

1                   **An ounce of prevention is worth a pound of cure:**  
2                   **managing macrophytes for nitrate mitigation in irrigated agricultural**  
3                   **watersheds**

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13  
14 **Abstract**

15 Although ubiquitous elements of agricultural landscapes, the interest on ditches and canals as  
16 effective filters to buffer nitrate pollution has been raised only recently. The aim of the present study  
17 was to investigate the importance of in-ditch denitrification supported by emergent aquatic vegetation  
18 in the context of N budget in agricultural lands of a worldwide hotspot of nitrate contamination and  
19 eutrophication, i.e. the lowlands of the Po River basin (Northern Italy). The effectiveness of N  
20 abatement in the ditch network (>18,500 km) was evaluated by extrapolating up to the watershed  
21 reach-scale denitrification rates measured in a wide range of environmental conditions. Scenarios of  
22 variable extents of vegetation maintenance were simulated (25%, 50% and 90%), and compared to  
23 the current situation when the natural development occurs in 5% of the ditch network length,  
24 subjected to mechanical mowing in summer.

25 Along the typical range of nitrate availability in the Po River lowlands waterways (0.5–8 mg N l<sup>-1</sup>),  
26 the current N removal performed by the ditch network was estimated in 3,300–4,900 t N yr<sup>-1</sup>,  
27 accounting for at most 11% of the N excess from agriculture. The predicted nitrate mitigation  
28 potential would increase up to 4,000–33,600 t N yr<sup>-1</sup> in case of vegetation maintenance in 90% of  
29 the total ditch length. Moreover, a further significant enhancement (57% on average) of this key  
30 ecosystem function would be achieved by postponing vegetation mowing at the end of the growing  
31 season.

32 The simulated outcomes suggest that vegetated ditches may offer new agricultural landscape  
33 management opportunities for effectively decreasing nitrate loads in surface waters, with potential  
34 improved water quality at the watershed level and in the coastal zones. In conclusion, ditches and  
35 canals may act as metabolic regulators and providers of ecosystem services if conservative  
36 management practices of in-stream vegetation are properly implemented and coupled to hydraulic  
37 needs.

38  
39 **Keywords:** nitrate pollution, ditch network, denitrification, vegetation management, Po River basin

## 40        **1. Introduction**

41        Denitrification, the reduction of nitrate (N-NO<sub>3</sub><sup>-</sup>) to nitrogen (N) gases under anaerobic conditions, is  
42        globally considered the main biogeochemical process responsible for permanent removal of  
43        anthropogenic N along the terrestrial-freshwater-estuarine continuum. Understanding the relative  
44        contributions of terrestrial and aquatic compartments to denitrification, and its temporal and spatial  
45        variability, remains a significant challenge in biogeochemistry (Burgin et al., 2013; Duncan et al.,  
46        2013; Anderson et al., 2014). Furthermore, landscape science aimed at developing best management  
47        practices for improving water quality in agricultural watersheds could benefit from increased  
48        knowledge on denitrification (Dollinger et al., 2015; Kalcic et al., 2018). Several studies have  
49        highlighted that an internal generation of large N loads in irrigated landscapes impacted by intensive  
50        agricultural activities may not necessarily result in high export to downstream aquatic ecosystems  
51        and coastal zones (Bartoli et al., 2012; Castaldelli et al., 2013; Romero et al., 2016). Both plot-scale  
52        and basin-scale observations demonstrated that the landscape potential capacity to buffer N increases  
53        in relation to the density of small-size aquatic ecosystems (e.g. wetlands, reservoirs, drainage ditches  
54        and drains) (Lassaletta et al., 2012; Powers et al., 2015; Hansen et al., 2018), and the availability of  
55        dissolved inorganic N forms in surface waters is negatively correlated to the recirculation degree of  
56        irrigation water (Hitomi et al., 2006; Törnqvist et al., 2015). Indeed, water management practices  
57        aimed at guaranteeing a supply for agricultural uses (i.e. flow regulation, water diversions), deeply  
58        affect the N delivery patterns from the agro-ecosystems to the terminal water bodies. In particular,  
59        during the vegetative period, water volumes diverted from the main waterways are redistributed,  
60        together with associated solutes, on the landscape via extensive networks of canals and ditches where  
61        increased flow-path lengths and hydraulic residence time offer high opportunity for N processing and  
62        losses (Barakat et al., 2016; Mortensen et al., 2016).

63        While the role of undisturbed headwater streams, wetlands and buffer zones in watershed N dynamics  
64        has been extensively investigated in the past (Peterson et al., 2001; Mayer et al., 2007; Passy et al.,  
65        2012; Hansen et al., 2018), the interest on agricultural ditches as effective N filters has been raised

66 only recently (McPhillips et al., 2016; Veraart et al., 2017; Speir et al., 2017; Schilling et al., 2018).  
67 Although ubiquitous elements of human-impacted watersheds, and usually accounting for the greatest  
68 part of the total length of waterways, agricultural ditches still remain largely understudied compared  
69 to other aquatic ecosystems (Pina-Ochoa and Alvarez-Cobelas, 2006) and are scarcely included in  
70 restoration programs compared to wetlands and vegetated buffer strips (Dalgaard et al., 2014;  
71 Dollinger et al., 2015; Faust et al., 2018). The usually low habitat complexity of these modified  
72 waterways is a direct consequence of human-driven alterations and management practices (e.g.  
73 homogeneous morphology of the riverbed, channelization, burial, artificial flow regime, reduction of  
74 riparian vegetation, dredging). This condition has been traditionally associated with reduced  
75 efficiencies in organic matter processing and N removal compared to natural systems, leading to the  
76 belief that these systems act simply as conduits for N (Pinay et al., 2002; Beaulieu et al., 2015; Moore  
77 et al., 2017). However, multiple features of agricultural ditches may support a high mitigation  
78 potential towards N-NO<sub>3</sub><sup>-</sup>: i) their being the first point of contact for diffuse and point N loads entering  
79 the hydrological network; ii) the occurrence of the three primary controls directly influencing  
80 occurrence and magnitude of denitrification, i.e. anoxic environment, availability of N-NO<sub>3</sub><sup>-</sup> and  
81 organic carbon; iii) the tight terrestrial-aquatic coupling resulting from their extensive and capillary  
82 distribution across the landscape; iv) the high opportunity for N microbial processing due to long  
83 hydraulic residence time and large ratio between biological active surfaces and in-stream water  
84 volumes carrying excess nutrients; v) the shallow water depth that potentially supports the role of  
85 aquatic vegetation as “ecosystem engineer” in regulating biogeochemical processes and providing the  
86 development of denitrification hotspots, such as biofilms on submerged portions and oxic-anoxic  
87 niches in organic matter-rich sediments; vi) the frequent recirculation of water through the landscape,  
88 which maximizes the interaction among water and bioreactive surfaces, especially during spring and  
89 summer period when high water temperatures (up to >25 °C in temperate zones) enhance microbial  
90 processes (McClain et al., 2003; Hines, 2006; Marion et al., 2014; Soana et al., 2017). All the above-

91 mentioned features make agricultural ditches and canals more similar to wetlands than to higher-order  
92 streams (Faust et al., 2018; Vymazal and Březinová, 2018).

93 Only a few attempts were made to assess the magnitude of catchment-scale denitrification in low-  
94 order waterways and drainage ditches, but modelling tools and geospatial approaches were often  
95 based on the up-scale of measurements of potential denitrification rates determined under optimal  
96 conditions of N-NO<sub>3</sub><sup>-</sup> and organic carbon availability, not always reflecting the actual *in situ* activity  
97 (Oehler et al., 2009; Christopher et al., 2017; O'Brien et al., 2017). Moreover, the potential for water  
98 quality improvement exerted by vegetation in the ditch network has never been assessed at the  
99 watershed scale. In-stream vegetation is an important interface between croplands and surface water  
100 bodies, thus its presence and abundance are considered key elements in determining the potentiality  
101 of the canal network to provide ecosystem services in general (Bolpagni et al., 2013; Boerema et al.,  
102 2014; Dollinger et al., 2015), and water purification in particular, due to a complex synergistic action  
103 with bacterial communities (Pierobon et al., 2013; Taylor et al., 2015; Vymazal and Březinová, 2018).

104 Nevertheless, aquatic vegetation is often considered only as a hindrance for water circulation by water  
105 management authorities, and thus regularly removed to preserve the hydraulic performance  
106 (Levavasseur et al., 2014).

107 The Po River catchment, the largest hydrographic system in Italy (652 km, more than 71,000 km<sup>2</sup>,  
108 about a quarter of the national territory), is one of the most densely populated and agriculturally  
109 productive areas in Europe, but also a paradigmatic case study for N pollution, eutrophication and  
110 related implications for environmental policies (Viaroli et al., 2018; Martinelli et al., 2018). The plain  
111 zone (46,000 km<sup>2</sup>), the largest Italian alluvial basin, is crossed by an extensive network of mostly  
112 artificial canals and ditches with irrigation, drainage, and flood control purposes. A comprehensive  
113 N budget has proven that the deltaic portion of the catchment, intensively cultivated and irrigated,  
114 acts as an effective N sink, buffering not only the N surplus leached from the croplands but also part  
115 of the N load generated by upstream agro-ecosystems and imported with drainage and irrigation water  
116 (Castaldelli et al., 2013). Multiple lines of evidences suggest that denitrification in vegetated ditches

117 accounts for the majority of N losses during water transit through the hydrological network (Pierobon  
118 et al., 2013; Castaldelli et al., 2015; Soana et al., 2018).

119 Our hypothesis is that in highly hydraulic-regulated and simplified, agricultural watersheds,  
120 landscape management may deeply affect the balance between N sources and sinks and thus, at a  
121 widely variable range, also the quality of outflows and N delivery to coastal areas. We investigated  
122 whether N excess in intensive agriculture impacted watersheds may be efficiently controlled by  
123 conservative management of in-stream macrophytes to promote denitrification. Our case study was  
124 the Po River system because it represents a worldwide hot spot of N-NO<sub>3</sub><sup>-</sup> contamination and  
125 eutrophication. We hypothesize that vegetated ditches may offer new management opportunities for  
126 effectively decreasing NO<sub>3</sub><sup>-</sup> loads in surface waters due to the intertwined action of macrophytes and  
127 microbial communities promoting N processing and sustaining their natural depuration capacity.

128 The effectiveness of N abatement in the ditch network was evaluated by combining reach-scale  
129 denitrification rates from previous studies in the area, measured across a wide range of environmental  
130 conditions, and GIS-based upscaling. The potentiality of the ditch network to buffer watershed-scale  
131 N-NO<sub>3</sub><sup>-</sup> pollution was quantified for four different levels of vegetation maintenance (5%, 25%, 50%  
132 and 90% of the total length) and two mowing timings, i.e. the *current management* where the cutting  
133 is performed in the middle of the summer and the *conservative management* where the cutting is  
134 postponed to the end of the growing season.

135

## 136 **2. Material and Methods**

### 137 *2.1 Study area description*

138 The study area included the Po River plain below 50 m above sea level (a.s.l.), an area of ~ 9100 km<sup>2</sup>  
139 (Fig. 1A, B), covering ~15% of the whole Po River basin but also the most intensively cultivated  
140 zone. We used a 20-m digital elevation model, provided by the Italian Ministry of the Environment  
141 and Protection of Land and Sea (<http://www.pcn.minambiente.it>) to generate a slope map from which  
142 the area laying within the 50 m sea-level curve was selected. The Po River crosses this area with its

143 300 km long final reach before the delta in the Adriatic Sea. This area has a humid subtropical climate  
144 (Type Cfa, according to Köppen classification), with a yearly average rainfall of 700-800 mm, mainly  
145 concentrated in autumn and spring (Joint Research Centre meteorological datasets,  
146 <http://eusoils.jrc.ec.europa.eu/library/Data/EFSA/>).

147 The landscape surrounding the river course is a typical over-exploited plain devoted to farming  
148 (mainly cows and pigs) and agricultural practices, resulting in >87% of the area cultivated by corn,  
149 wheat and temporary grassland as main crops (6<sup>th</sup> Agricultural Census, National Institute for  
150 Statistics, 2010, <http://dati-censimentoagricoltura.istat.it>), while approximately 6% of the surface is  
151 classified as urbanized land (~1.8 million inhabitants, ~11% of the total population in the Po River  
152 catchment, average density 200 inhabitants km<sup>-2</sup>; 15<sup>th</sup> Population and housing census, National  
153 Institute for Statistics, 2011, <http://dati.istat.it>), and <3% as forest and semi natural area (Corine Land  
154 Cover inventory 2012, level 1; Fig. 1C). Following the enactment of the European Water Framework  
155 Directive (2000/60 CE), about half of the whole study area was declared “vulnerable to nitrates from  
156 agricultural sources”. More than 270 municipalities (surface from 6 to 405 km<sup>2</sup>) are included totally  
157 or partly within the study area, belonging to nine Italian provinces, three in the Lombardy Region  
158 (Lodi, Cremona and Mantova), five in the Emilia-Romagna Region (Piacenza, Parma, Reggio Emilia,  
159 Modena and Ferrara), and one in the Veneto Region (Rovigo).

160 The area is homogenous in terms of source of the irrigation water (i.e. Po River) and chemical quality  
161 of surface waters. During the growing season (May–September), water is diverted mainly from the  
162 Po River into some main canals to irrigate croplands by an extensive open-earth ditch network of  
163 >18,500 km (Fig. 1D; average density ~3 km per km<sup>2</sup> of utilised agricultural land), managed by  
164 fourteen local land reclamation authorities. Flat topography, low soil permeability and slopes of a few  
165 cm per km generate low water velocities (up to 10 cm s<sup>-1</sup>) and bottom sediments are usually a  
166 combination of muddy sand or muddy silt.

167 Heavy management practices of the ditch network (i.e. dredging, mechanical mowing, chemical  
168 weeding) has led to the complete disappearance of the submerged vegetation (Piccoli and Gerdol,

169 1983). The voluntary introduction, widespread diffusion and establishment of the exotic grass carp  
170 (*Ctenopharyngodon idella*, Valenciennes 1844) contributed as well to the biological control of  
171 submerged plants and to the turbid, phytoplankton-dominated status of most watercourses (Milardi  
172 et al., 2015). Bank mowing is performed twice a year, in the middle of the summer (i.e. August) to  
173 facilitate water flow during the period of highest water demand for irrigation, and before the period  
174 of intense rainfall (i.e. October) to reduce flood risk. Natural propagation and evolution of helophytes  
175 are strongly affected by routine management practices that have been in place since 1990s and as a  
176 result, vegetation is maintained only in isolated stretches of the ditch network with low flood risk,  
177 representing about 5% of the total length. Macrophyte stands are generally not monospecific but  
178 composed by two main species, *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha latifolia* L.,  
179 and to a lesser extent also by *Glyceria maxima* Hartm. At peak biomass, standing stocks range from  
180 230 to 550 g of dry biomass per m<sup>2</sup> (Pierobon et al., 2013). Before the introduction of mechanical  
181 vegetation mowing (in the 1980s), vegetation cover was supposed to be 90% of the total length, with  
182 the exclusion of the deeper canals used also for navigation and where depth, sediment resuspension  
183 and turbidity prevented both submerged and emergent macrophytes to develop.

184

## 185 2.2 Denitrification rates in vegetated and unvegetated ditches

186 Measurements of N-NO<sub>3</sub><sup>-</sup> removal rates (kg km<sup>-1</sup> d<sup>-1</sup>) obtained for vegetated and unvegetated ditches  
187 by reach-scale in-out N-NO<sub>3</sub><sup>-</sup> budgets along the irrigation period (May–September), corresponding  
188 to the macrophyte growing season, were synthesized from previous sampling campaigns (Pierobon  
189 et al., 2013; Castaldelli et al., 2015). These experimental activities were conducted on several  
190 drainage ditches belonging to the hydrological network of the Po di Volano basin, a deltaic reclaimed  
191 alluvial area (~2,600 km<sup>2</sup>; Fig. A1, Appendix A), representing about one third of the total area  
192 under investigation in the present study. The studied sites were representative of the dominant  
193 waterways type of the whole Po River lowlands, in terms of chemico-physical features (i.e. dissolved  
194 inorganic N and organic carbon availability), substrate, hydraulic regime, routine management

195 practices and, if present, emergent aquatic vegetation.  $\text{N-NO}_3^-$  removal rates were estimated from  
196 changes in  $\text{N-NO}_3^-$  loads along the selected reach by adopting a Lagrangian sampling scheme  
197 during stable weather and flow conditions. Details about experimental design, analytical methods and  
198 calculations of  $\text{N-NO}_3^-$  removal rates are reported in Pierobon et al. (2013) and Castaldelli et al.  
199 (2015).

200 As vegetation presence and  $\text{N-NO}_3^-$  availability were considered the main factors affecting ditch N  
201 dissipation capacity, predictive relationships between incoming water  $\text{N-NO}_3^-$  concentrations and  
202 daily reach-scale  $\text{N-NO}_3^-$  removal rates were built employing the previous acquired datasets,  
203 separately for vegetated (data from Pierobon et al., 2013 and Castaldelli et al., 2015) and unvegetated  
204 ditches (data from Pierobon et al., 2013). Specifically, water  $\text{N-NO}_3^-$  concentrations were plotted  
205 against experimental values of  $\text{N-NO}_3^-$  daily removal rates and the obtained regression was used to  
206 predict  $\text{N-NO}_3^-$  removal in vegetated ditch sediments ( $\text{Dr}_v$ ) as a function of water  $\text{N-NO}_3^-$  availability  
207 spanning the typical range found in N-polluted artificial waterways of the Po River lowlands (0.5–8  
208  $\text{mg L}^{-1}$ ). Since this dataset did not include direct measurements of  $\text{N-N}_2$  production rates, we provided  
209 independent evidences supporting the hypothesis of denitrification being the main process  
210 responsible for  $\text{N-NO}_3^-$  dissipation in vegetated sediments. First, our previous study (Pierobon et al.,  
211 2013) demonstrated that plant N uptake and sequestration in biomass represent a small fraction of the  
212 total  $\text{N-NO}_3^-$  consumption (<5%). This statement was further supported by presenting dataset of  $\text{N-}$   
213  $\text{N}_2$  production rates and corresponding  $\text{N-NO}_3^-$  removal rates obtained by the simultaneous  
214 application of the  $\text{N}_2$  open channel method and reach-scale  $\text{N-NO}_3^-$  budget in vegetated ditch  
215 sediments (Castaldelli et al., 2015; Castaldelli et al., 2018, Soana et al., 2018). The  $\text{N}_2$  open channel  
216 approach, proposed by Laursen and Seitzinger (2002), provides the direct estimate of whole-system  
217 denitrification in running waters by measuring the variation of its end-product, i.e.  $\text{N}_2$ , within a water  
218 parcel moving from upstream to downstream. A model-based approach is used to solve for  
219 denitrification rates, correcting the variations of  $\text{N}_2$  for atmospheric exchanges during transport,  
220 according to concentration gradients and channel morphology (e.g. width and depth). This approach



221 allows to estimate *in situ* net N<sub>2</sub> fluxes (i.e. total denitrification, including denitrification coupled to  
222 nitrification, minus N<sub>2</sub> fixation) under natural conditions, at a scale comparable to the reach-scale N-  
223 NO<sub>3</sub><sup>-</sup> budgets. Details about experimental design, analytical methods and calculations of N-N<sub>2</sub>  
224 production rates are reported in Castaldelli et al. (2015). Descriptive statistics of the used datasets are  
225 reported in Appendix A (Figs. A2, A3 and A4).

226 Daily denitrification rates for unvegetated sediments (i.e. N-N<sub>2</sub> production rates, Dr<sub>UV</sub>, mg m<sup>-2</sup> d<sup>-1</sup>)  
227 were estimated according to a simple diffusion-reaction model proposed by Christensen et al. (1990)  
228 for N-NO<sub>3</sub><sup>-</sup>-rich streams, and previously tested in unvegetated sediments of several shallow slow-  
229 flow aquatic environments of the Po River Plain (Bartoli et al., 2008; Pinardi et al., 2009; Racchetti  
230 et al., 2011):

$$231 \quad Dr_{UV} = SOD \cdot 0.8 \cdot \left[ \sqrt{1 + 0.82 \cdot \frac{N - NO_3^-}{DO} \cdot \frac{1}{0.8}} - 1 \right]$$

232 Where:

233 SOD = Sediment Oxygen Demand (mg m<sup>-2</sup> d<sup>-1</sup>)

234 N-NO<sub>3</sub><sup>-</sup> = concentrations of water column nitrate (mg L<sup>-1</sup>)

235 DO = concentrations of water column dissolved oxygen (mg L<sup>-1</sup>)

236 0.8 = ratio between the activities per unit of volume in the denitrification and oxygen respiration  
237 zones, found to be relatively constant in stream sediments and biofilms (Nielsen et al., 1990)

238 0.82 = ratio between the diffusion coefficients of N-NO<sub>3</sub><sup>-</sup> and DO (Christensen et al., 1990; Nielsen  
239 et al., 1990).

240

241 The predicted rates represent denitrification of N-NO<sub>3</sub><sup>-</sup> diffusing from the water column into anoxic  
242 sediments, which constitutes the dominant source of NO<sub>3</sub><sup>-</sup> required for denitrification when  
243 concentrations are generally higher than 0.5 mg L<sup>-1</sup>, as demonstrated for sediments across a wide  
244 range of freshwater and marine ecosystems (Pina-Ochoa and Alvarez-Cobelas, 2006; Seitzinger et

245 al., 2006). Furthermore, nitrification in organic-rich muddy sediments may be severely limited by  
246 oxygen availability, thus being only a minor source of  $\text{N-NO}_3^-$  for coupled denitrification (Racchetti  
247 et al., 2011; Soana et al., 2015).  $\text{N-N}_2$  production rates obtained on a surface basis were converted  
248 into values expressed per unit of ditch length ( $\text{kg km}^{-1} \text{d}^{-1}$ ) by considering an average ditch width of  
249 3 m (Pierobon et al., 2013; Castaldelli et al., 2015).

250 Water quality data of the ditch network (i.e. T-water temperature, DO,  $\text{N-NO}_3^-$ ,  $\text{BOD}_5$ - Biochemical  
251 Oxygen Demand) were provided by the Regional Agency for the Environmental Protection (ARPA)  
252 of Lombardy and Emilia-Romagna Regions (monthly data, from 2009 to 2014,  
253 <http://www.arpalombardia.it/Pages/Acqua.aspx>; <https://www.arpae.it/index.asp?idlivello=112>).

254 Stations on artificial waterways (N=70) located within the 50 m a.s.l. area were selected from the  
255 ARPA surface water monitoring network, which include natural systems, such as streams, rivers and  
256 lakes. The equation by Christensen et al. (1990) was applied to all ARPA surveys on ditch network  
257 for which datasets of water T ( $^\circ\text{C}$ ), DO ( $\text{mg L}^{-1}$ ) and  $\text{N-NO}_3^-$  ( $\text{mg L}^{-1}$ ) were concomitantly available.  
258 Descriptive statistics of the water quality datasets are reported in Appendix A (Table A1). A range of  
259 daily denitrification rates was calculated for each month from January to March and from August to  
260 December.

261 Experimental measurements of SOD for ditch environments of the Po River plain are limited to  
262 summer months (Castaldelli et al., 2015; Soana et al., 2017) and seasonal evolution is lacking. Thus,  
263 following Soana et al. (2011), reasonable estimates of SOD ditch sediments were calculated as a  
264 function of water T by applying the equation obtained from a large dataset of SOD values measured  
265 in a vast array of shallow eutrophic environments having muddy bottoms similar to slow-flow  
266 agricultural waterways (Pinaridi et al., 2009; Racchetti et al., 2011; Pinaridi et al., 2011; Ribauda et  
267 al., 2011; Soana et al., 2015) (Appendix A, Fig. A5).

268

269 *2.3. Scaling  $\text{N-NO}_3^-$  removal rates to the ditch network: scenarios of vegetation maintenance*

270 In order to extrapolate N-NO<sub>3</sub><sup>-</sup> removal measurements to the entire study area, a detailed map of the  
 271 canal and ditch network (Fig. 1D) was created in QGIS 2.18 by merging vector data obtained by the  
 272 geoportals of the Italian regions included in the study area (Lombardy Region,  
 273 <http://www.geoportale.regione.lombardia.it/>; Emilia-Romagna Region  
 274 <http://geoportale.regione.emilia-romagna.it/it>; and Veneto Region, <http://idt.regione.veneto.it>) and of  
 275 the Po River Basin Authority within the framework of the Water Management Plan of the Po River  
 276 Basin (<http://pianoacque.adbpo.it/piano-di-gestione-2015/>).

277 Four scenarios of in-stream vegetation maintenance were simulated, 5% (current level), 25%, 50%  
 278 and 90% (historic level) of total ditch length, while the remaining length was assumed as unvegetated.  
 279 For each scenario, two options of vegetation management were assessed, i.e. the *current management*  
 280 where the mowing is performed in the middle of the summer (effect of vegetation on N dynamics for  
 281 120 days, from April to July), and the *conservative management* where the mowing is postponed to  
 282 the end of the growing season (effect of vegetation on N dynamics for 210 days, from April to  
 283 October). The potential N-NO<sub>3</sub><sup>-</sup> removal capacity of the ditch network was thus tested in eight  
 284 different conditions (four extensions of in-stream vegetation x two options of vegetation  
 285 management) and compared to the N budget in the surrounding agricultural lands.

286 The following equation was used to predict the N-NO<sub>3</sub><sup>-</sup> removal capacity (t yr<sup>-1</sup>) of the ditch network  
 287 considering the *current management* of the vegetation:

$$288 \quad NR_{cur} = ((100 - V\%) \cdot L \cdot \sum (Dr_{UV} \cdot n) + V\% \cdot L \cdot Dr_V \cdot N_{cur}) * \frac{1}{1000}$$

289 where:

290 V% = scenario of vegetation maintenance (5%, 25%, 50%, 90% of total ditch length)

291 L = total ditch length (km)

292 Dr<sub>UV</sub> = daily rates in unvegetated ditch sediments calculated by the model proposed by Christensen  
 293 et al. (1990) (kg km<sup>-1</sup> d<sup>-1</sup>) for each month from January to March and from August to December

294 n = number of days of each month (from January to March and from August to December)

295  $Dr_V$  = daily rates of N-NO<sub>3</sub><sup>-</sup> removal (kg km<sup>-1</sup> d<sup>-1</sup>) in vegetated ditch sediments calculated as a function  
296 of water N-NO<sub>3</sub><sup>-</sup> availability in the range 0.5–8 mg L<sup>-1</sup> by employing the predictive relationship  
297 explained in paragraph 2.2

298  $N_{cur}$  = 120, numbers of days with vegetation maintenance in the *current management* (d yr<sup>-1</sup>)

299

300 The following equation was used to predict the N-NO<sub>3</sub><sup>-</sup> removal capacity (t yr<sup>-1</sup>) of the ditch network  
301 considering the *conservative management* of the vegetation:

$$302 \quad NR_{cons} = ((100 - V\%) \cdot L \cdot \sum (Dr_{UV} \cdot n) + V\% \cdot L \cdot Dr_V \cdot N_{cons}) \cdot \frac{1}{1000}$$

303 where:

304  $V\%$  = scenario of vegetation maintenance (5%, 25%, 50%, 90% of total ditch length)

305  $L$  = total ditch length (km)

306  $Dr_{UV}$  = daily rates in unvegetated ditch sediments calculated by the model proposed by Christensen  
307 et al. (1990) (kg km<sup>-1</sup> d<sup>-1</sup>) for each month from January to March and from November to December

308  $n$  = number of days of each month (from January to March and from November to December)

309  $Dr_V$  = daily rates of N-NO<sub>3</sub><sup>-</sup> removal (kg km<sup>-1</sup> d<sup>-1</sup>) in vegetated ditch sediments calculated as a function  
310 of water N-NO<sub>3</sub><sup>-</sup> availability in the range 0.5–8 mg N L<sup>-1</sup> by employing the predictive relationship  
311 explained in paragraph 2.2

312  $N_{cons}$  = 210, numbers of days with vegetation maintenance in the *conservative management* (d yr<sup>-1</sup>)

313

314 To determine the likely variation of the potential N-NO<sub>3</sub><sup>-</sup> removal capacity of the entire ditch network,  
315 the interquartile range (first and fourth quartiles as inferior and superior extremes) for N removal in  
316 unvegetated condition was combined with the interquartile range for N removal in presence of  
317 vegetation. Thus, for each scenario of in-stream vegetation and condition of water N-NO<sub>3</sub><sup>-</sup>  
318 availability, best-case and worst-case situations were estimated.

319

320 *2.4. Calculation of N budget in agricultural land*

321 A N balance in agricultural land of the investigated area was compiled for the year 2010 by integrating  
322 official census data in a nutrient budgeting approach previously applied to some sub-basins of the Po  
323 River system (Soana et al. 2011; Bartoli et al., 2012; Castaldelli et al. 2013; Pinardi et al., submitted)  
324 and other Italian temporary rivers (De Girolamo et al. 2017). N budget ( $\text{t yr}^{-1}$ ) was estimated as the  
325 net difference between N inputs and N outputs across the Utilised Agricultural Area (UAA) and  
326 calculated as follow:

327 
$$\text{N budget} = N_{\text{Man}} + N_{\text{Fert}} + N_{\text{Fix}} + N_{\text{Dep}} - N_{\text{Harv}} - N_{\text{Vol}} - N_{\text{Den}}$$

328 where:

329  $N_{\text{Man}}$  = N in livestock manure applied to UAA ( $\text{t yr}^{-1}$ )

330  $N_{\text{Fert}}$  = synthetic N fertilizer applied to UAA ( $\text{t yr}^{-1}$ )

331  $N_{\text{Fix}}$  = agricultural  $\text{N}_2$  fixation associated with N fixing crops ( $\text{t yr}^{-1}$ )

332  $N_{\text{Dep}}$  = atmospheric N depositions on UAA ( $\text{t yr}^{-1}$ )

333  $N_{\text{Harv}}$  = N exported from UAA with crop harvest ( $\text{t yr}^{-1}$ )

334  $N_{\text{Vol}}$  =  $\text{NH}_3$  volatilization ( $\text{t yr}^{-1}$ )

335  $N_{\text{Den}}$  = denitrification in UAA ( $\text{t yr}^{-1}$ )

336

337 UAA was summarised as arable land (cereals, industrial crops, and fresh vegetables), grassland  
338 (temporary and permanent pasture for fodder production), and permanent woody crops. N budgets  
339 were first calculated at the municipal scale, i.e. the smallest administrative unit at which official  
340 agricultural statistics are available. Municipality-level N budgets were then weighted for the  
341 percentage of each municipality surface included within the 50 m a.s.l. boundaries, and finally  
342 summed up to obtain the total budget of the study area. Input, output, and surplus of each municipality  
343 as well as the large scale N balance were expressed in unit of mass per time ( $\text{t yr}^{-1}$ ), and on a per-area  
344 basis, after normalization for the corresponding UAA ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ).

345 N surplus represents the excess N unused by crops that remains in the soil, i.e. an indicator of the N  
346 use efficiency in the agricultural system. Being net of losses to the atmosphere, it is also a proxy of  
347 the potential source of diffuse pollution for surface and ground waters, via runoff and leaching, to be  
348 compared to the N retention capacity ascribed to the ditch network under different scenarios of  
349 vegetation restoration. A detailed description of data sources, budget equations and uncertainty  
350 assessment of N budget is reported in Appendix B.

351

## 352 *2.5. Statistical analyses*

353 Differences between vegetated and unvegetated sites in N-NO<sub>3</sub><sup>-</sup> removal rates, chemico-physical  
354 (water T, N-NO<sub>3</sub><sup>-</sup>, DO) and hydraulic (discharge) features obtained from experimental activities were  
355 assessed by the non-parametric Mann-Whitney test, due to lack of variance homogeneity for most of  
356 the datasets. A multiple regression model of reach-scale N-NO<sub>3</sub><sup>-</sup> removal rates vs. water T, N-NO<sub>3</sub><sup>-</sup>  
357 and DO and discharge was established, separately for vegetated and unvegetated sites. Critical level  
358 was p=0.05 and statistical analyses were conducted with SigmaPlot 11.0 (Systat Software, Inc., CA,  
359 USA).

360

## 361 **3. Results and discussion**

### 362 *3.1. Water quality of the ditch network and reach-scale N-NO<sub>3</sub><sup>-</sup> removal rates in vegetated and* 363 *unvegetated sediments*

364 Table 1 reports the main chemico-physical and hydraulic features of vegetated and unvegetated  
365 ditches of the Po River lowlands for which experimentally measured values of reach-scale N-NO<sub>3</sub><sup>-</sup>  
366 removal rate are available from previous studies (Pierobon et al., 2013, Castaldelli et al., 2015). Ditch  
367 inflow water was highly variable in term of N-NO<sub>3</sub><sup>-</sup> and DO concentrations, but quite homogeneous  
368 in temperature, reflecting the typical quality of the Po River lowland waterways during the irrigation  
369 period. The reported parameters were not significantly different between vegetated and unvegetated  
370 sites (p>0.05). In the regression analyses, only N-NO<sub>3</sub><sup>-</sup> was found to significantly explain N-NO<sub>3</sub><sup>-</sup>

371 removal rate variability, indicating that its availability was a key factor controlling N retention  
372 processes in the ditch network when water temperature generally exceeds 20°C. This evidence  
373 highlights the absence of saturation in N removal along the measured N-NO<sub>3</sub><sup>-</sup> concentration range  
374 and supports the up-scale simulations conducted for different conditions of water N-NO<sub>3</sub><sup>-</sup> availability.  
375 Reach-scale N-NO<sub>3</sub><sup>-</sup> removal rates were predicted by incoming N-NO<sub>3</sub><sup>-</sup> concentrations for both  
376 vegetated (linear regression,  $y=(1.939\pm 0.221)x-(0.371\pm 0.601)$ ;  $R^2=0.647$ , slope:  $p<0.0001$ ,  
377 intercept:  $p>0.05$ ,  $N=44$ ) (Fig. 2A) and unvegetated ( $y=(0.364\pm 0.053)x+(0.289\pm 0.095)$ ;  $R^2=0.441$ ,  
378 slope:  $p<0.0001$ , intercept:  $p>0.05$ ,  $N=61$ ) ditch sediments (Fig. 2B). Daily N-NO<sub>3</sub><sup>-</sup> removal was  
379 significantly higher in vegetated ditches than in unvegetated ones ( $p<0.001$ ), with rates ranging from  
380 0.01 to 20.52 kg km<sup>-1</sup> d<sup>-1</sup> (median value 1.94 kg km<sup>-1</sup> d<sup>-1</sup>), and from 0.02 to 3.10 kg km<sup>-1</sup> d<sup>-1</sup> (median  
381 value 0.50 kg N km<sup>-1</sup> d<sup>-1</sup>), respectively.

382 Summing up experimental activities performed in the studied area, the capacity of agricultural canals  
383 and ditches to control N pollution is maximised if the following features are simultaneously met: low  
384 water depth (<30-40 cm), low water flow (3-6 cm s<sup>-1</sup>), concentrations of dissolved inorganic N higher  
385 than 0.5 mg L<sup>-1</sup>, and availability of dissolved organic matter (e.g. BOD<sub>5</sub> > 5 mg L<sup>-1</sup>). In these  
386 conditions, if emergent vegetation is present, a reduction of up to 40% of the incoming N load  
387 throughout the irrigation period can be reached in a 1 km-long stretch. Otherwise, in the same chemo-  
388 physical conditions but in absence of vegetation, N loads behave almost conservatively. Reach-scale  
389 N-NO<sub>3</sub><sup>-</sup> consumption and N-N<sub>2</sub> production rates in vegetated ditch sediments, measured by applying  
390 simultaneously in-out N-NO<sub>3</sub><sup>-</sup> budget and N<sub>2</sub> open channel method were positive correlated  
391 ( $R^2=0.9224$ , slope:  $p<0.0001$ , intercept:  $p>0.05$ ,  $N=39$ ), proving that denitrification of water column  
392 N-NO<sub>3</sub><sup>-</sup> was the main reaction responsible for its dissipation, a key process in the context of  
393 eutrophication-related issues (Fig. 3).

394 Official monitoring surveys of the whole ditch network showed water features typical of eutrophic  
395 freshwater environments of temperate zones (Fig. 4), with concentrations of N-NO<sub>3</sub><sup>-</sup> and BOD<sub>5</sub>  
396 constantly higher than 1.5 mg L<sup>-1</sup> and 3.0 mg L<sup>-1</sup>, respectively, along the year, and temperature >20°C

397 maintained during the whole late spring-summer period when the concomitant elevated sediment  
398 oxygen consumption may create favourable conditions for denitrification to occur. Water temperature  
399 displayed a clear seasonal cycle, with the highest values recorded in late summer (August, media  
400 values 25.9°C) and minimum values in the middle of the winter (January, median value, 5.9°C),  
401 resulting in a marked seasonal variation of over 20°C (Fig. 4A). N-NO<sub>3</sub><sup>-</sup> revealed an approximately  
402 inverse seasonal pattern to temperature, with median values constantly lower than 3 mg L<sup>-1</sup> from  
403 April to October (Fig. 4B). From winter to summer N-NO<sub>3</sub><sup>-</sup> median concentrations more than halved,  
404 passing from a maximum in January of 5.5 mg L<sup>-1</sup> to a minimum in August of 1.51 mg L<sup>-1</sup>. A slight  
405 decrease from winter to summer was detected also for DO concentrations although they never  
406 dropped below 6.6 mg L<sup>-1</sup> (Fig. 4C). Median BOD<sub>5</sub> concentrations varied between 3.0 and 4.8 mg L<sup>-1</sup>  
407 without a clear seasonal pattern (Fig. 4D).

408 Using monitoring data of N-NO<sub>3</sub><sup>-</sup> and DO and SOD obtained as a function of temperature,  
409 denitrification rates of water column N-NO<sub>3</sub><sup>-</sup> were calculated according to the Christensen model  
410 (Christensen et al., 1990; Fig. 5). Ditch network expressed the highest N-N<sub>2</sub> production rates in the  
411 middle summer (July, median value 0.53 kg km<sup>-1</sup> d<sup>-1</sup>). The lowest rates were observed in winter  
412 months (0.25-0.34 kg km<sup>-1</sup> d<sup>-1</sup>, from December to February), despite the highest N-NO<sub>3</sub><sup>-</sup> availability  
413 at this time of the year because the water temperature was low. However, denitrification did not show  
414 a pronounced seasonal pattern, with the lowest median winter rates being about half of the highest  
415 summer rates. With the exclusion of winter months, N-N<sub>2</sub> production rates were constantly higher  
416 than 0.40 kg km<sup>-1</sup> d<sup>-1</sup> in the rest of the year and a clear increase in the spring/summer shift followed  
417 by a decrease at the onset of autumn was not observed. This is probably due to the lower N-NO<sub>3</sub><sup>-</sup>  
418 availability when bacterial activity might be promoted by water temperature (Fig. 4A, B). For the  
419 irrigation period (May–September), N-N<sub>2</sub> production rates calculated according to the Christensen  
420 model (monthly median values varying from 0.47 to 0.53 kg km<sup>-1</sup> d<sup>-1</sup>) overlapped the range of N-  
421 NO<sub>3</sub><sup>-</sup> removal rates experimentally measured by reach-scale N-NO<sub>3</sub><sup>-</sup>-budgets in selected unvegetated  
422 ditches (range: 0.02–3.10 kg km<sup>-1</sup> d<sup>-1</sup>; median value: 0.50 kg km<sup>-1</sup> d<sup>-1</sup>), while resulted significantly



423 lower than the experimental values obtained for vegetated ditches (range: 0.01–20.52 kg km<sup>-1</sup> d<sup>-1</sup>;  
424 median value: 1.94 kg km<sup>-1</sup> d<sup>-1</sup>) (Fig 2, Fig. 5).

425 Shallow aquatic ecosystems colonised by rooted macrophytes are usually considered hotspots of  
426 denitrification due to the development of multiple biological active surfaces both in the water column  
427 and in the rhizosphere (Pierobon et al., 2013; Taylor et al., 2015). Submerged portions like stems and  
428 leaves provide physical support for the growth of epiphytic biofilms, complex matrices of bacteria,  
429 microalgae and debris, that promote reactions of nutrient retention and transformations whose kinetics  
430 are usually maximised due to the constant renewal of the water in contact with them (Toet et al.,  
431 2003; Soana et al., 2018). Due to the ability to transport and release oxygen in the sediment, rooted  
432 plants allow the establishment of a mosaic of oxic and anoxic microniches. This extensive  
433 development of oxic-anoxic interfaces promotes the aerobic degradation of the organic matter and  
434 the coupling of aerobic and anaerobic processes, e.g. denitrification coupled to nitrification, that  
435 remove N from the system (Vila-Costa et al., 2016; Roley et al., 2018). The influence of vegetation  
436 on denitrification occurs also by increasing the availability of labile organic compounds which act as  
437 substrates for heterotrophic denitrifying communities, such as radical exudates, decaying plant litter  
438 and suspended particles trapped as a consequence of low hydrodynamics among the vegetation stands  
439 (Hang et al., 2016; Srivastava et al., 2017).

440

441 *3.2. Predicted N-NO<sub>3</sub><sup>-</sup> removal in the ditch network: scenarios of vegetation maintenance and*  
442 *comparison to agricultural N surplus*

443 The removal of macrophytes from the ditch network causes a simplification of the agricultural  
444 landscapes in terms of denitrification hotspots, due to the loss of multiple interfaces among water,  
445 sediment and vegetation itself, a basic requirement for the microbial processes underlying the  
446 depuration capacity (Boerema et al., 2014; Vymazal and Březinová, 2018). Considering the typical  
447 range of N-NO<sub>3</sub><sup>-</sup> availability (0.5–8 mg L<sup>-1</sup>) in the Po River lowlands waterways, the current N-NO<sub>3</sub><sup>-</sup>  
448 removal performed by the ditch network, where vegetation cover is limited to 5% of its extension,

449 was estimated to vary between 3,300 and 4,900 t yr<sup>-1</sup> (Fig. 6A). This buffer capacity represents an  
450 irrelevant fraction of the dissolved inorganic N load that the Po River exports to the Adriatic Sea, on  
451 average ~110,000 t yr<sup>-1</sup> (Viaroli et al., 2018). The predicted N-NO<sub>3</sub><sup>-</sup> mitigation potential of the ditch  
452 network would increase up to 4,000–33,600 t yr<sup>-1</sup> in case of restoring vegetation on 90% of its  
453 extension, albeit maintaining the current management practice, i.e. the mowing operations performed  
454 in the middle of the summer (Fig. 6A). Differently, a more conservative management of vegetation  
455 with the cutting postponed to the end of the growing season, enhances the N-NO<sub>3</sub><sup>-</sup> mitigation potential  
456 of the ditch network in terms of 1-26% and 16-168%, for the 5 and 90% scenarios, respectively (Fig.  
457 6B). Supposing the highest N-NO<sub>3</sub><sup>-</sup> availability commonly found in the Po River lowlands waterways  
458 (up to 8 mg L<sup>-1</sup>), the ditch network would at present express a potential N-NO<sub>3</sub><sup>-</sup> removal of 6,200 t  
459 yr<sup>-1</sup>, if only its 5% extension where vegetation is present was managed conservatively. This key  
460 ecosystem function would increase almost tenfold (up to 56,600 t yr<sup>-1</sup>) in case of restoring vegetation  
461 on 90% of the total length like in the past. This amount almost equals the N load needing treatment  
462 (supposing a depuration target of 75% of the incoming N) produced annually by the whole Po River  
463 basin population of over 16 million inhabitants.

464 The N budget revealed a N surplus in the agricultural soils of the investigated area (~46,000 t yr<sup>-1</sup>),  
465 due to N inputs (~163,000 t yr<sup>-1</sup>) exceeding N outputs (~117,000 t yr<sup>-1</sup>). Total inputs were almost  
466 equally divided among synthetic fertilizers (34%), livestock manure (30%), and biological fixation  
467 (33%), with atmospheric depositions accounting for the remaining 3% (Table 2). Total N input in the  
468 municipalities of the studied area ranged from 112 to 817 kg ha<sup>-1</sup> yr<sup>-1</sup> and overall the average input  
469 rate was 260 kg ha<sup>-1</sup> yr<sup>-1</sup>. Livestock manure was produced mainly by cattle (60%) and swine (32%)  
470 farming, sustained by large portions of agricultural lands devoted to fodder crops (mainly N-fixing  
471 alfalfa, 25% of total agricultural surfaces), maize (23%), and other cereals (26%). The main N output  
472 term was crop harvest, mainly as feed for livestock which accounted for over 81% of the total N  
473 removal from agricultural lands. The remaining portion was estimated to be lost as N gases to the  
474 atmosphere by ammonia volatilization (10%) and denitrification (9%). Total N output in the

475 municipalities of the studied area ranged from 123 to 362 kg ha<sup>-1</sup> yr<sup>-1</sup> and overall the average output  
476 rate was 186 kg ha<sup>-1</sup> yr<sup>-1</sup>.

477 More than one quarter (~28%) of total N input remained in the agricultural soils as N surplus,  
478 representing a risk of N runoff and potential pollution of aquatic ecosystems. However, N input and  
479 output patterns varied spatially within the area, together with the resulting N surplus (on average 74  
480 kg ha<sup>-1</sup> yr<sup>-1</sup>), reflecting the heterogeneous distribution of agricultural and farming activities across the  
481 territory, in specific three different agro-environments (Fig. C1, Appendix C). The first is the zone  
482 on the Po River hydrographic left side (Fig. 1B) where livestock manure was the biggest N source in  
483 most of the municipalities. This area was characterized by a dramatically high density of cows and  
484 pigs (equivalent livestock units: up to 15-20 per ha of agricultural surfaces) generating N inputs up  
485 to 500-600 kg ha<sup>-1</sup> yr<sup>-1</sup> that, despite the widespread occurrence of high N-demanding crops (i.e.  
486 maize), were generally in excess compared to uptake and accumulation in crop biomass. The second  
487 is the Po River hydrographic right side zone (Fig. 1B), with the exclusion of the deltaic sub-basin of  
488 the Po di Volano, where the alfalfa dominated among crops resulting in N input from biological  
489 fixation. The last is the Po di Volano sub-basin (Fig. A1, Appendix A) characterized by low livestock  
490 densities and cereals as major crops amended almost exclusively by synthetic fertilizers.

491 In the current management situation of vegetation on 5% of ditch length which is cut in the middle  
492 of the growing season, N abatement performed by ditch network accounted for only 7-11% of the N  
493 surplus (Fig. 6A). This percentage would increase by only a small amount (7-13%) if vegetation was  
494 managed conservatively. Differently, for the 90% vegetation scenario with the current management,  
495 the N-NO<sub>3</sub><sup>-</sup> mitigation potential of the ditch network represented from 9% to 73% of the N excess in  
496 agricultural lands (Fig. 6A), due to the presence of vegetation in ditch stretches characterised by  
497 different degrees of N-NO<sub>3</sub><sup>-</sup> contamination. If vegetation would be maintained with a conservative  
498 management on 90% of ditch length having N-NO<sub>3</sub><sup>-</sup> concentrations > 6 mg L<sup>-1</sup>, N abatement would  
499 potentially overcome the N surplus by about 20% (Fig. 6B) and reduce the dissolved inorganic N  
500 load exported annually by the Po River to the Northern Adriatic Sea.

501

#### 502 **4. Management and research perspectives**

503 The outcomes of the up-scale model presented here highlight that routine ditch network management  
504 practices involving aquatic vegetation removal deeply affect its capacity to process  $\text{N-NO}_3^-$ , with  
505 cascading implications for N dynamics at the watershed scale. One of the most valuable ecosystem  
506 service provided by macrophytes, i.e. the mitigation of  $\text{N-NO}_3^-$  pollution, may play a considerable  
507 role in water quality improvement in agricultural landscapes. Thus proper management of the  
508 interfaces between croplands and surface water bodies may potentially reduce an appreciable amount  
509 of the N surplus generated by farming activities and ultimately delivered to the coastal zones. Even  
510 if tightly intertwined by water management practices, agricultural surfaces and river networks are  
511 commonly managed and investigated separately, with consequent limitations in solving nutrient  
512 excess issues across agricultural landscapes. An increasing amount of research is promoting the use  
513 of vegetated ecological ditches (eco-ditches) as an agricultural best management practice. Eco-ditches  
514 have been proven to be effective in reducing diffuse and point source pollution in several human-  
515 impacted catchments in China and the United States. Eco-ditches are engineered systems that are  
516 constructed *ex novo* or transformed from conventional agricultural drainage ditches. They are  
517 designed to mimic the depuration processes occurring in surface-flow wetlands and are considered  
518 an attractive alternative to traditional technologies for wastewater treatments (Chen et al., 2015;  
519 Xiong et al., 2015; Faust et al., 2018; Kalcic et al., 2018). In Italy, vegetated ditches have never been  
520 implemented as an agricultural best management practice (Provolo et al., 2016), as the study of  
521 pollutant mitigation in this type of aquatic ecosystem is just beginning. A few studies have  
522 investigated how processes and functions ascribed to the ditch networks may affect hydrochemical  
523 quality and ecosystem quality (e.g. biodiversity, resilience) on a broader scale (Bolpagni et al., 2013;  
524 Castaldelli et al., 2015; Otto et al., 2016; Soana et al., 2018). In recent years, some regions have tried  
525 to fill this knowledge gap by developing guidelines for ecological restoration of agricultural ditches.  
526 However, the qualitative nature of the provided indications and in particular the lack of

527 parameterization regarding the self-depurative processes have led to a poor level of implementation  
528 of the suggested interventions, limited to a few canal stretches in the form of pilot actions (Bischetti  
529 et al., 2008; Emilia-Romagna Region, 2012).

530 In agricultural impacted watersheds, restoration or construction of wetlands is often impracticable  
531 due to limitations in available area and funds (Verhoeven, 2014; Hansen et al., 2018). However,  
532 ditches and canals are already present on croplands and their management could be aimed at  
533 harmonizing hydraulic functionality as well as ecological issues. Actions targeting N-NO<sub>3</sub><sup>-</sup> mitigation  
534 and sustainable agricultural practices might for example consider how to manipulate those  
535 widespread landscape elements through a design aimed at promoting biogeochemical processing, in  
536 particular creating conditions that maximize denitrification (e.g. organic matter availability, high  
537 water retention times) (Soana et al., 2017; Vymazal and Březinová, 2018; Schilling et al., 2018).

538 Uncertainty exists in the up-scale presented here, likely due to the high spatial and temporal  
539 heterogeneity of biogeochemical processes, which in turn are affected by several hydrological,  
540 geomorphological, and biological drivers. Nevertheless, scenario investigations clearly showed that  
541 vegetation restoration in ditch network of the Po River lowlands could be an effective tool to decrease  
542 N-NO<sub>3</sub><sup>-</sup> loads in surface waters, but only if implemented at a larger scale with respect to the present  
543 situation. Restoring vegetation on 90% of the ditch length, as prior to the introduction of mechanical  
544 mowing, appears unrealistic. However, the denitrification potential could be increased by identifying  
545 ditch stretches with appropriate chemico-physical features (e.g. Castaldelli et al., 2018; Soana et al.,  
546 2018) and low hydraulic risk. Here, interventions like reshaping and reduction of the slope banks  
547 could be carried out to restore suitable sites for macrophyte maintenance throughout the growing  
548 season. For example, our simulations demonstrated that a removal of up to 35-40% of the N surplus  
549 generated in the surrounding agricultural lands could be expected if vegetation is maintained in one  
550 quarter of the total ditch length. In order to preserve water transport capacity, canal sections would  
551 also be enlarged accordingly to the increase in hydraulic impedance due to the presence of vegetation.  
552 These actions would ultimately create a mosaic of heterogeneous habitats, and increase the water

553 retention time and the surface area for microbial biofilms, which would maximize the depuration  
554 potential. Moreover, frequency and extension of the vegetation cutting could be planned to avoid  
555 complete and simultaneous removal of vegetation in all ditches, using maintenance practices that  
556 could ensure denitrification functionalities are maintained in time and space. Lastly, the effectiveness  
557 of restoration actions would be maximized if they are placed where the vegetated ditches intercept N  
558 surplus derived from manure or synthetic fertilizers since these are more prone to be leached towards  
559 surface waters than the organic N pool from N-fixing crops.

560 Further research is necessary to gain insights into the mechanisms underlying the depurative capacity  
561 of canals and ditches. The mitigation potential of vegetated ditch towards N has been less extensively  
562 studied compared to vegetated wetlands and the relative contribution of denitrification has been  
563 scarcely investigated, especially when assessed by the direct measurement of N<sub>2</sub> production (Taylor  
564 et al., 2015; Castaldelli et al., 2015; Speir et al., 2017). A more quantitative understanding of the  
565 processes accounting for N retention in vegetated ditches is crucial to develop management strategies  
566 to reduce eutrophication. Studies where the relative contributions of permanent N dissipation  
567 processes (nitrification coupled denitrification) and temporary storage mechanisms (plant uptake) are  
568 simultaneously assessed are still scarce for vegetated ditched (Castaldelli et al., 2015; Soana et al.,  
569 2017; Vymazal and Březinová, 2018). Conventional biogeochemical techniques (e.g. isotope pairing,  
570 acetylene inhibition) based on intact sediment core, microcosm or slurry approaches have been  
571 recently adopted to measure denitrification in ditches and have contributed to the understanding of  
572 specific controls of the process (Kröger et al., 2014; Soana et al., 2017; Veraart et al., 2017). However,  
573 severe uncertainty arises in scaling up data from the laboratory to the watershed, in particular  
574 measurements of potential denitrification activity where the structural integrity of sediment with  
575 associated biogeochemical gradients is altered and the direct influence of root activity is eliminated.  
576 Robust datasets should be collected, not only spanning a variety of environmental conditions (e.g.  
577 hydraulic parameters, solute concentrations, plant type), but also at spatial and temporal scales  
578 relevant to N pollution issues and appropriate to be extrapolated up to the watershed level. The

579 parameterization of the N-NO<sub>3</sub><sup>-</sup> mitigation capacity should be obtained by the application of whole-  
580 reach approach (e.g. N<sub>2</sub> open-channel) that capture small-scale spatial and temporal heterogeneity of  
581 environmental conditions and metabolic processes occurring in shallow aquatic ecosystems, such as  
582 vegetated ditches, where multiple riverine habitats and interfaces exist (i.e. biofilms, oxic-anoxic  
583 microniches in the rhizosphere) (Castaldelli et al., 2015; Taylor et al., 2015; Soana et al., 2017). The  
584 actual efficiency of small artificial waterways to buffer N pollution can be really appreciated if they  
585 are considered as a whole, i.e. sediment, water, vegetation, biofilms and their multiple interactions.  
586 Reach-scale methods integrate water column and benthic compartment dynamics and overcomes the  
587 limitations inherent in the upscaling of results from the laboratory to the field (e.g. measurements  
588 performed over small surfaces, incubation artifacts, etc.) (Reisinger et al., 2016; O'Brien et al., 2017).  
589 The scientific outcomes will be instrumental to produce predictive tools and management guidelines  
590 aimed at maximizing the natural depuration capacity of the ditch network in agricultural landscapes.

591

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810

811 **Tables**

812

813 **Table 1.** Main chemico-physical and hydraulic features of vegetated and unvegetated ditches of the  
814 Po River lowlands for which experimentally measured values of reach-scale  $\text{N-NO}_3^-$  removal rate are  
815 available. These datasets were acquired in previous experimental campaigns (sampling events along  
816 the irrigation period:  $N=44$  for vegetated sites and  $N=61$  for unvegetated sites) reported by Pierobon  
817 et al. (2013) and Castaldelli et al. (2015), and were used to build the  $\text{N-NO}_3^-$  concentrations– $\text{N-NO}_3^-$   
818 removal relationships shown in Fig. 2.

819

	<b>Vegetated ditches</b>		<b>Unvegetated ditches</b>	
	Median value	Range	Median value	Range
Water T (°C)	23	17 – 31	23	16 – 29
$\text{N-NO}_3^-$ ( $\text{mg L}^{-1}$ )	1.17	0.01 – 7.94	1.20	0.71 – 7.05
DO ( $\text{mg L}^{-1}$ )	7.23	3.34 – 13.86	7.68	6.23 – 8.24
Discharge ( $\text{L s}^{-1}$ )	31	5 – 158	39	3 – 171

820

821 **Table 2.** N budget in the lowland of the Po River below 50 m a.s.l. Data are expressed as tons of N  
 822 produced or consumed per year ( $\text{t yr}^{-1}$ ) in the whole area and normalized for the utilized agricultural  
 823 area ( $\text{kg ha}^{-1} \text{yr}^{-1}$ ). The percentage of each input or output with respect to the total is reported in  
 824 brackets.

INPUT	N budget			
	Average ( $\text{t yr}^{-1}$ )	Min – Max ( $\text{t yr}^{-1}$ )	Average ( $\text{kg ha}^{-1} \text{yr}^{-1}$ )	Min – Max ( $\text{kg ha}^{-1} \text{yr}^{-1}$ )
Livestock manure	48,838 (30)	39,554 – 58,123	77.8	63.0 – 92.6
Synthetic fertilizers	55,245 (34)	54,140 – 56,350	88.1	86.3 – 89.8
Biological fixation by N-fixing crops	45,815 (28)	29,486 – 65,395	73.0	47.0 – 104.2
Biological fixation by natural surfaces*	7,971 (5)	4,042 – 12,297	12.7	6.4 – 19.6
Atmospheric deposition	5,019 (3)	4,266 – 5,772	8.0	6.8 – 9.2
<b><math>\Sigma</math> input</b>	<b>162,889</b>	131,488 – 197,937	<b>259.6</b>	209.6 – 315.5
<b>OUTPUT</b>				
Harvest by feed crops	70,231 (60)	44,832 – 100,859	111.9	71.5 – 160.8
Harvest by food crops	24,305 (21)	15,649 – 34,679	38.7	24.9 – 55.3
$\text{NH}_3$ volatilization	11,763 (10)	1,430 – 24,986	18.7	2.3 – 39.8
Denitrification in agricultural soils	10,408 (9)	4,685 – 17,171	16.6	7.5 – 27.4
<b><math>\Sigma</math> output</b>	<b>116,707</b>	66,596 – 177,695	<b>186.0</b>	106.1 – 283.2
<b><math>\Sigma</math> input – <math>\Sigma</math> output = Surplus</b>	<b>46,182</b>	20,242 – 64,893	<b>73.6</b>	32.3 – 103.4

825\*The term includes N fixed by permanent grassland and pastures and by non-symbiotic process in arable land and permanent crops.

826 **Figure captions**

827 **Fig. 1.** Study area: A) location of the Po River basin in Europe; B) location of the Po plain below 50  
828 m a.s.l. within the Po River basin; C) land use (Corine Land Cover inventory 2012) and D)  
829 hydrographic network of the studied area (Bing Aerial Maps Baselayer for QGIS,  
830 [www.bing.com/maps](http://www.bing.com/maps)).

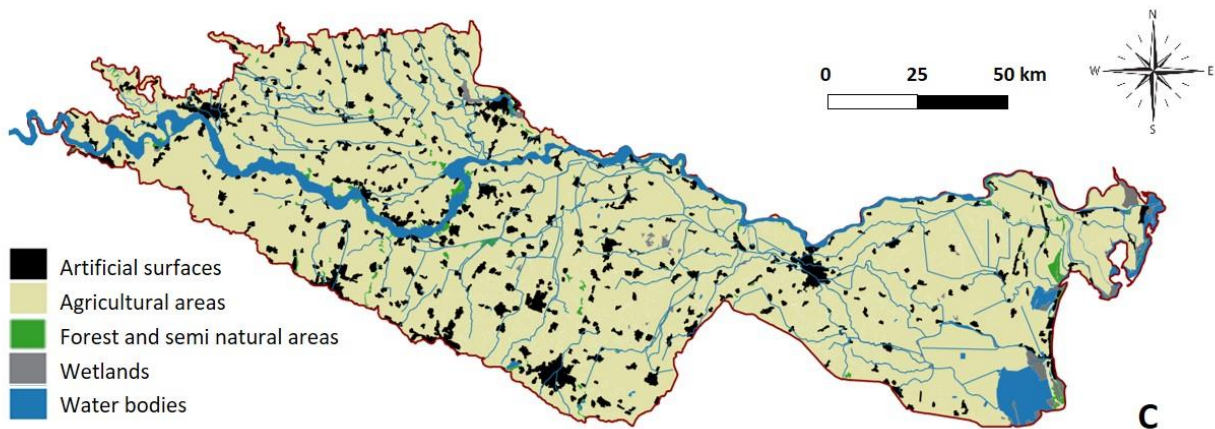
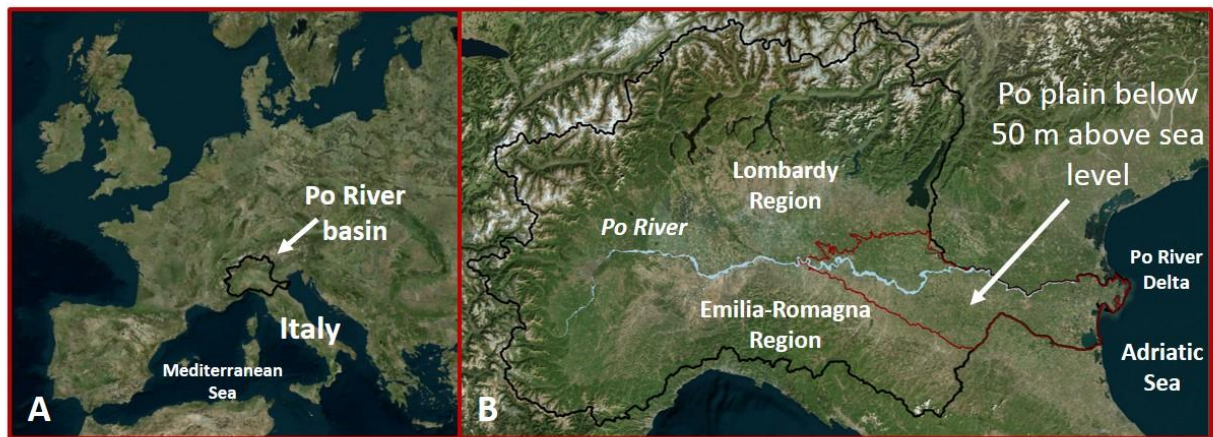
831 **Fig. 2.** Relationship between incoming N-NO<sub>3</sub><sup>-</sup> concentrations (mg L<sup>-1</sup>) and reach-scale N-NO<sub>3</sub><sup>-</sup>  
832 removal rates (kg km<sup>-1</sup> d<sup>-1</sup>) measured along the irrigation period in vegetated (panel A) and  
833 unvegetated ditches (panel B) of the Po River lowlands. The regression line (solid line) is presented  
834 along with the 95% confidence interval (dotted line). Data from Pierobon et al. (2013) and Castaldelli  
835 et al. (2015).

836 **Fig. 3.** Log-log relationship between reach-scale N-NO<sub>3</sub><sup>-</sup> removal and N-N<sub>2</sub> production rates (g km<sup>-1</sup>  
837 d<sup>-1</sup>) in vegetated ditch sediments obtained by the simultaneous application of in-out NO<sub>3</sub><sup>-</sup> budget and  
838 N<sub>2</sub> open channel method. The regression line (solid line) is presented along with the 95% confidence  
839 interval (dotted line). Data from Castaldelli et al. (2015), Castaldelli et al. (2018) and Soana et al.  
840 (2018).

841 **Fig. 4.** Seasonal variations of water T (°C, panel A), N-NO<sub>3</sub><sup>-</sup> (mg L<sup>-1</sup>, panel B), DO (mg L<sup>-1</sup>, panel  
842 C), and BOD<sub>5</sub> (mg L<sup>-1</sup>, panel D) in the ditch network of the Po River lowlands. Box and Whisker  
843 plots include monthly data (period 2009-2014) for 70 stations located within the 50 m a.s.l. area and  
844 belonging to official surface water monitoring network of the Regional Agency for the Environmental  
845 Protection of Lombardy and Emilia-Romagna Regions. Central horizontal line in the box is the  
846 median, top and bottom boxes are 25<sup>th</sup> and 75<sup>th</sup> percentiles, and whiskers are 10<sup>th</sup> and 90<sup>th</sup> percentiles.  
847 Outliers are showed as open circles.

848 **Fig. 5.** Seasonal variations of N-N<sub>2</sub> production rates (kg km<sup>-1</sup> d<sup>-1</sup>) predicted by the model proposed  
849 by Christensen et al. (1990) for the ditch network of the Po River lowlands. Box and Whisker plots  
850 include the predicted rates for 70 stations located within the 50 m a.s.l. area and belonging to official  
851 surface water monitoring network of the Regional Agency for the Environmental Protection of  
852 Lombardy and Emilia-Romagna Regions. Christensen model was applied for all the official monthly  
853 surveys (period 2009-2014) for which values of water T, DO and N-NO<sub>3</sub><sup>-</sup> were concomitantly  
854 available. Central horizontal line in the box is the median, top and bottom boxes are 25<sup>th</sup> and 75<sup>th</sup>  
855 percentiles, and whiskers are 10<sup>th</sup> and 90<sup>th</sup> percentiles. Outliers are showed as open circles.

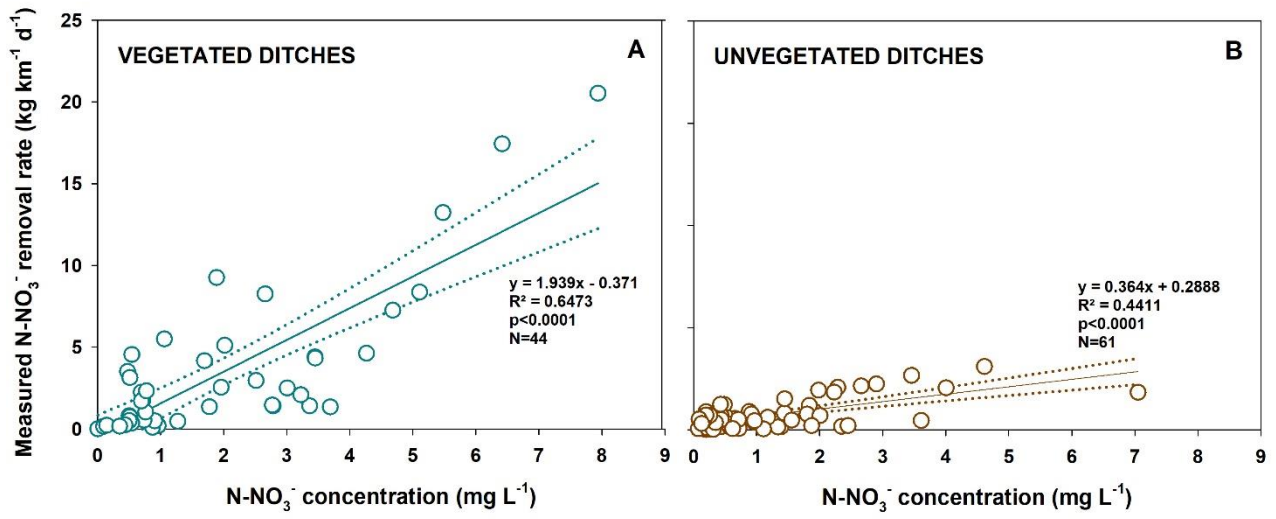
856 **Fig. 6.** Predicted N-NO<sub>3</sub><sup>-</sup> removal (t yr<sup>-1</sup>) with vegetation restoration on variable extension of the ditch  
857 network (5%, 25%, 50% and 90% of the total ditch network length). Different water N-NO<sub>3</sub><sup>-</sup>  
858 availability together with two options of vegetation management were simulated, i.e. the *current*  
859 *management* (mowing performed in the middle of the summer, panel A) and the *conservative*  
860 *management* (mowing performed at the end of the growing season, panel B). The corresponding  
861 percentage of N surplus in the agricultural land is also reported.



862

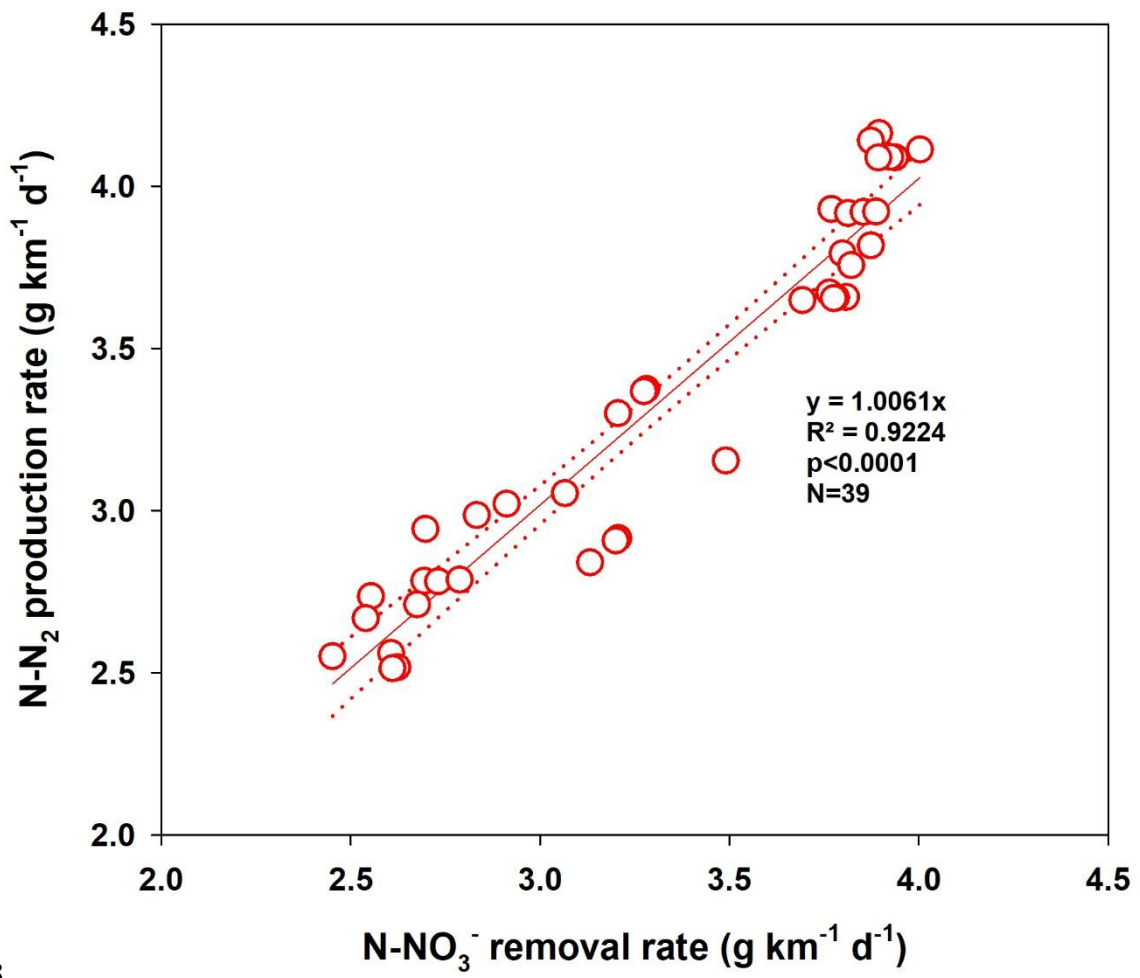
863 Fig. 1



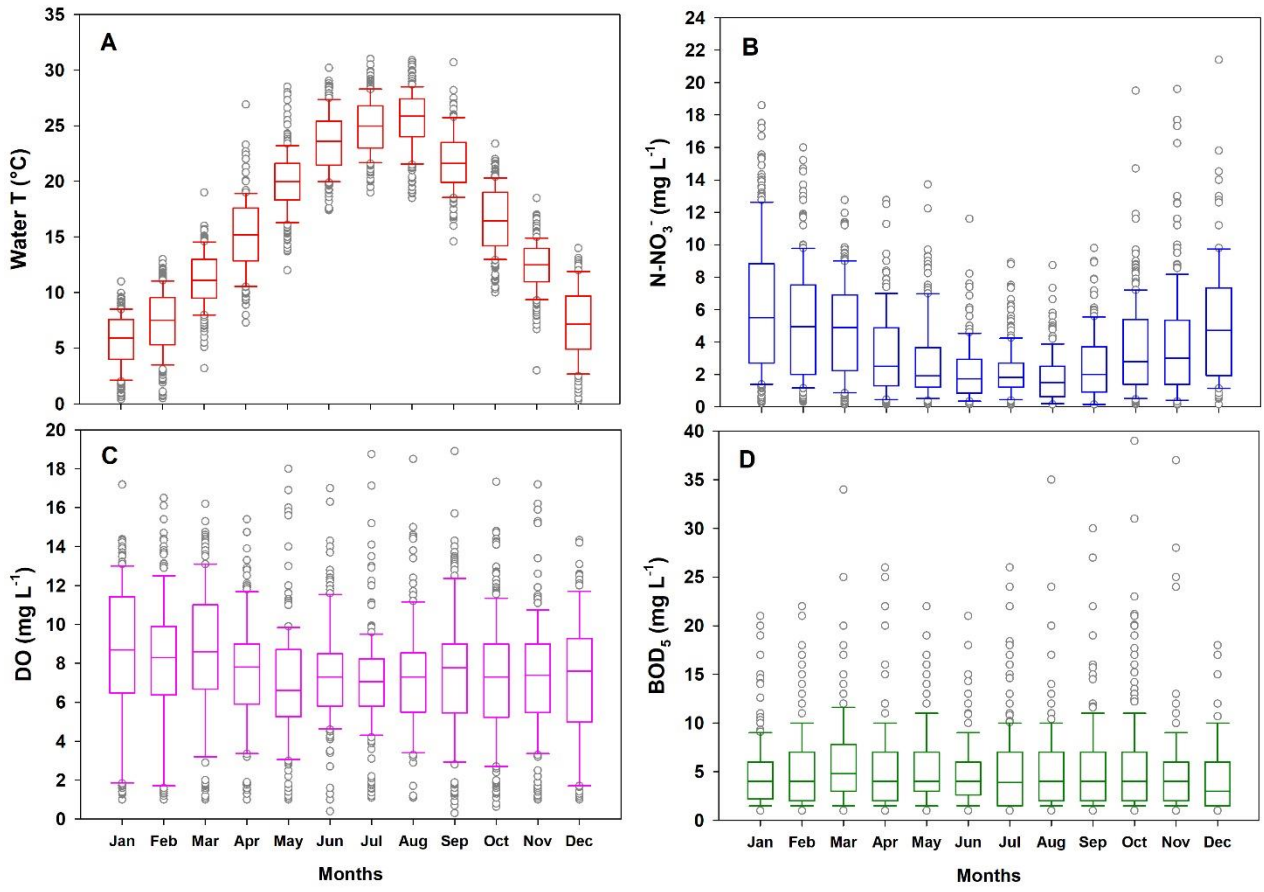


864

865 Fig. 2

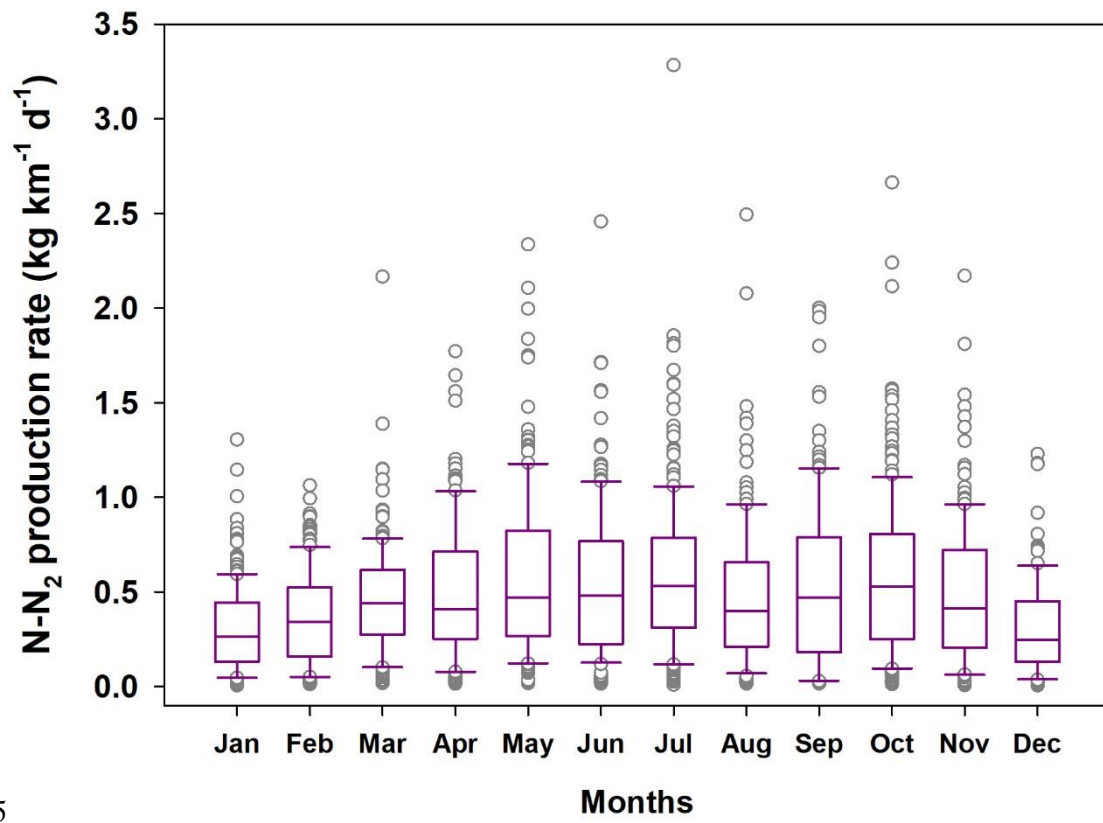


866 Fig. 3

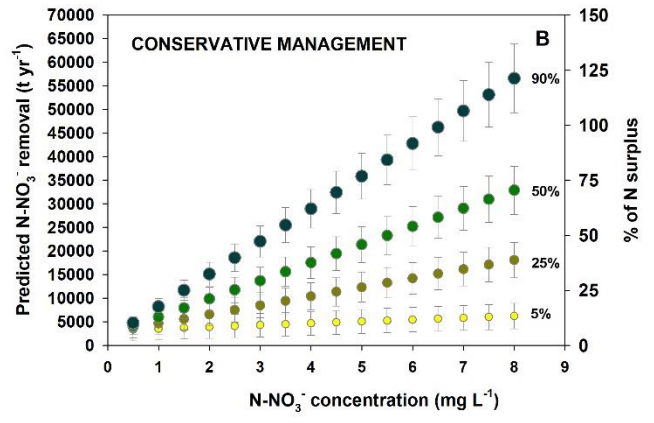
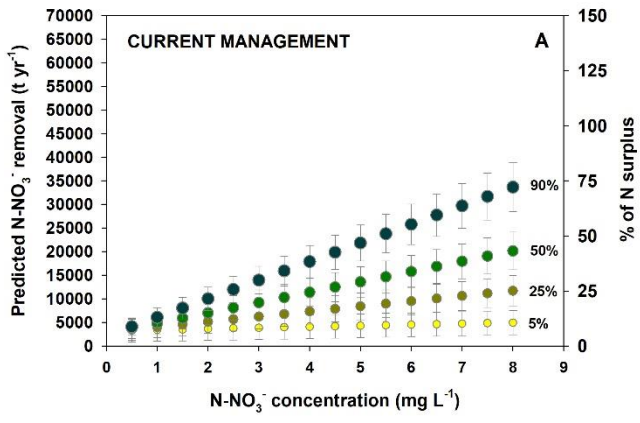


867

868 Fig. 4



869 Fig. 5



870  
871 Fig. 6