds, W.K., 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8(4), 442–453.

546 Birgand, F., Skaggs, R.W., Chescheir, G.M., Gilliam, J.W., 2007. Nitrogen removal in streams of
547 agricultural catchments – a literature review. Crit. Rev. Env. Sci. Tech. 37, 487–499.

548 Borin, M., Salvato, M., 2012. Effects of five macrophytes on nitrogen remediation and mass balance
549 in wetland mesocosms. Ecol. Eng. 46, 34–42.

550 Bower, C.E., Holm-Hansen, T., 1980. A salicylate-hypochlorite method for determining ammonia in
551 seawater. Can. J. Fish. Aquat. Sci. 37(5), 794–798.

552 Brix, H., Lorenzen, B., Morris, J.T., Schierup, H.H., Sorrell, B.K., 1994. Effects of oxygen and nitrate
553 on ammonium uptake kinetics and adenylate pools in *Phalaris arundinacea* L. and *Glyceria maxima*
554 (Hartm.) Holmb. Proc. R. Soc. Edinburgh. Section B. Biological Sciences 102, 333–342.

555 Bukaveckas, P.A., Isenberg, W.N., 2013. Loading, transformation, and retention of nitrogen and
556 phosphorus in the tidal freshwater James River (Virginia). Estuar. Coasts 36(6), 1219–1236.

557 Castaldelli, G., Soana, E., Racchetti, E., Pierobon, E., Mastrocicco, M., Tesini, E., Bartoli, M., Fano,
558 E.A., 2013. Nitrogen budget in a lowland coastal area within the Po River Basin (Northern Italy):
559 multiple evidences of equilibrium between sources and internal sinks. Environ. Manag. 52, 567–580.

560 Castaldelli, G., Soana, E., Racchetti, E., Vincenzi, F., Fano, E. A., Bartoli, M., 2015. Vegetated canals
561 mitigate nitrogen surplus in agricultural watersheds. Agric. Ecosyst. Environ. 212, 253–262.

562 Cox, B. A., 2003. A review of dissolved oxygen modelling techniques for lowland rivers. Sci. Total
563 Environ. 314, 303–334.

564 Dalsgaard, T., Nielsen, L.P., Brotas, V., Viaroli, P., Underwood, G.J.C., Nedwell, D.B., Sundbäck,
565 K., Rysgaard, S., Miles, A., Bartoli, M., Dong, L., Thornton, D.C.O., Ottosen, L.D.M., Castaldelli,
566 G., Risgaard-Petersen, N., 2000. Protocol Handbook for NICE-Nitrogen Cycling in Estuaries: A
567 Project Under the EU Research Programme. Marine Science and Technology (MAST III). National
568 Environmental Research Institute, Silkeborg, Denmark, 62 pp.

569 Dollinger, J., Dagès, C., Bailly, J. S., Lagacherie, P., Voltz, M., 2015. Managing ditches for
570 agroecological engineering of landscape. A review. Agron. Sustain. Dev. 35(3), 999–1020.

571 Duncan, J.M., Groffman, P.M., Band, L.E., 2013. Towards closing the watershed nitrogen budget:
572 Spatial and temporal scaling of denitrification. J. Geophys. Res-Biogeo. 118(3), 1105–1119.

573 Gardner, J.R., Fisher, T.R., Jordan, T.E., Knee, K.L., 2016. Balancing watershed nitrogen budgets:
574 accounting for biogenic gases in streams. Biogeochemistry 127, 231–253.

575 Groffman, P.M., Bain, D..J., Band, L.E., Belt, K.T., Brush, G.S., Grove, J. M., Pouyat, R.V.,
576 Yesilonis, I.C., Zipperer, W. C., 2003. Down by the riverside: urban riparian ecology. Front. Ecol.
577 Environ. 1(6), 315–321.

578 Haider, H., Ali, W., Haydar, S., 2013. Evaluation of various relationships of reaeration rate coefficient
579 for modeling dissolved oxygen in a river with extreme flow variations in Pakistan. Hydrol. Process.
580 27, 3949–3963.

581 Hang, Q., Wang, H., Chu, Z., Ye, B., Li, C., Hou, Z., 2016. Application of plant carbon source for
582 denitrification by constructed wetland and bioreactor: review of recent development. Environ. Sci.
583 Pollut. R. 23(9), 8260–8274.

584 Harrison, J.A., Matson, P.A., Fendorf, S.E., 2005. Effects of a diel oxygen cycle on nitrogen
585 transformations and greenhouse gas emissions in a eutrophied subtropical stream. Aquat. Sci. 67(3),
586 308–315.

587 Hefting, M.M., van den Heuvel, R.N., Verhoeven, J.T., 2013. Wetlands in agricultural landscapes for
588 nitrogen attenuation and biodiversity enhancement: Opportunities and limitations. Ecol. Eng. 56, 5–
589 13.

590 Iseyemi, O.O., Farris, J.L., Moore, M.T., Choi, S.E., 2016. Nutrient Mitigation Efficiency in
591 Agricultural Drainage Ditches: An Influence of Landscape Management. B. Environ. Contam. Tox.
592 96(6), 750–756.

593 Jähne, B, Munnich, K.O., Bosinger, R., Dutzi, A., Huber, W., Libner, P., 1987. On parameters
594 influencing air–water exchange. J. Geophys. Res. 92, 1937–1949.

595 Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, J.B., Bennett, G.E., Cornwell, J.C., 1994.
596 Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in
597 environmental water samples. Anal. Chem. 66(23), 4166-4170.

598 Karjalainen, H., Stefansdottir, G., Tuominen, L., Kairesalo, T., 2001. Do submersed plants enhance
599 microbial activity in sediment? Aquat. Bot. 69, 1–13.

600 Laini, A., Bartoli, M., Castaldi, S., Viaroli, P., Capri, E., Trevisan, M., 2011. Greenhouse gases (CO_2,
601 CH_4 and N_2O) in lowland springs within an agricultural impacted watershed (Po River Plain, northern
602 Italy). Chem. Ecol. 27(2), 177–187.

603 Laursen, A.E., Seitzinger, S.P., 2002. Measurement of denitrification in rivers: an integrated, whole
604 reach approach. Hydrobiologia 485, 67–81.

605 Laursen, A.E., Seitzinger, S.P., 2004. Diurnal patterns of denitrification, oxygen consumption and
606 nitrous oxide production in rivers measured at the whole-reach scale. Fresh. Biol. 49, 1448–1458.

607 Li, F., Pan, Y., Xie, Y., Chen, X., Deng, Z., Li, X., Hou, Z., Tang, Y., 2016. Different roles of three
608 emergent macrophytes in promoting sedimentation in Dongting Lake, China. Aquat. Sci. 78(1), 159–
609 169.

610 Lunstrum, A., Aoki, L.R., 2016. Oxygen interference with membrane inlet mass spectrometry may
611 overestimate denitrification rates calculated with the isotope pairing technique. Limnol. Oceanogr-
612 Meth. 14, 425–431.

613 Lupon, A., Martí, E., Sabater, F., Bernal, S., 2016. Green light: gross primary production influences
614 seasonal stream N export by controlling fine-scale N dynamics. Ecology 97(1), 133–144.

615 Marion, A., Nikora, V., Puijalon, S., Bouma, T., Koll, K., Ballio, F.. Tait, S., Zaramella, M.,
616 Sukhodolov, A., O'Hare, M., Wharton, G., Aberle, J., Tregnaghi, M., Davies, P., Nepf, H., Parker,

617 G., Statzner, B., 2014. Aquatic interfaces: a hydrodynamic and ecological perspective. J. Hydraul.
618 Res. 52, 744–758.

619 Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O. Jr, Hamilton, S.K., Peterson, B.J., Tank, J.L.,
620 Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E.G., Gregory, S.V., Grimm,
621 N.B., Johnson, S.L., Mcdowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P.,
622 Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Johnson, L.T., Niederlehner, B.R.,
623 O'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J., Thomas, S.M., 2008. Stream denitrification
624 across biomes and its response to anthropogenic nitrate loading. Nature 452, 202–205.

625 Nielsen, L.P., 1992. Denitrification in sediment determined from nitrogen isotope pairing. FEMS
626 Microbiol. Lett. 86(4), 357-362.

627 Nizzoli, D., Welsh, D.T., Longhi, D., Viaroli, P., 2014. Influence of *Potamogeton pectinatus* and
628 microphytobenthos on benthic metabolism, nutrient fluxes and denitrification in a freshwater littoral
629 sediment in an agricultural landscape: N assimilation versus N removal. Hydrobiologia 737, 183–
630 200.

631 Pellerin, B. A., Downing, B. D., Kendall, C., Dahlgren, R. A., Kraus, T. E., Saraceno, J., Spencer,
632 R.G.M., Bergamaschi, B. A., 2009. Assessing the sources and magnitude of diurnal nitrate variability
633 in the San Joaquin River (California) with an in situ optical nitrate sensor and dual nitrate isotopes.
634 Fresh. Biol. 54(2), 376–387.

635 Picek, T., Čížková, H., Dušek, J., 2007. Greenhouse gas emissions from a constructed wetland—
636 plants as important sources of carbon. Ecol. Eng. 31(2), 98–106.

637 Pierce, S.C., Kröger, R., Pezeshki, R., 2012. Managing artificially drained low-gradient agricultural
638 headwaters for enhanced ecosystem functions. Biology 1, 794–856.

639 Pierobon, E., Castaldelli, G., Mantovani, S., Vincenzi, F., Fano, E.A., 2013. Nitrogen removal in
640 vegetated and unvegetated drainage ditches impacted by diffuse and point sources of pollution. Clean
641 Soil Air Water 41, 24–31.

642 Pinay, G., Peiffer, S., De Dreuzy, J.R., Krause, S., Hannah, D.M., Fleckenstein, J.H., Sebilo, M.,
643 Bishop, K., Hubert-Moy, L., 2015. Upscaling nitrogen removal capacity from local hotspots to low
644 stream orders' drainage basins. Ecosystems 18, 1101–1120.

645 Racchetti, E., Longhi, D., Ribaudo, C., Soana, E., Bartoli, M. Nitrogen uptake and coupled
646 nitrification–denitrification in riverine sediments with benthic microalgae and rooted macrophytes.
647 Aquatic Sciences, in press.

648 Reisinger, A.J., Tank, J.L., Hoellein, T.J., Hall, R.O., 2016. Sediment, water column, and open-
649 channel denitrification in rivers measured using membrane-inlet mass spectrometry. Journal of
650 Geophysical Research: Biogeosciences 121(5), 1258-1274.

651 Roley, S.S., Tank, J.L., Stephen, M.L., Johnson, L.T., Beaulieu, J.J., Witter, J.D., 2012. Floodplain
652 restoration enhances denitrification and reach-scale nitrogen removal in an agricultural stream. Ecol.
653 Appl. 22(1), 281–297.

654 Romero, E., Garnier, J., Billen, G., Peters, F., Lassaletta, L., 2016. Water management practices
655 exacerbate nitrogen retention in Mediterranean catchments. Sci. Total Environ. 573, 420–432.

656 Sacchi, E., Acutis, M., Bartoli, M., Brenna, S., Delconte, C. A., Laini, A., Pennisi, M., 2013. Origin
657 and fate of nitrates in groundwater from the central Po plain: insights from isotopic
658 investigations. Appl. Geochem 34, 164–180.

659 Schaller, J.L., Royer, T.V., David, M.B., Tank, J.L., 2004. Denitrification associated with plants and
660 sediments in an agricultural stream. J. N. Am. Benthol. Soc. 23(4), 667–676.

661 Schwarzenbach R., Gschwend P.M., Imboden, D.M., 1993. Environmental Organic Chemistry,
662 Wiley, NewYork.

663 Seitzinger, S., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C.,
664 Drecht, G.V., 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol. Appl. 16(6),
665 2064–2090.

666 Soana, E., Bartoli, M., 2014. Seasonal regulation of nitrification in a rooted macrophyte (*Vallisneria*
667 *spiralis* L.) meadow under eutrophic conditions. Aquat. Ecol. 48(1), 11–21.

668 Soana, E., Naldi, M., Bonaglia, S., Racchetti, E., Castaldelli, G., Brüchert, V., Viaroli, P., Bartoli,
669 M., 2015. Benthic nitrogen metabolism in a macrophyte meadow (*Vallisneria spiralis* L.) under
670 increasing sedimentary organic matter loads. Biogeochemistry 124(1-3), 387–404.

671 Srivastava, J. K., Chandra, H., Kalra, S.J., Mishra, P., Khan, H., Yadav, P., 2016. Plant–microbe
672 interaction in aquatic system and their role in the management of water quality: a review. Appl. Water
673 Sci. 1–12.

674 Stelzer, R.S., Scott, J.T., Bartsch, L.A., Parr, T.B., 2014. Particulate organic matter quality influences
675 nitrate retention and denitrification in stream sediments: evidence from a carbon burial experiment.
676 Biogeochemistry 119(1-3), 387–402.

677 Taylor, J.M., Moore, M.T., Scott, J.T., 2015. Contrasting nutrient mitigation and denitrification
678 potential of agricultural drainage environments with different emergent aquatic macrophytes. J.
679 Envir. Qual. 44(4), 1304–1314.

680 Tournebize, J., Chaumont, C., Mander, Ü., 2017. Implications for constructed wetlands to mitigate
681 nitrate and pesticide pollution in agricultural drained watersheds. Ecol. Eng. In press

682 Viaroli, P., Nizzoli, D., Pinardi, M., Soana, E., Bartoli, M., 2015. Eutrophication of the Mediterranean
683 Sea: a watershed—cascading aquatic filter approach. Rend. Lincei 26(1), 13–23.

684 Vilain, G., Garnier, J., Tallec, G., Tournebize, J., 2012. Indirect N_2O emissions from shallow
685 groundwater in an agricultural catchment (Seine Basin, France). Biogeochemistry, 111(1-3), 253-
686 271.

687 Wanninkhof, R., 1992. Relationship between gas exchange and wind speed over the ocean. J.
688 Geophys. Res. 97, 7373–7381.

689 Weiss, R.F., 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep-Sea
690 Res. 17(4), 721-735.

691 Zhao, Y., Xia, Y., Li, B., Yan, X., 2014. Influence of environmental factors on net N_2 and N_2O
692 production in sediment of freshwater rivers. Environmental Science and Pollution Research, 21(16),
693 9973-9982.

694 **Table 1.** Morphometric characteristics and water and sediment features of the two investigated
695 ditches. Discharge, velocity and transit time are reported as average ± standard deviation of three
696 cross sections along each ditch. Depths are measured in the central portion of each ditch. Gas transfer
697 velocity (k600) is calculated as described in section 2.2. Water physical and chemical parameters,
698 measured at the upstream station, are average values ± standard deviation of the dark and light
699 samplings in each period. Sediment features were measured on 4 cores sampled in each ditch.

			V	**U**
Morphometric parameters		Length (m)	380	330
		Width (m)	2.8	3.2
		k600 (cm h^{-1})	1.2-4.6	0.91-6.38
	May	Discharge (L s^{-1})	40 (8)	66 (11)
		Velocity (cm s^{-1})	6 (1)	19 (3)
		Depth (cm)	30-40	10-15
		Transit time (min)	115 (23)	30 (5)
	July	Discharge (L s^{-1})	10 (2)	14 (3)
		Velocity (cm s^{-1})	2 (1)	9 (2)
		Depth (cm)	10-20	5-10
		Transit time (min)	270 (55)	58 (12)
	September	Discharge (L s^{-1})	106 (23)	147 (5)
		Velocity (cm s^{-1})	10 (2)	20 (1)
		Depth (cm)	40-50	25-30
		Transit time (min)	72 (16)	28 (1)
Chemical - physical parameters	May	T (°C)	15.62 (0.75)	15.05 (0.05)
		Conductivity (µS cm^{-1})	665 (6)	651 (3)
		O_2 saturation (%)	84 (14)	74 (7)
		NO_3^- (µM)	395 (2)	386 (6)
		N_2 (µM)	593 (18)	612 (5)
		N_2 saturation (%)	103 (2)	105 (1)
	July	T (°C)	16.37 (0.48)	17.07 (0.71)
		Conductivity (µS cm^{-1})	634 (11)	566 (57)
		O_2 saturation (%)	77 (26)	78 (3)
		NO_3^- (µM)	355 (6)	376 (32)
		N_2 (µM)	586 (16)	576 (8)
		N_2 saturation (%)	103 (2)	103 (1)
	September	T (°C)	16.58 (0.93)	16.12 (0.60)
		Conductivity (µS cm^{-1})	621 (4)	604 (3)
		O_2 saturation (%)	94 (33)	84 (21)
		NO_3^- (µM)	389 (6)	376 (4)
		N_2 (µM)	573 (15)	591 (10)
		N_2 saturation (%)	101 (1)	104 (1)
Sediment features		Density (g mL^{-1})	1.46 (0.16)	1.84 (0.04)
		Porosity	0.66 (0.09)	0.36 (0.03)
		Organic matter (%)	4.22 (1.67)	0.73 (0.06)

700

701 **Table 2.** Upstream hourly NO_3^- loads (average ± standard deviation) and average percentages of NO_3^-
702 removal ($\Delta<0$) in light and dark conditions; Δ is calculated as (downstream load – upstream
703 load)/upstream load x 100. The percentage of NO_3^- with respect to DIN (dissolved inorganic nitrogen)
704 is also reported.
705

		V		U	
		Dark	*Light*	*Dark*	*Light*
May	**Upstream NO_3^- load** (mol N h^{-1})	157 (12)	156 (34)	91 (17)	92 (16)
	Δ load	-3.90%	-0.37%	-1.96%	-0.28%
	NO_3^-:DIN	99.89%	99.70%	99.95%	99.28%
July	**Upstream NO_3^- load** (mol N h^{-1})	13 (2)	13 (2)	19 (6)	20 (6)
	Δ load	-20.89%	-16.33%	-3.82%	-3.81%
	NO_3^-:DIN	99.57%	99.50%	99.92%	99.94%
September	**Upstream NO_3^- load** (mol N h^{-1})	146 (32)	150 (34)	197 (7)	201 (7)
	Δ load	-1.50%	-0.61%	-0.23%	-0.29%
	NO_3^-:DIN	99.75%	99.92%	99.91%	99.96%

706

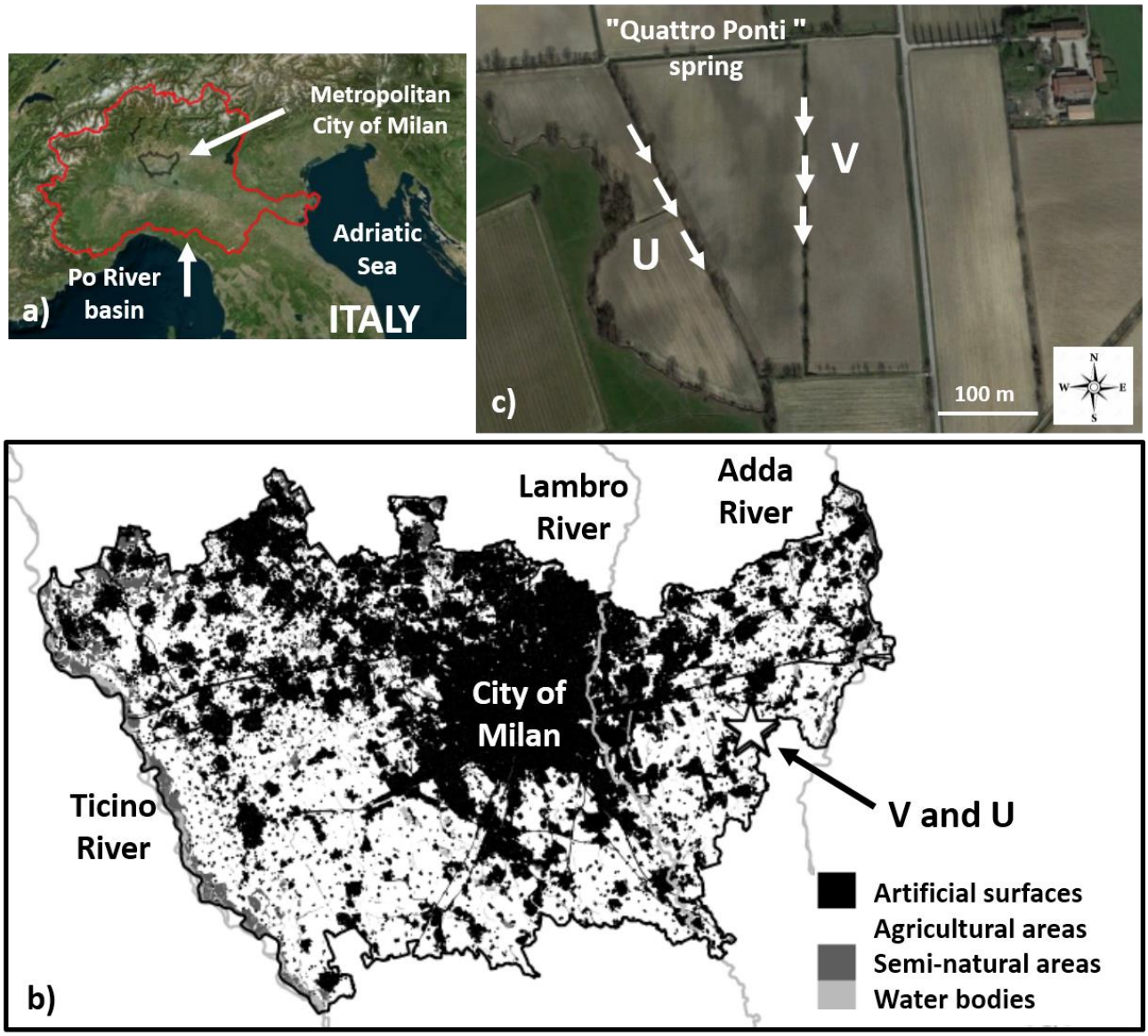
Figure captions

Figure 1. Location of the two investigated ditches: a) the Po River basin and the Metropolitan City of Milan (Northern Italy); b) the land use of the Metropolitan City of Milan (DUSAF cartographic database - Land Use of Agricultural and Forest Land, Lombardy Region, 2012); c) the spring "Quattro Ponti" and the two ditches (V and U), with the flow path indicated. Geographic data showed in the maps were downloaded from the Lombardy Region Geoportal (http://www.geoportale.regione.lombardia.it/).

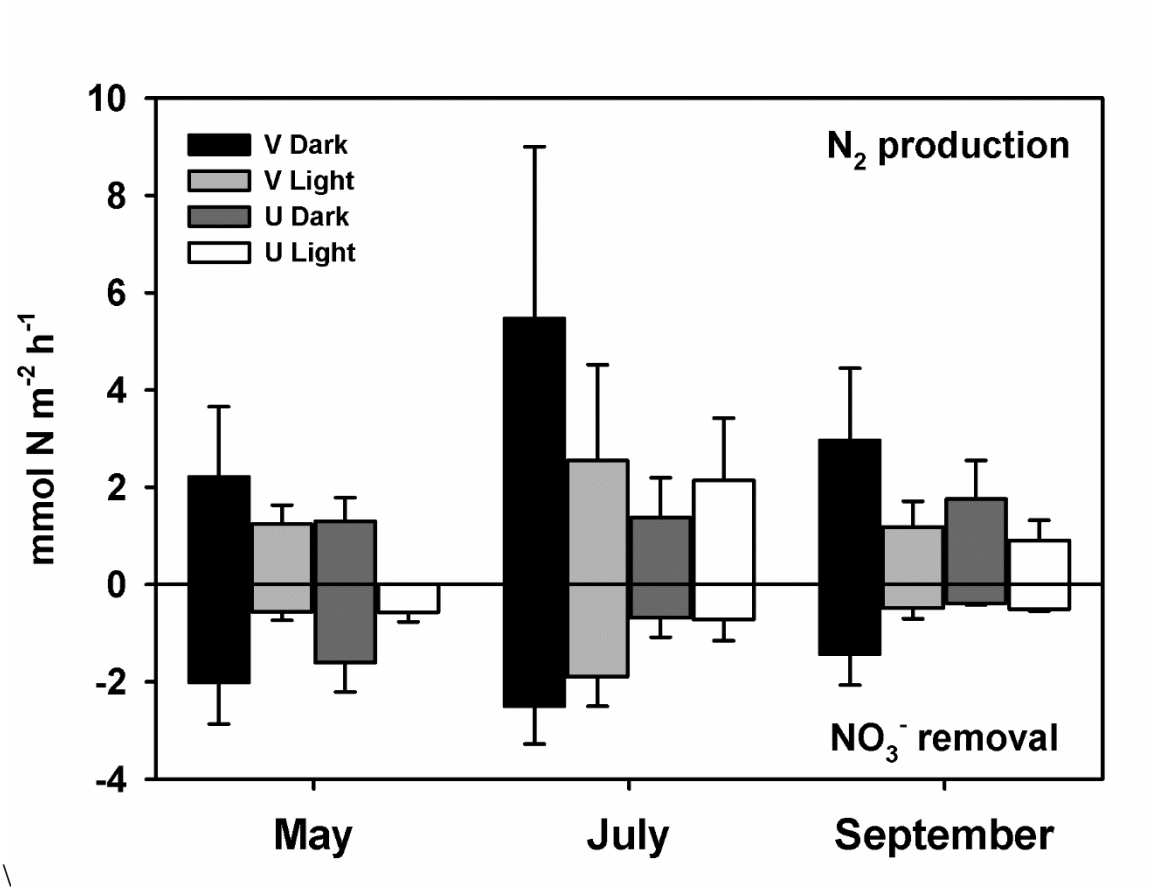
Fig. 2. Hourly rates of NO_3^- removal (box below) and N_2 production (box at the top) measured in the two investigated ditches throughout the study period (average±standard deviation). Rates obtained at the reach-scale in light and dark conditions are compared. In May at U, N_2 production was null for light sampling.

Fig. 3. Benthic fluxes of O_2, NH_4^+, NO_3^-, N_2, and denitrification rates measured via incubations of sediment cores sampled in the two ditches (average±standard deviation). NO_3^- fluxes include also NO_2^- contribution never exceeding 2%. Denitrification rates determined by IPT (isotope pairing technique) are split in the contribution of Dw (denitrification of water column NO_3^-) and Dn (denitrification of NO_3^- produced by nitrification in the oxic sediment layer).

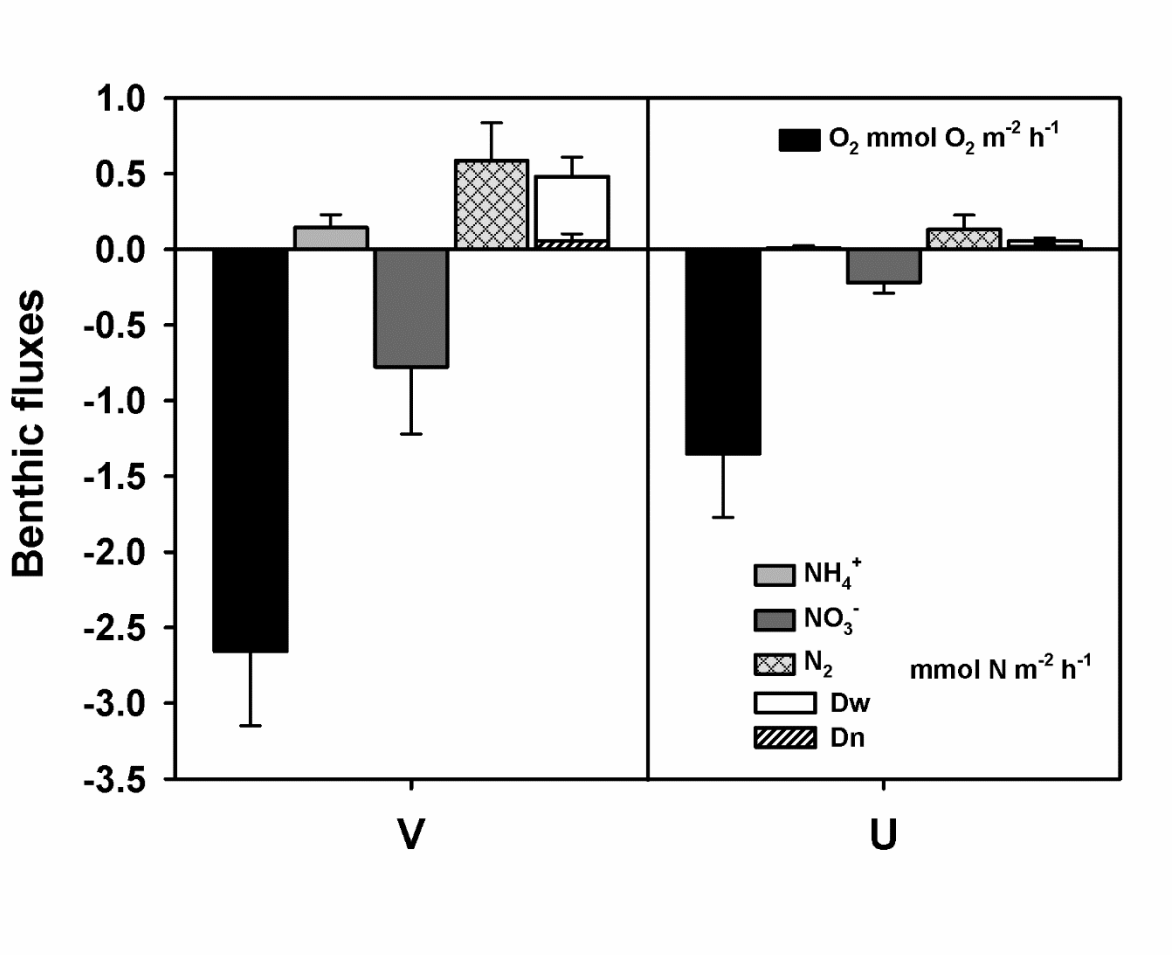
Fig. 4. Daily rates of NO_3^- removal (box below) and N_2 production (box at the top) measured in the two investigated ditches throughout the study period (average±standard deviation). Rates obtained at the reach-scale (in May, July, and September) and by core incubations (in May) are compared.


Metropolitan
City of Milan
Adriatic
Sea
Po River
basin
a)
ITALY
"Quattro Ponti "
spring
V
U
c)
100 m
N
W
E
S
Lambro
River
Adda
River
City of
Milan
Ticino
River
V and U
Artificial surfaces
Agricultural areas
Semi-natural areas
Water bodies
b)

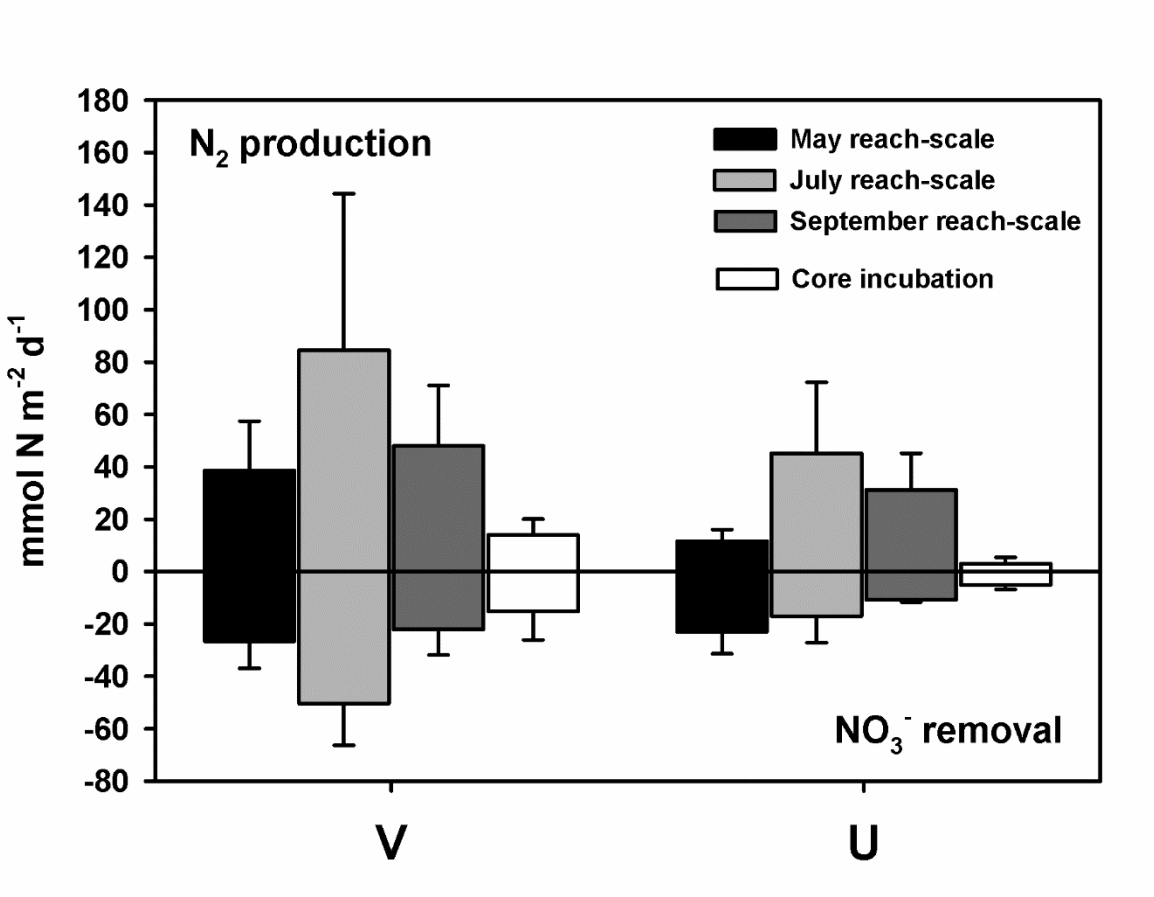
726
727 Fig. 1


10
8
6
4
2
0
-2
-4
mmol N m-2 h-1
V Dark
V Light
U Dark
U Light
N2 production
NO3- removal
May
July
September

728 \
729 Fig. 2


Benthic fluxes
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
V
U
O2 mmol O2 m-2 h-1
NH4+
NO3-
N2
Dw
Dn
mmol N m-2 h-1

730
731 Fig. 3


180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
mmol N m-2 d-1
N2 production
May reach-scale
July reach-scale
September reach-scale
Core incubation
NO3- removal
V
U

732 Fig. 4.