Vegetated canals mitigate nitrogen surplus in agricultural watersheds

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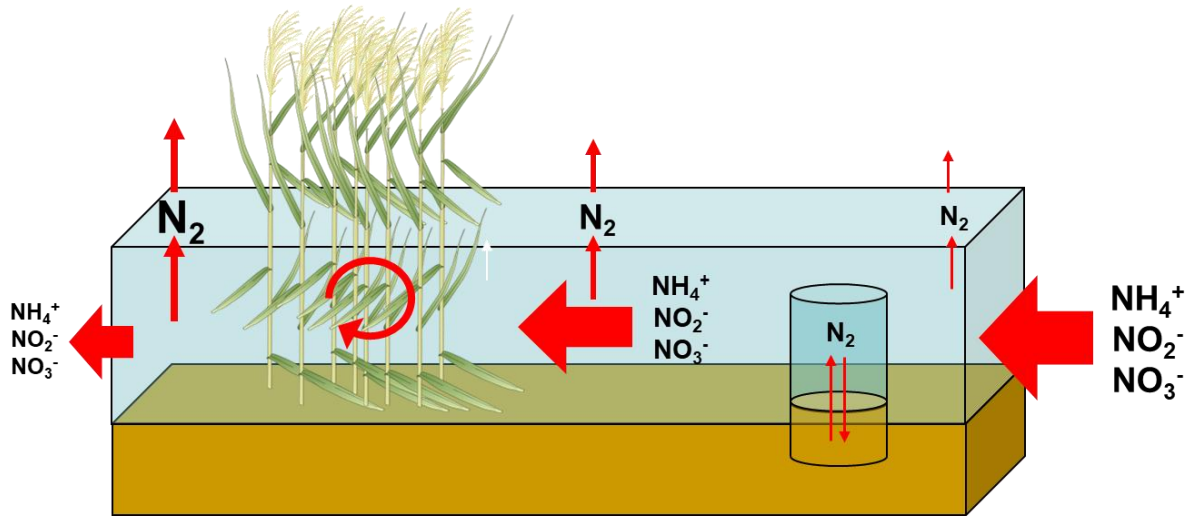
Graphical abstract caption

Measurements of nitrogen fluxes performed in the investigated ditches at the reach-scale and in sediment cores



Abstract

Within irrigated agricultural watersheds, canal networks may play a crucial role as nitrogen (N) sink. This is due to the intertwined action of macrophytes and microbial communities occurring in the dense net of small watercourses. We hypothesize that vegetated canals may buffer relevant fractions of excess N from agriculture via microbial denitrification, and that vegetation provides multiple interfaces that greatly support the activity of bacteria. To test these hypotheses, we measured net dinitrogen (N_2) fluxes in bare sediments and at the reach-scale in vegetated ditches. As study areas we selected canals subjected to diffuse N pollution, laying in a lowland sub-basin of the Po River (northern Italy). Denitrification was evaluated on the basis of changes in dissolved N_2:Ar, measured by Membrane Inlet Mass Spectrometry. Complementary data were obtained via upstream-downstream inorganic N balances and intact core incubations targeting sedimentary N fluxes. Denitrification was the major pathway for N removal, with rates at the reach-scale (5 to 25 mmol N m^{-2} d^{-1}) up to one order of magnitude higher than in sediment alone (3 to 7 mmol N m^{-2} d^{-1}). Results highlighted that N uptake by macrophyte stands was quantitatively small; however, aquatic vegetation provided multiple interfaces for microbial growth and N-related processes. Our data suggest that 1 ha of vegetated canal may remove between 150 and 560 kg N yr^{-1}. In the study area, an average canal density of ~0.05 linear km ha^{-1} of agricultural land has the potential to buffer 5 to 17% of the excess N from agriculture (~60 kg N ha^{-1} yr^{-1}).

The results of this study suggest the central role of emergent vegetation in promoting microbial N-transformation and canal self-depuration. Innovative management of canal networks should couple hydraulic needs with the maintenance of emergent vegetation.

1. Introduction

In agricultural watersheds, the role of the ditch network as provider of ecosystem services (e.g. nutrient cycling, biodiversity, habitat, water depuration) is generally not considered in the current management practices (Herzon and Helenius, 2008; Pierce et al., 2012; Dollinger et al., 2015). This contrasts with the Millennium Ecosystem Assessment (2005), which aims at identifying, protecting and restoring multiple services provided by ecosystems, including agricultural ones. When considering biogeochemical functions, nitrogen (N) reduction capacity of the ditch network is an understudied and underestimated term of lowland basin N budgets if compared to waterways in natural watersheds (Mulholland et al., 2008; von Schiller et al., 2009). Small watercourses such as canals and ditches may be very effective in retaining N temporarily, or removing it permanently via denitrification due to their strict interaction with terrestrial systems, extensive and capillary distribution and high metabolic activity (Birgand et al., 2007).

An interesting hypothesis is that in agricultural catchments the ditch networks may have a large potential as N metabolic regulator and buffer against excess N (Lassaletta et al., 2012). This is due to some peculiarities that promote N processing (e.g. long water residence time, frequent water recirculation on the landscape, elevated primary production, and multiple interfaces). The ditch network may assume a considerable role in controlling N dynamics especially during the irrigation period, when large water volumes are withdrawn from the main water bodies, delivered to the territory and drained. In temperate areas, the irrigation period overlaps the vegetative phase and the warm season, when high water temperatures (up to >25°C) enhance microbial processes. This represents the moment when N transformations may be presumably the highest, in terms of both retention in primary producer biomass and permanent loss via denitrification (Bernot and Dodds, 2005; Piña-Ochoa and Álvarez-Cobelas, 2006). Primary producers (e.g. benthic microalgae, epiphytes, submerged and emergent macrophytes) have been shown to strongly affect N-related microbial transformations (Eriksson, 2001; Forshay and Dodson, 2011; Kreiling et al., 2011; O'Brien et al., 2013; Soana and Bartoli, 2014). Benthic vegetation can inhibit or stimulate N loss via denitrification

coupled to nitrification in the rhizosphere. Inhibition generally occurs in net autotrophic aquatic environments where inorganic N is limiting (Risgaard-Petersen and Jensen, 1997; Ottosen et al., 1999; Risgaard-Petersen, 2004), while stimulation can occur where N is abundant and the plant-microbe community competition is attenuated (Nizzoli et al., 2014; Soana et al., 2015). A side effect of in-stream N removal is the production of the greenhouse gas nitrous oxide (N_2O) as an intermediate of several N cycle processes (Reay et al., 2003). Comparative assessment of ecosystem services and disservices performed by running waters in agricultural basins is still an open question (Burgin et al., 2013).

The Po Plain, the most densely urbanized, industrialized and farmed territory of Italy, is a paradigmatic study area to analyse N paths along the terrestrial-coastal zone continuum. The Po di Volano Basin lays at the interface between the Po Plain and the deltaic lagoons of the north Adriatic Sea. Dominant land use is agriculture, mainly sustained by chemical fertilizers. A comprehensive N budget has shown that this watershed exports to the coastal zone less N than the amount entering into the hydrological network (Castaldelli et al., 2013). This means that besides the excess N leached from arable lands, a fraction of inputs from upstream ecosystems is also metabolized. Denitrification in the ditch network likely provides this relevant ecosystem service (Pierobon et al., 2013). The delivery of nutrients to the coastal zone during summer months is particularly undesirable as they may favour eutrophication-related phenomena as algal blooms and anoxia, with considerable economic impact on fisheries and tourism (de Jonge et al., 2002).

This paper reports new insights on N removal in the secondary canal network of an intensively-cropped basin by means of the dinitrogen (N_2) open-channel method, applied for the first time in Europe and validated by comparison with the mass balance of N species at the reach-scale and the incubations of intact sediments. The occurrence of multiple interfaces and three dimensional environments both in the water column (i.e. algal and bacterial biofilms on vegetation) and in the pore water (i.e. oxic-anoxic interfaces in the rhizosphere of aquatic plants and in macroinvertebrate burrows) (Hines, 2006; Marion et al., 2014) makes the study of N processes within small canals

difficult. Here, sediment core incubations may provide rates that are likely large underestimates of *in situ* ones, suggesting that alternative methods, such as open-channel, are needed (Laursen and Seitzinger, 2002). We hypothesize that vegetated canals may buffer relevant fractions of excess N from agriculture via microbial denitrification, and that vegetation provides multiple interfaces that greatly support the activity of microbial communities. This is a relevant issue both in heavily exploited watersheds, where agriculture dominates the landscape, and in developing countries where the increase of food demand will be strictly connected to the intensification of agricultural practices and hydraulic networks.

2. Material and Methods

2.1. Study area

The study was carried out in the Po di Volano Basin (~2,600 km^2, Fig. 1), an intensively-cropped watershed of the lower Po floodplain and with low population density, concentrated in the town of Ferrara (~130,000 inhabitants) and other minor towns, mostly (>90%) served by wastewater treatment plants. The hydrological structure of the territory is the result of a long-term reclamation completed in the 1960s, and includes a capillary network of canals (>8,200 km) serving for both drainage and irrigation. As aquatic vegetation is considered an impediment for water circulation, it is mechanically removed during routine management practices of canals and ditches. The widespread diffusion of the allochthonous grass carp contributes as well to the biological control of submerged aquatic plants and to the turbid, phytoplankton-dominated status of most watercourses. As a result, emergent macrophytes are maintained only in rare, isolated stretches of the canal network and are mainly represented by *Phragmites australis*, *Typha latifolia* and *Glyceria maxima*.

Three reaches (labeled C1, C2, and C3) were selected, according to several pilot surveys, with the aim of having homogenous replicates of the dominant waterway type of this territory. They were independent but located in the same agricultural area, in proximity to one another and fed by the same water. The function of these ditches is generally mixed and they serve both for drainage and irrigation

121 in spring and summer. These reaches, as with most of the entire hydrological network, are fed by the
122 nitrate-rich Po di Volano, a former Po River branch, which at present is canalized.
123
124 *2.2. Experimental design*
125 Experimental activities were performed in summer 2013 (from June 18th to July 3rd) during stable
126 hydrological and meteorological conditions. In this period, the three selected canals had a constant
127 flow (Table 1) and were characterized by emergent vegetation with peak biomass and N loads
128 consisting mainly of nitrate (Pierobon et al., 2013). For each ditch, two sampling stations (upstream
129 and downstream) were identified and three experimental approaches were adopted: 1) N_2 and N_2O
130 open-channel method; 2) reach-scale DIN (dissolved inorganic nitrogen) mass balance; and 3)
131 incubation of intact sediment cores (Fig. 2). Finally, the N amount stored in vegetation biomass was
132 estimated from the standing stock and N elemental composition of the dominant species of each
133 investigated ditch. Data were obtained from previous experimental campaigns performed in the same
134 ditch network (Pierobon et al., 2013). Samplings for the application of the N_2 and N_2O open-channel
135 method and the reach-scale DIN mass balance were performed both in the dark and light to investigate
136 the effect of photosynthetic processes on N dynamics in these highly productive ecosystems. Intact
137 core incubations provide a measure of the benthic N fluxes (DIN and N_2) in sediment alone. On the
138 other hand, whole-reach approaches allow the inclusion of the whole set of biotic and abiotic
139 processes and dynamics that cannot be detected in laboratory experiments. The net effect of
140 vegetation presence on in-stream N metabolism can be distinguished if two systems (vegetated and
141 unvegetated) are compared.
142
143 *2.3. N_2 and N_2O open-channel method*
144 Whole-reach denitrification was quantified by measuring the variation in dissolved N_2 concentrations
145 of the water column, as proposed by Laursen and Seitzinger (2002). The method provides the direct
146 estimate of denitrification by measuring the concentration of N_2 along the canal water path from

147 N_2:Ar analysed by Membrane Inlet Mass Spectrometry (MIMS). A model-based approach is used to
148 solve for denitrification rates, correcting the variations of N_2 during downstream transport for
149 atmospheric exchanges, according to concentration gradients and channel morphology (width and
150 depth). Both N_2 and Ar concentrations are assumed to change during downstream transport as a
151 function of temperature-dependent re-equilibration with the atmosphere. However, N_2 concentration
152 may also change as a function of metabolic activity (denitrification), and its production is determined
153 by quantifying accumulation in water after correction for atmospheric exchanges. This approach
154 allows for the estimate of *in situ* net N_2 fluxes (i.e. total denitrification, including coupled
155 nitrification/denitrification, minus N_2 fixation) under natural conditions, at a scale comparable to the
156 reach DIN mass balance. Dinitrogen production by anammox (Anaerobic Ammonium Oxidation) is
157 also included but probably has a minor relevance in eutrophic freshwater environments (Zhou et al.,
158 2014). Positive N_2 fluxes suggest net denitrification, while negative fluxes suggest net N_2 fixation
159 (Fulweiler et al., 2008). Using the same approach given by Laursen and Seitzinger (2004) for N_2
160 fluxes, we applied the open-channel method to model N_2O fluxes, under the same assumption that
161 N_2O concentrations may vary during transport due to atmospheric exchanges and metabolic activity.
162 The selected reaches were sampled from access points located near bridges using a two-station
163 (upstream and downstream) Lagrangian approach. The ditch segments chosen had uniform
164 morphology and no lateral surface water input or output. Clay sediments are common in these
165 environments and ground-surface water exchange was previously verified to be irrelevant (Pierobon
166 et al., 2013). At each reach, water samples for dissolved gases (N_2 and N_2O) and inorganic nitrogen
167 analyses were collected from the same parcel as it moved downstream. Wet section area and discharge
168 were used to calculate mean velocity and thus travel time of the water parcel between sampling
169 locations. Depth and velocity were measured at vertical transects equally spaced. The number of
170 transects ranged from a minimum of 7 to a maximum of 15, depending upon the ditch width. We used
171 a current meter (Open Stream Current Velocity Meter, 2100, Swoffer Instruments Inc, Seattle, WA,
172 USA) mounted on a measuring pole with centimetric resolution, equipped with a modified propeller

for low flow conditions. Temperature was measured with a multiparametric probe (Ocean Seven 316, Idronaut, Brugherio, Italy). Because of the shallowness of the ditches, the whole water column was sampled in triplicate by means of a 10-litre bucket and processed for gas and nutrient analyses (see paragraph 2.4). Samples for N_2:Ar and N_2O analysis were collected using a glass syringe and transferred into 12-ml glass-tight vials (Exetainer, Labco, High Wycombe, UK), flushed at least 3 times the vial volume and preserved by adding 100 µl of $ZnCl_2$ saturated solution. Dissolved N_2:Ar was measured using a MIMS (Bay Instruments, Easton, MD, USA) at the laboratory of Aquatic Ecology, University of Ferrara. Dinitrogen concentration was calculated from the measured N_2:Ar multiplied by the equilibrium Ar concentration at the *in situ* water temperature, determined from solubility equation (Weiss, 1970). Indeed, replicate samples (n=10) had a lower coefficient of variation among N_2:Ar measurements (~0.04%) than among N_2 measurements (~0.4%). Nitrous oxide was measured by gas chromatography (Trace GC, 2000 Series, Thermo Finnigan, San Jose, CA, USA) equipped with an ECD detector.

According to the approach proposed by Laursen and Seitzinger (2002), net N_2 and N_2O fluxes at the reach-scale were simulated at 1-min time steps. The following model input parameters were used: measured N_2 and N_2O concentrations and water temperature at upstream and downstream stations, average depth and width of each reach, gas transfer velocity (k600), Schmidt number coefficient (2/3 for surfaces without waves; Jähne et al., 1987), and travel time of the water parcel from upstream to downstream, calculated from average current velocity (Baulch et al., 2010). Gas transfer velocities of the three investigated reaches were calculated by means of two different approaches. As suggested by Schwarzenbach et al. (1993), when the ratio stream velocity to stream depth is higher than 0.03 s^{-1}, benthic turbulence is generally considered the primary driver of gas exchanges. This criteria was fulfilled for C1 and C2, where the oxygen reaeration coefficient (KO_2, 20°C, d^{-1}) was calculated by means of a set of empirical equations, as reported by Genereux and Hemond (1992) and recently reviewed by Haider et al. (2013), using average current velocity and water depth as the only variables affecting reaeration. The transfer velocity for oxygen (kO_2, 20°C, cm h^{-1}) was obtained by multiplying

each reaeration coefficient by the correspondent water depth, assuming a well-mixed water column. The gas transfer velocity was finally normalized to a Schmidt number of 600 (k600, for CO_2 at 20 °C, cm h^{-1}) (Jähne et al., 1987; Wanninkhof, 1992) as requested by the model. Differently, the wind-based models were more suitable for C3, where the ratio stream velocity to stream depth was ~0.01 s^{-1} and the benthic turbulence was considered negligible (Baulch et al., 2010). Several authors developed empirical relationships where k600 falls in the range 0.74-2.07 cm h^{-1} (Cole and Caraco, 1998; Guérin et al., 2007) for water bodies subjected to almost null wind speed, as usually occurs in the Po valley. Within the models, estimates of k600 were converted to values for N_2 and N_2O based on Schmidt numbers of these gases calculated for *in situ* water temperatures, according to the polynomial fit given by Wanninkhof (1992). For each site, a range of N_2 and N_2O production or consumption rates was generated from simulations run by varying the values of k600 (minimum, maximum and average). Uncertainty in modelled net N_2 and N_2O fluxes was estimated by taking into account the variability of gas determination and depth measurements. Hourly rates were multiplied by the average number of light (16) and dark (8) hours in the summer period and summed to obtain daily values.

2.4. Reach-scale DIN (dissolved inorganic nitrogen) mass balance

Triplicate subsamples for dissolved inorganic nitrogen (NH_4^+, NO_3^- and NO_2^-) determination were collected, filtered *in situ* (Whatman GF/F), cooled, transported to the lab within a few hours, and frozen at -20°C until analyses. Ammonium was determined on a double beam UV-VIS spectrophotometer (V-550, Jasco Co., Tokyo, Japan) using salicylate and hypochlorite in the presence of sodium nitroprussiate (Bower and Holm-Halsen, 1980). Nitrite and nitrate were measured on a Technicon Autoanalyser II (Armstrong et al., 1967). Detection limits were 0.5 µM, 0.1 µM, and 0.4 µM for NH_4^+, NO_2^-, and NO_3^-, respectively. Precision ranged between ±3% and ±5% for the three nutrient analyses. Dissolved inorganic nitrogen mass balance was determined as the difference in DIN load (calculated by multiplying concentrations and water flow) between upstream and

downstream sampling stations (Burns, 1998) both in dark and light. Potential errors in flow measurements and variability in replicates for NH_4^+, NO_2^-, and NO_3^- determination (summed to compute the DIN concentration) affect reach-scale mass balances. Uncertainty was estimated following the error-propagation principles (Taylor, 1982). Net DIN loss or gain along each selected segment was divided by the corresponding canal surface to obtain areal rates. Daily rates were calculated from hourly rates as previously described for reach-scale N_2 fluxes.

2.5. Incubation of intact sediment cores

A total of 9 intact sediment cores (Plexiglas liners, i.d. 4.5 cm, height 20 cm) were collected manually from each ditch and incubated in the laboratory for dark benthic flux measurement of gases (O_2, N_2) and dissolved inorganic N forms (NO_3^-, NO_2^-, NH_4^+). Sampling, pre-incubation and incubation procedures were performed according to standard protocols (Dalsgaard et al., 2000; Smith et al., 2006; Racchetti et al., 2011). A two-hour, start-end batch incubation was performed maintaining the cores at *in situ* temperature. Oxygen was measured with a microsensor (OX-500, Unisense, Science Park Aarhus, Denmark) directly inside the cores at the beginning and end of the incubation. Water samples for N_2:Ar ratio and DIN measurements were collected and analysed as previously described (see paragraphs 2.3 and 2.4). Hourly dark fluxes of O_2, N_2, NH_4^+, NO_3^- and NO_2^- were determined from the rate of change in concentrations with time and expressed as rate per square meter. Since light penetration was generally limited by high water turbidity, we assumed that sediment surface was always in the dark and we calculated daily fluxes by multiplying hourly rates by 24.

2.6. Statistical analyses

The effects of factors *site* and *light condition* (light/dark) on N_2 concentrations and reach-scale net N_2 fluxes (for average values of k600) were tested by means of a two-way ANOVA. Differences among sites in benthic fluxes measured by core incubations were tested via one-way ANOVA and a pairwise multiple comparison procedure (Holm–Sidak method). Untransformed 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. N_2 and N_2O open-channel method

The three canals had a width ranging from 2 to 4 meters, they were shallow (at most 0.6 m) and had a slope of a few cm per km, such as to generate low water velocities, up to ~8 cm s^{-1} (Table 1). The length of the three experimental sites varied due to the need to identify homogeneous reaches without significant water inputs or outputs, or other factors likely affecting dissolved gas dynamics (e.g. presence of weirs and culverts). Emergent vegetation was dominated by *Phragmites australis* and *Typha latifolia* at C1 and C2, and by *Glyceria maxima* at C3. The lowest flow velocity detected at C3 was due to the widespread presence of dense canopies of *Glyceria maxima*.

Predicted k600 values as a function of current velocity and water depth for C1 and C2 are reported in Table 2. Minimum and maximum values were obtained by applying the equations proposed by Isaacs et al. (1969) and Bennett and Rathbun (1972), respectively. Due to low current velocity and wind speed, we considered the range of k600 values reported for stagnant water as reasonable for C3. Water temperature decreased from upstream to downstream in all the investigated reaches during the night samplings, while the contrary was true in the light (Table 2). For each reach, dissolved N_2 concentrations were lower in light compared to dark condition ($p<0.01$). With a few exceptions, N_2 concentrations were higher than expected equilibrium values in all sampling sites and both in dark and light ($p<0.05$). Dinitrogen saturation ranged from 99.58% (C3, downstream, dark) to 101.61% (C2, downstream, dark). All sites exhibited supersaturation of N_2O both in dark and light. Nitrous oxide concentrations varied from 45 to 103 nM, corresponding to a saturation of 549% (C3, dark) and 1290% (C2, dark), respectively. Model input parameters are listed in Tables 1 and 2.

Dinitrogen concentrations measured in the dark at downstream sampling stations always exceeded concentrations predicted by atmospheric re-equilibration, suggesting net N_2 production. The highest

277 and lowest dark denitrification rates were estimated in C1 (2.57 to 10.21 mmol N m^{-2} h^{-1}) and C3
278 (0.28 to 0.64 mmol N m^{-2} h^{-1}), respectively (Fig. 3). In the light, a production of N_2 was detected only
279 at C1 and C3 by adopting in the model the mean and maximum k600 values. At C2, negative net N_2
280 fluxes (-0.53 to -1.86 mmol N m^{-2} h^{-1}) were estimated with the whole set of k600 values, suggesting
281 net N_2 fixation. The ratio of reach-scale net dark to light N_2 production varied from 1.6 (C3, maximum
282 k600) to 3.6 (C3, average k600). The two-way ANOVA suggests that differences between whole-
283 reach net N_2 fluxes depend on the factors *site* and *light condition*.
284 A dark production of N_2O was detected in all reaches. Rates varied according to the k600 chosen
285 between 5.4 and 48.5, 0 and 12.3 and 0.3 and 2.8 µmol N m^{-2} h^{-1} at C1, C2 and C3, respectively. In
286 the light, net N_2O efflux was detected only at C1 (2.4 to 37.8 µmol N m^{-2} h^{-1}) and C3 (1.1 to 3.6 µmol
287 N m^{-2} h^{-1}), while at C2 N_2O exchange was negligible. Rates of N_2O represented on average 1–8‰ of
288 the correspondent N_2 production rates.
289
290 *3.2. Reach-scale DIN mass balance*
291 Dissolved inorganic nitrogen concentrations measured at the upstream station of all canals were
292 similar (31 to 54 µM of NO_3^-+NO_2^- and 0.5 to 3 µM of NH_4^+, pooled data), and reflected the chemistry
293 of the Po di Volano, the common water source. Slight differences were due to the presence of a reach
294 of variable length between the main channel and the experimental sections. Incoming DIN loads
295 varied between ~ 3 and ~18 mol N h^{-1} according to different discharge values and NO_2^-+NO_3^- was
296 the dominant dissolved nitrogen form (on average 93 to 99% of the total DIN) (Table 3). In all reaches
297 the DIN load measured downstream was lower than that measured upstream by 10 to 37%, suggesting
298 in-stream N loss or retention, both in dark and light. At C1 and C3 dark DIN removal rates were
299 significantly higher than light ones, by a factor 1.8-3.5.
300
301 *3.3. Incubation of intact sediment cores*

Benthic oxygen consumption varied between 2629±431 (C2) and 3614±741 µmol O_2 m^{-2} h^{-1} (C1) (Fig. 4). Measured rates were significantly higher at C1 ($p<0.01$) than C2 and C3, even if the variability among replicates was large. Ammonium was always released to the water column, with significantly higher values measured at C1 (300±206 µmol N m^{-2} h^{-1}) than at the other two sites ($p<0.01$; Fig. 4). Conversely, rates of sediment nitrate consumption (averaging -162 µmol N m^{-2} h^{-1}, pooled data) and net N_2 fluxes (averaging ~200 µmol N m^{-2} h^{-1}, pooled data) were not significantly different among sites ($p>0.05$). Nitrite fluxes were directed from the sediment to the water column but they were one order of magnitude lower than ammonia regeneration rates.

3.4. Nitrogen fluxes: sediment cores vs reach-scale

Fluxes of DIN and N_2 measured in laboratory incubations of sediment core were one order of magnitude lower than those detected *in situ* at the reach-scale, with the exception of rates at C3 (Fig. 5). Macrophyte biomass peaked in the study period, so net uptake and N accumulation in new plant tissues were at minimum and irrelevant if compared to DIN budgets and N_2 fluxes. Also the amount of N stored into the biomass of emergent vegetation was a small pool if compared to reach-scale fluxes of inorganic nitrogen (Fig. 5). At C1 and C2, total N in the plants standing stock represented <2% and <7% of the daily N_2 fluxes and less than 5% and 4% of the correspondent daily DIN mass balance, respectively, suggesting slow N turnover in these plant biomass. Reach-scale DIN consumption was consistent with the net N_2 production at C1 and C3 calculated with minimum and average k600, respectively. Differently, at C2 a greater DIN consumption compared to N_2 production was detected, regardless the value of k600 used in the models.

4. Discussion

4.1. Emergent vegetation as a key component in N transformations

Results from this study confirm the two working hypotheses: small canals may buffer diffuse nitrate pollution from agriculture via microbial denitrification, and vegetation greatly supports the activity

328 of bacteria. Denitrification rates obtained with the N_2 open-channel approach were higher than those
329 calculated via mass balances or measured via core incubations in similar watercourses in the summer
330 period (Table 4). Moreover, they were within reported ranges obtained with the same modelling
331 approach in lotic environments having greater discharge or more permeable sediments, and over a
332 wide range of temperatures (Table 5). Such outcomes have two main implications. The first deals
333 with the great denitrification potential that vegetated canals have, which is a relevant ecosystem
334 function in agricultural contexts. The second is methodological: the reach-scale approach provides
335 much more reliable estimates of the whole set of processes occurring in aquatic ecosystems
336 characterised by multiple interfaces, such as vegetated canals. The simultaneous measurement of N_2
337 and DIN fluxes, both at the whole-reach and at sediment core scales, demonstrates how a dominant
338 fraction of the microbial activity occurs in the rhizosphere and in the water column, likely associated
339 to epiphytic organisms. Our data suggest that vegetation supports the activity of bacteria, as the net
340 DIN removal was comparable to the production of N_2, excluding a relevant influence of the plant
341 assimilation on nitrogen loads. Indeed, at peak biomass the macrophyte growth and N uptake
342 decrease, but plants sustain the activity of denitrifiers.
343 The small size of the ditches and the presence of dense stands of emergent macrophytes lead to a
344 large ratio between multiple interfaces and water volume, amplifying the influence of biofilm activity
345 on water chemistry. Wetzel and Søndergaard (1998) suggested that 1 m^2 of vegetated area can be
346 equivalent to over 24 m^2 of interfaces where biofilm can develop and promote nutrient retention and
347 transformations. Within submerged stands, rates of particle retention, DIN uptake, ammonification,
348 nitrification and denitrification by periphyton may largely exceed rates ascribed to microbial
349 communities in surface sediment or to the plants alone (Sirivedhin and Gray, 2006). In dense
350 macrophyte meadows, denitrification in biofilms may be promoted by elevated oxygen consumption,
351 especially in the dark (Enrich-Prast and Esteves, 1998; Eriksson 2001). Our results are consistent
352 with such findings. The investigated ditches were metabolically active both light and dark, but higher
353 dark rates of nitrate consumption and N_2 production suggest the dominant role of heterotrophic and

354 dissimilative over autotrophic and assimilative activities. Diurnal patterns of N removal previously
355 described in other aquatic ecosystems are usually mediated by oxygen dynamics, related to
356 respiratory or photosynthetic processes (Christensen and Sorensen, 1986; Laursen and Seitzinger,
357 2004; Desmet et al., 2011). In the dark, elevated community respiration reduces oxygen availability
358 in water and sediment. The thinner diffusional path length of water nitrate across the oxic sediment
359 layer enhances its removal via denitrification (Risgaard-Petersen and Jensen, 1997; Nizzoli et al.,
360 2014). In the light the situation is reversed as photosynthetic oxygen production increases the
361 thickness of the oxic sediment layer and the nitrate diffusional path length, deepening the
362 denitrification zone and depressing denitrification rates. As a result, net N_2 fluxes and N removal
363 measured in terms of upstream-downstream budgets were higher in dark conditions. Generally, a
364 major fraction of removed nitrate was converted into molecular nitrogen while N_2O production was
365 irrelevant.
366 Negative reach-scale N_2 fluxes measured at C2 in the light suggest the dominance of N-fixation over
367 denitrification. Autotrophic cyanobacteria in benthic, floating, or periphytic mats are usually
368 responsible for biological fixation in freshwater aquatic ecosystems (Šantrůčková et al., 2010). In
369 shallow environments, periphyton communities can be highly productive and alter nutrient cycling
370 not only in oligotrophic conditions (Wetzel, 1996). Nitrogen was not a limiting nutrient at the
371 investigated sites. However, the low current velocity typical of ditches may reduce solute
372 replenishment into diffusive boundary layers, and thus the availability of nutrients to be assimilated
373 or transformed. Moreover, N can become limiting where it is rapidly consumed such as in the
374 rhizosphere microniches (Soana and Bartoli, 2014). Under such circumstances, N_2 fixation at both
375 the sediment-water interface and in biofilms associated with dense plant stands, may become a
376 relevant path importing N into the system (Kunza and Hall, 2014).
377
378 *4.2.Methodological considerations*

379 The open-channel N_2 method is particularly suitable in low-gradient shallow waterways, such as
380 canals and ditches, subjected to low wind speeds. Here, N_2 can accumulate and be accurately detected
381 due to multiple concomitant elements, such as large sediment surface/water volume ratio, high
382 metabolic activity, minimal gas exchange (low velocity and turbulence), high water retention time
383 and irrelevant groundwater input (Laursen and Seitzinger, 2002; Baulch et al., 2010). Since all
384 methods to measure denitrification have inherent bias, the N_2:Ar methodology was compared to
385 reach-scale DIN mass budget. The latter in fact sets a limit to the maximum amount of dissolved
386 nitrogen actually removed from the system.
387 A successful application of the N_2 open-channel approach is limited by the ability to distinguish
388 between physical or biologically-mediated N_2 fluxes. Calculation of gas exchanges with the
389 atmosphere (i.e. reaeration coefficients) is thus a critical step in open-channel measurements,
390 from the classical Odum oxygen method to the more innovative N_2 reach-scale approach.
391 Several methods exist for measuring the reaeration coefficients (e.g. volatile gas tracer addition,
392 night-time drop of oxygen concentrations, and empirical formulas based on hydraulic features),
393 each with their own assumptions and sources of uncertainties (Izagirre et al., 2007). The use of
394 volatile tracers is usually an accurate but time-consuming and expensive technique, especially
395 in monitoring programmes and when many sites are investigated simultaneously. Several
396 mathematical formulas have been proposed, but their performance is often uneven and
397 contradictory, and none of them is suited across a wide range of hydrological conditions (Cox,
398 2003). Thus, the identification of the appropriate equation is usually a challenging step. The
399 approach adopted in the present study was conservative, as the whole set of depth–velocity
400 functions was applied with the aim of providing a range of k600 values, likely including the true
401 value of each investigated reach. However, the comparison between the upstream-downstream
402 DIN budget and N_2 open-channel can help in identify the most realistic reaeration coefficient
403 for each of the investigated reach. For example, at C1 the true k600 is probably close to the

lower extreme of the calculated range, while at C3 the true k600 is closer to the upper extreme. Future applications of the N_2 open-channel method in the investigated sites should include also the direct measurement of the reaeration coefficients, in order to make N metabolism estimates more accurate. In perspective, a simple and cheap option would be the coefficient calculation from the analyses of the night-time drop in oxygen concentration (Izagirre et al., 2007).

4.3. Canal network as sink of excess agricultural N

While forested catchments are characterized by low N inputs and elevated N retention, agricultural watersheds are characterized by large excess N whose fate is debated (Howarth et al., 2012; Billen et al., 2013; Burgin et al., 2013). Denitrification in the canal network may represent a relevant N sink, but this element of the agricultural landscape is seldom considered in management practices (Kellogg et al., 2010; Lassaletta et al., 2012; Castaldelli et al., 2013). Common management techniques to control agricultural N surplus include the limitation of fertilizer addition and the treatment of wastes from animal holding facilities (Christianson et al., 2014; Garnier et al., 2014). Vegetated wetlands have been extensively used to mitigate agricultural runoff, but they were mostly reclaimed in human-impacted watersheds (Racchetti et al., 2011). Properly managed vegetated ditches may represent an alternative to wetlands, providing environmental benefits. Ditch development dates back hundreds year ago and, even if artificial, they are now integral and ubiquitous components of the agricultural landscape strongly intertwined into the watershed dynamics. These ecosystems often represent the only aquatic habitats on farmland and could become the new target of restoration actions. In the Po Plain, the over 50,000 km of major canals and twice as much developed ditch network, if correctly managed, may have a large potential to provide multiple ecosystem services, among which the abatement of nutrient loads annually delivered to the Adriatic Sea. A proper management of the ditch system could ultimately be incorporated in "precision agriculture" practices based on a more accurate use of water and fertilizers, considering the differential capacity of different areas to be polluted or to buffer excess N. Within the framework of the Water Directive (2000/60/EU), areas with a high

density of canals might be considered as potential hot spots of denitrification where high N input and surplus may be efficiently conveyed to the atmosphere as N_2. Agriculture and food production are linked to water availability, but farming activities impact water quantity and quality (Zalidis et al., 2002; Gordon et al., 2010). The potential role of managed ecotones (such as the environments of the ditch system) in N mitigation acquires relevance in the context of increasing agricultural production maintaining water quality (MEA, 2005). Outcomes from this and other analogous studies are relevant contributions when planning the exploitation of alluvial plains into irrigated and fertilized areas (http://www.eu-water.eu/transnational_str.shtml).

The upscale of denitrification rates measured in vegetated sediments (5 to 25 mmol N m^{-2} d^{-1}) for the vegetative period of emergent macrophytes (approximately 120 d $year^{-1}$), results in a theoretical N abatement varying from 150 to 560 kg N ha^{-1} $year^{-1}$. The N removal potential of vegetated canals, on areal basis is from 2 to nearly 10 times higher than the average surplus generated by the agriculture in the Po di Volano Basin (~60 kg N ha^{-1} yr^{-1}, Castaldelli et al., 2013). The average spatial density of the canal network in the investigated catchment is about 0.05 linear km ha^{-1} of agricultural land. This means that a canal surface of ~0.02 ha (average width 4 m) is associated with each hectare of agricultural land. The vegetated canals may therefore buffer up 5 to 17% of the N surplus generated in the adjacent agricultural land, while a much lower amount can be removed in canals devoid of vegetation. These calculations are based on the constancy of rates, measured on a single occasion, for the whole vegetative period and should therefore be considered with some caution; but we are more prone to consider our results as conservative. Our sampling was in fact performed under stable conditions typical of midsummer (peak plant biomass, steady hydrological flows, constant temperature and nutrient regime). At peak biomass, plant growth is at minimum and N uptake limited to the maintenance of basic metabolism. We speculate about more relevant plant assimilation in the spring and early summer when macrophytes are actively growing, likely resulting in higher rates of N retention.

4.4. Towards new research and management of the canal network in agricultural watersheds

Irrigation and drainage canals are extremely heterogeneous in terms of hydraulic parameters (flow, velocity, water level fluctuations), solute concentrations, turbidity, benthic features (quality and quantity of organic matter, texture), and biological communities. They may be viewed as *in situ* laboratories, offering experimental alternatives difficult to reproduce in other contexts. Their small dimension and simplified and constant sections remove some degree of variability found in other natural systems and may allow for investigation of biogeochemical processes regulation in relation to each single variable (e.g. type of vegetation, substrate, nutrient availability, presence/absence of benthic communities, etc.) or a combination of them. Such semi-artificial environments represent the majority of hydrological networks in intensively farmed areas, i.e. where the largest N loads are generated. Thus, for remediation purposes it is strategic to adopt policies for increasing their depuration capacity. With this aim, future research should address the functions regulating N processing and transport and their spatial and temporal variability, to produce management guidelines aimed at maximizing the natural depuration capacity of the drainage network while maintaining the hydraulic efficiency. Current management is aimed at reducing flood risk and supplying water for irrigation practices during summer months. Most ditches are subjected to yearly maintenance operations to preserve hydraulic performance, such as removal of aquatic and riparian vegetation, dredging and drying during the non-irrigation period. Future investigations should focus on how current management affects the canals metabolic capacity toward N and how this impacts N dynamics at the watershed scale. The main challenge is to investigate the site-specific carrying capacity towards N and identify management criteria of the ditch network able to combine production, flood prevention, and water quality goals. The general perspective is finally to define a new role for the water authorities as provisions of multiple environmental benefits for the community.

In conclusion, the results of this study suggest with an integrated methodological and analytical approach, the central role of emergent vegetation in supporting N-related microbial functions and

canals self-depuration. Future management of the canal networks should merge these relevant ecosystem services with hydraulic needs.

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681 **Table 1.** General features of the three investigated reaches.

	C1	**C2**	**C3**
Length (m)	880	1200	475
Average depth (m)	0.3	0.6	0.5
Width (m)	2.1	2.4	4.0
Average velocity (cm s^{-1})	8.7	6.6	1.1
Discharge (l s^{-1})	53	93	21
Transit time (min)	168	309	746
Riverbed surfarce (m^2)	1,850	2,920	1,900

682

683 **Table 2.** Model inputs used to estimate net N_2 and N_2O fluxes in the three investigated reaches
684 (average±standard deviation, n=3). Minimum, maximum and average values of k600 are reported. IN
685 and OUT correspond to upstream and downstream stations, respectively. Measured concentrations of
686 dissolved gases and equilibrium concentrations based on temperature at each sampling station are
687 reported.

Reach	**C1**	**C2**	**C3**
k600 (cm h^{-1})	2.12 - 10.67 (4.92)	1.17 - 5.82 (3.13)	0.74 - 2.07 (1.41)
DARK			
T - IN (°C)	26.60±0.10	23.43±0.15	22.17±0.06
T - OUT (°C)	26.10±0.10	22.87±0.06	21.43±0.06
Measured [N_2] - IN (µM)	478.77±0.78	504.43±1.32	513.20±0.51
Measured [N_2] - OUT (µM)	482.03±0.80	511.68±0.51	514.09±0.52
Equilibrium [N_2] - IN (µM)	472.69±0.77	498.65±1.31	509.69±0.51
Equilibrium [N_2] - OUT (µM)	476.55±0.79	503.55±0.50	516.40±0.53
Measured [N_2O] - IN (nM)	87.05±4.30	102.96±2.01	60.83±1.81
Measured [N_2O] - OUT (nM)	63.54±2.48	84.64±3.08	46.75±1.83
Equilibrium [N_2O] - IN (nM)	7.26±0.02	7.99±0.04	8.32±0.02
Equilibrium [N_2O] - OUT (nM)	7.37±0.02	8.13±0.02	8.52±0.02
LIGHT			
T - IN (°C)	29.33±0.06	25.07±0.06	24.33±0.15
T - OUT (°C)	30.00±0.01	26.20±0.01	25.30±0.10
Measured [N_2] - IN (µM)	456.65±0.35	487.16±0.47	491.65±1.27
Measured [N_2] - OUT (µM)	450.51±0.01	477.72±0.01	486.10±0.81
Equilibrium [N_2] - IN (µM)	452.46±0.35	485.09±0.47	491.05±1.27
Equilibrium [N_2] - OUT (µM)	447.72±0.01	476.06±0.01	483.15±0.80
Measured [N_2O] - IN (nM)	80.42±9.53	96.33±2.32	47.15±1.67
Measured [N_2O] - OUT (nM)	46.48±12.90	58.28±3.21	45.28±1.30
Equilibrium [N_2O] - IN (nM)	6.76±0.01	7.59±0.01	7.76±0.04
Equilibrium [N_2O] - OUT (nM)	6.66±0.01	7.35±0.01	7.54±0.02

688

689

690 **Table 3.** Dissolved inorganic nitrogen inputs and outputs in the three investigated canals measured
691 in dark and light. IN and OUT correspond to upstream and downstream stations, respectively. Δ% is
692 calculated as (Load OUT- Load IN)/Load IN and expressed as a percentage. Areal rates are obtained
693 by dividing (Load OUT- Load IN) by the corresponding canal surface.

		NO_2^-+ NO_3^- load IN mol N h^{-1}	**NH_4^+ load IN** mol N h^{-1}	**NO_2^-+ NO_3^- load OUT** mol N h^{-1}	**NH_4^+ load OUT** mol N h^{-1}	**Δ DIN load** mol N h^{-1}	**Δ%**	**Areal Δ DIN** mmol N m^{-2} h^{-1}
C1	DARK	12.17± 2.57	0.18±0.04	8.97±1.89	0.27±0.06	-3.11±0.66	-25%	-1.68±0.36
	LIGHT	8.53±0.18	0.18±0.03	7.47±1.58	0.41±0.09	-0.84±0.18	-10%	-0.46±0.10
C2	DARK	19.14±4.04	0.83±0.18	12.51±2.64	1.03±0.20	-6.43±1.35	-32%	-2.20±0.46
	LIGHT	18.60±3.93	0.14±0.03	11.63±2.45	0.31±0.06	-6.86±1.44	-37%	-2.35±0.50
C3	DARK	3.03±0.64	0.23±0.05	2.18±0.46	0.27±0.06	-0.82±0.17	-25%	-0.43±0.09
	LIGHT	2.60±0.55	0.14±0.02	2.15±0.45	0.08±0.02	-0.46±0.10	-17%	-0.24±0.05

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695 **Table 4.** Nitrogen removal rates measured in selected canals and ditches of agricultural catchments around the world.

Location	N removal rate mmol N m^{-2} h^{-1}	T °C	Discharge m^3 s^{-1}	Vegetation	Depth m	$[NO_3^-]$ µM	Method	Reference
Canal receiving waste effluent, Denmark	2.72 0.24-0.51	20	<0.1	No	<1.2	20-700	Reach-scale N mass balance NO_3^- fluxes, core incubations	van Kessel, 1977 van Kessel, 1977
Agricultural canal, USA	0.60-2.38	5-30	<0.2	Yes	1.3-1.8	7-700	Reach-scale N mass balance	Birgand, 2005
Agricultural canal, Italy	0.05-0.10	12-25	-	No	0.5	10-80	Isotope Pairing Technique, core incubation	Racchetti et al., 2011
Drainage ditches, China	0.03-0.74	10-25	-	No	0.5-0.8	10-180	N_2 fluxes, core incubations	Li et al., 2013
Drainage ditches, Italy	0.36-3.60	22-23	0.02-0.06	No/Yes	-	80-150	Reach-scale N mass balance	Pierobon et al., 2013
Experimental drainage ditch, China	1.20-3.60	*	-	Yes	0.8	400-1500	Reach-scale N mass balance	Zhang et al., 2013
Drainage ditches, USA	0.03-0.32	**	-	No	-	230-460	N_2 fluxes, core incubations	Kröger and others, 2014
Drainage ditches, Italy	0.14-0.28	21-30	0.02-0.09	No	0.3-0.6	30-50	N_2 fluxes, core incubations	This study
Drainage ditches, Italy	0.47-4.24			Yes			N_2 open-channel	This study

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697 * Measurements were performed in different plant growth stages (early, mid and late growing season).

698 ** Measurements were performed in spring, summer and winter.

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Table 5. Denitrification rates measured with the N_2 open-channel method in selected lotic environments around the world.

Location	Denitrification rate mmol N m^{-2} h^{-1}	T °C	Discharge m^3 s^{-1}	Depth m	$[NO_3^-]$ µM	Reference
Iroquois River, USA	3.4-8.5	18-24	8-19	1.5-2	-	Laursen and Seitzinger 2002
Sugar Creek, USA	0.27	22	1.6	0.4	-	
Milstone River, USA	1.9-15.8	7-12	3-10	1-1.2	-	
Iroquois River, USA	2.6-15.9	9-26	-	-	730-907	
Sugar Creek, USA	3.6-10.5	16-28	-	-	980-1020	Laursen and Seitzinger 2004
Milstone River, USA	0.3-2.7	10-17	-	-	90-170	
South Platte River	7.83	12	12	0.5	440	McCutchan et al., 2003
Changjiang River, China	2.82-5.74	6-26	20600-51000	12-18	<100	Yan et al., 2004
South Platte River, USA	0-9.16	4-30	10-130	0.3-0.7	215-430	Pribyl et al., 2005
South Platte River, USA	0.30-10.12	7-23	-	-	280-430	McCutchan et al., 2008
Connecticut River, USA	0.17-0.23	24	<50	1.3	<20	Smith et al., 2008
Jiulong River Estuary, China	0.06-5.58	14-30	260-585	-	10-200	Wu et al., 2013
West River, China	0.50-1.53	15-30	23-308	0.6-3	80-890	Chen et al., 2014
North River, China	0.07-3.51	15-30	45-640	0.6-3	100-370	Chen et al., 2014

Figure captions

Fig. 1. Hydrological network of the Po di Volano Basin with the indication of the three sampled reaches (C1, C2, and C3).

Fig. 2. Experimental set-up adopted for each investigated site.

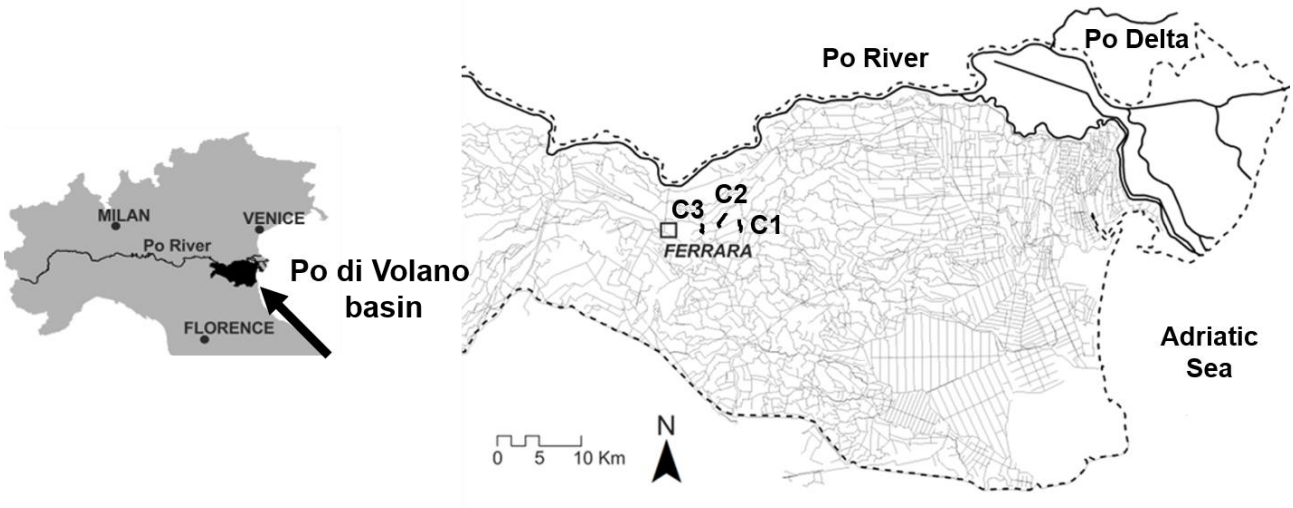
Fig. 3. Reach-scale N_2 fluxes estimated in the three investigated canals.

Fig. 4. Benthic fluxes of O_2, N_2 and dissolved inorganic nitrogen forms measured via incubations of sediment cores sampled from the three investigated ditches (average±standard deviation, n=9).

Fig. 5. Fluxes of dissolved nitrogen species in the three investigated ditches. Rates measured in bare sediments and at the reach-scale are reported (note the different ordinate scales in the three graphs).[1]

[1] In C3, reach-scale net N_2 flux obtained with the minimum k600 value resulted almost null and is not visible in the graph.

713 Fig.



714

715 Fig.1

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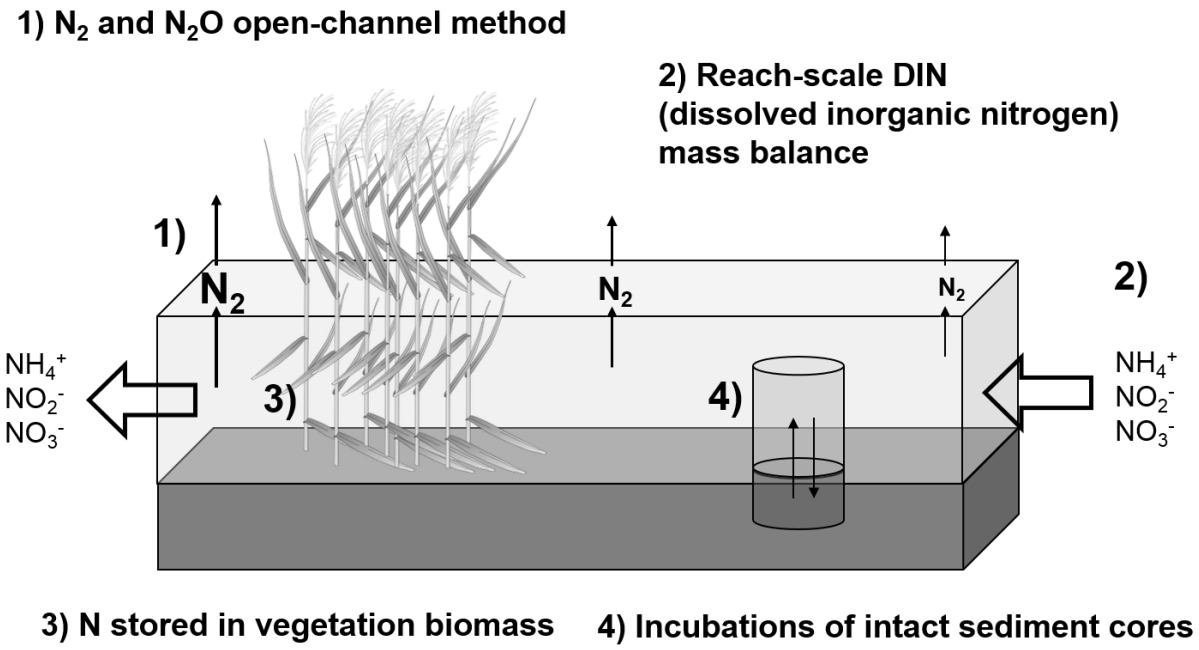
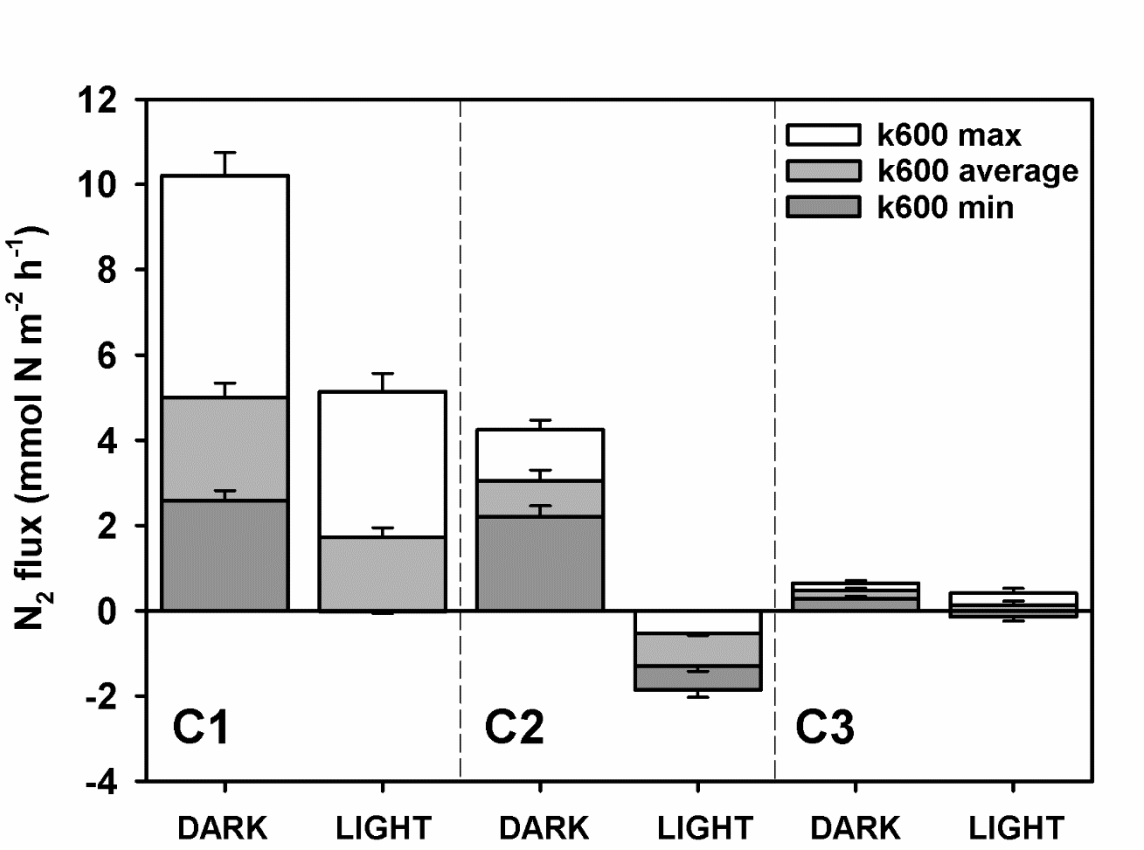

1) N_2 and N_2O open-channel method
2) Reach-scale DIN (dissolved inorganic nitrogen) mass balance
1)
N_2
N_2
N_2
2)
NH_4^+
NO_2^-
NO_3^-
3)
4)
NH_4^+
NO_2^-
NO_3^-
3) N stored in vegetation biomass
4) Incubations of intact sediment cores

Fig. 2



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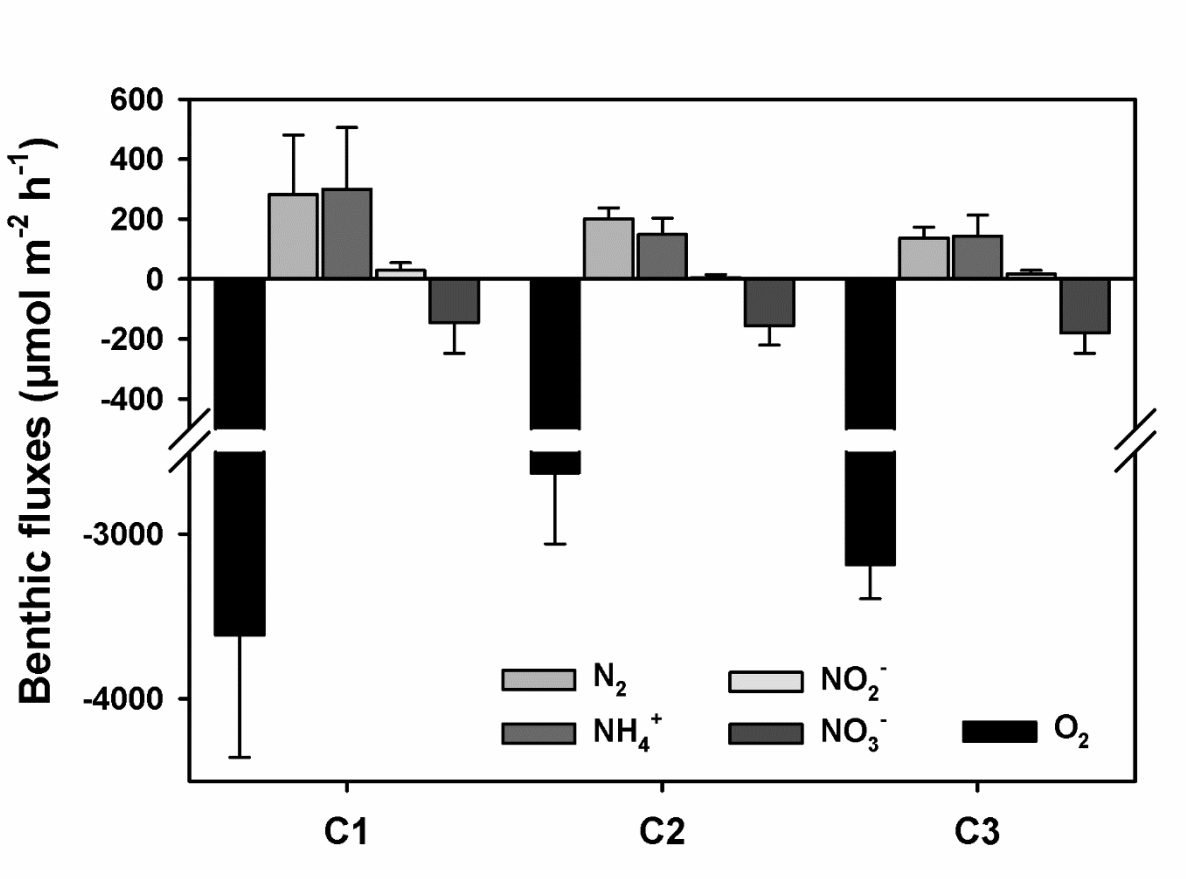
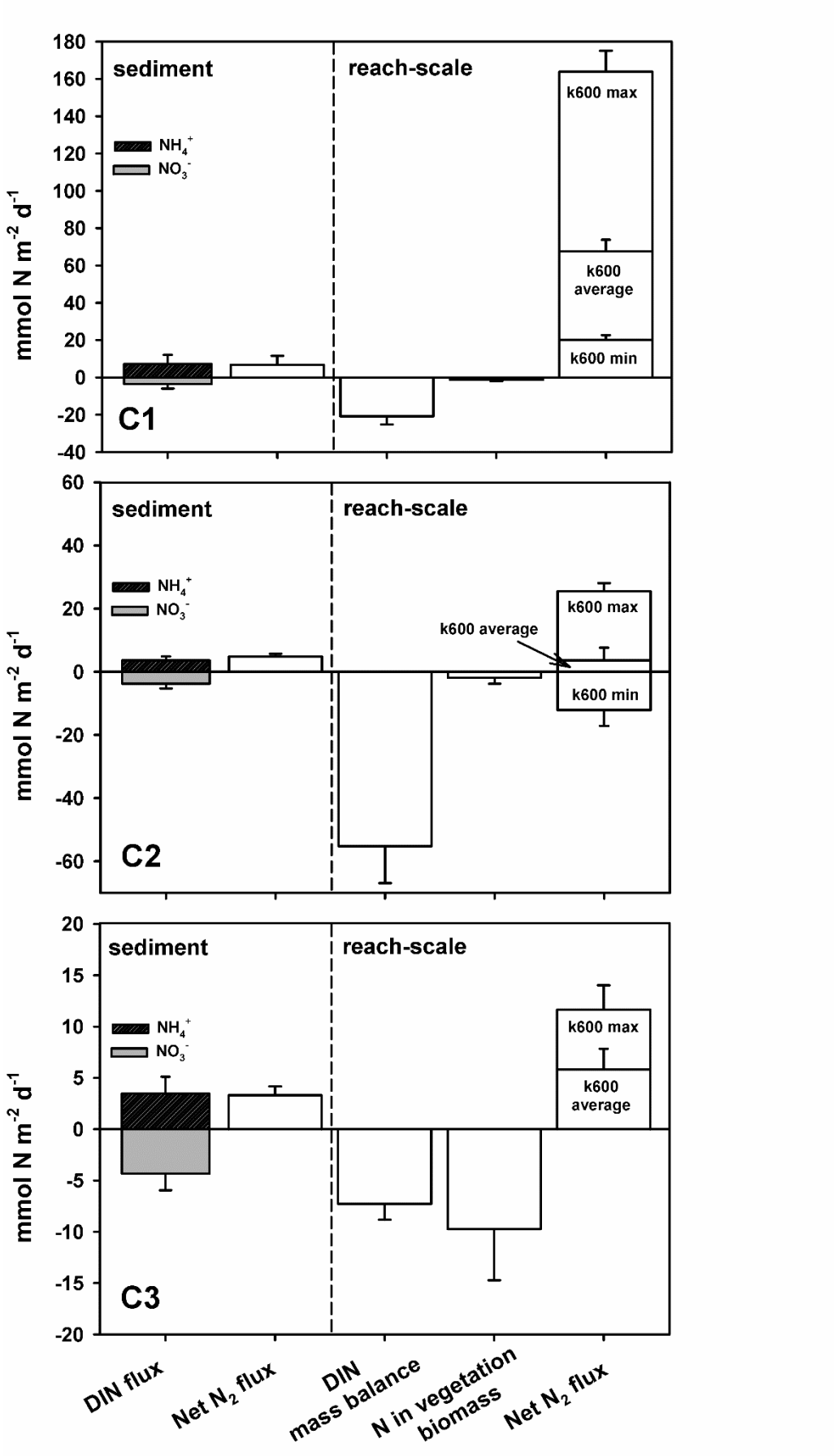
722 Fig. 3

723


Benthic fluxes (μmol m-2 h-1)
600
400
200
0
-200
-400
-3000
-4000
N2
NO2-
NH4+
NO3-
O2
C1
C2
C3

724

725 Fig. 4


sediment
reach-scale
k600 max
k600 average
k600 min
NH4+
NO3-
mmol N m-2 d-1
C1
C2
C3
k600 average
DIN flux
Net N2 flux
DIN mass balance
N in vegetation biomass
Net N2 flux

726

727 Fig. 5