Journal Pre-proofs

Could a freshwater fish be at the root of dystrophic crises in a coastal lagoon?

Marco Milardi, Elisa Soana, Duane Chapman, Elisa Anna Fano, Giuseppe Castaldelli

PII: S0048-9697(19)35085-5
DOI: https://doi.org/10.1016/j.scitotenv.2019.135093
Reference: STOTEN 135093

To appear in: *Science of the Total Environment*

Received Date: 11 September 2019
Revised Date: 18 October 2019
Accepted Date: 19 October 2019

Please cite this article as: M. Milardi, E. Soana, D. Chapman, E. Anna Fano, G. Castaldelli, Could a freshwater fish be at the root of dystrophic crises in a coastal lagoon?, *Science of the Total Environment* (2019), doi: https://doi.org/10.1016/j.scitotenv.2019.135093

Could a freshwater fish be at the root of dystrophic crises in a coastal lagoon?

Marco Milardi[1,3]*, Elisa Soana[1], Duane Chapman[2], Elisa Anna Fano[1], Giuseppe Castaldelli[1]

1 – Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

2 – U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO, United States of America

3 – Fisheries New Zealand - Tini a Tangaroa, Ministry for Primary Industries - Manatū Ahu Matua, Wellington, New Zealand

* - Corresponding author: marco.milardi@unife.it

Abstract

Eutrophication has a profound impact on ecosystems worldwide. Grass carp *Ctenopharyngodon idella*, an herbivorous fish, has been introduced to control aquatic plant overgrowth caused by eutrophication, but could have other, potentially detrimental, effects.

We used the Po di Volano basin (south of the Po River delta, northern Italy) as a test case to explore whether grass carp effects on canal aquatic vegetation could be at the root of historical changes in N loads exported from the basin to the Goro Lagoon. We modeled the aquatic vegetation production and standing crop, its denitrification potential, and its consumption by introduced grass carp. We then examined whether changes in historical nitrogen loads matched the modeled losses of the drainage network denitrification function or other changes in agricultural practices.

Our results indicate that introduced grass carp could completely remove submerged vegetation in the Po di Volano canal network, which could – in turn – lead to substantial loss of the denitrification function of the system, causing in an increase in downstream nitrogen loads.

A corresponding increase, matching both timing and magnitude, was detected in historical nitrogen loads to the Goro Lagoon, which were significantly different before and after the time of modeled collapse of the denitrification function. This increase was not clearly linked to watershed use or agricultural practices, which implies that the loss of the denitrification function through grass carp overgrazing could be a likely explanation of the increase in downstream nitrogen loads.

Perhaps for the first time, we provide evidence that a freshwater fish introduction could have caused long-lasting changes in nutrient dynamics that are exported downstream to areas where the fish is not present.

Keywords: invasive species; denitrification; grass carp; nitrogen loads; pre-post approach; species introductions; submerged vegetation

Introduction

The human-mediated nutrient enrichment of natural ecosystems, commonly known as eutrophication, has a profound impact on ecosystems worldwide (Garnier et al., 2010, Paerl, 2009). Eutrophication affects freshwater ecosystems more directly, because they are closely connected to agricultural landscapes, but eventually nutrients are carried by rivers to estuarine, coastal and gulf areas, where they can also cause problems (e.g. the Gulf of Mexico, the Baltic Sea, Lake Erie: Rönnberg and Bonsdorff, 2004, Rabalais et al., 2002, Kane et al., 2014). Eutrophication can have a direct effect on algal and aquatic vegetation communities, and cause loss of species and linked ecosystem services (Glibert, 2017). Nutrient loading to freshwaters is strongly influenced by human population within the watershed and by intensity and type of farming practices (Billen et al., 2013). However, eutrophication could arise not only from increases in nutrient loading, but also from changes within the watersheds, when these alter the watersheds' capacity to metabolize nutrient loads (Beaulieu et al., 2015, Pinay et al., 2002).

Among watershed changes, the loss of natural buffers against the accumulation of excessive nitrogen (N) loads could be of paramount importance (Hansen et al., 2018, Hill, 2019). Denitrification, the reduction of nitrate (NO_3^-) to nitrogen gas (mostly N_2) performed by microbial communities under anaerobic conditions, is one of the most important mechanisms of N removal and can take place wherever an anoxic environment, and availability of substrates (NO_3^- and organic carbon) allow it. The presence of aquatic vegetation boosts N processing by providing carbon and creating oxic-anoxic interfaces in the rhizosphere, thus increasing the suitable habitats available to the denitrifying microbiota and resulting in higher denitrification rates compared to bare sediment (2–4 folds higher for sediments covered by submerged vegetation; see e.g. Pinardi et al., 2009, Alldred and Baines, 2016). Aquatic vegetation is thus a key component of the buffer capacity of wetlands (Choudhury et al., 2018, Bastviken et al., 2009) or agricultural ditches (Vymazal and Březinová, 2018, Castaldelli et al., 2015). Therefore, human actions that disrupt aquatic vegetation could reduce denitrification buffers and further contribute to eutrophication. For example, in-stream vegetation can be mechanically removed to increase the flow of water through rivers and canals (Levavasseur et al., 2014, Pierobon et al., 2013). However, in-stream vegetation could also be lost through biological control (i.e. the stocking of an herbivorous species to control vegetation growth, Lodge (1991)).

Grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) is a prime example of such an herbivorous fish; originally from Asia, it has been widely introduced to control excessive or undesirable aquatic vegetation (Kelly et al., 2011, Wittmann et al., 2014). The "ecosystem engineering" capabilities of grass carp can provoke unwanted effects through altering the abundance of submerged vegetation, decreasing available spawning habitats for native species, increasing turbidity and ultimately favoring other introduced species (Milardi et al., 2018a). Furthermore, an increase of several dissolved N species has been observed following vegetation control with grass carp, partly as a result of sediment resuspension and fecal matter deposition (reviewed in e.g. Dibble and Kovalenko, 2009). These effects not only cascade through the food web, but as long as the population survives (through long-life, continued stocking or natural recruitment) and spreads (through natural or aided dispersion) these effects can be magnified and have basin-wide consequences (Rabalais et al., 2002, Rönnberg and Bonsdorff, 2004). These ecosystem shifts might not have been investigated in the past but could be reconstructed through modeling with modern techniques rooted in historical data (see e.g. Milardi et al., 2016, Milardi et al., 2019b), arguably widening and strengthening our understanding of overlooked ecosystem processes.

Grass carp was first introduced in Western Europe in the 1980s (FAO, 2016), as a mean of aquatic weed control. In Italy, the first introduction to the wild was at the end of the 1980s (Melotti et al., 1987), in the Po di Volano basin, in the southern part of the Po River delta, where it has recently been found to reproduce naturally (Milardi et al., 2015). Large-scale grass carp introduction at the end of the 1980s coincided with the onset of well-studied onset of massive algal blooms and anoxia in the Goro Lagoon, downstream of the Po di Volano (Pugnetti et al., 1992, Viaroli et al., 1995, Viaroli et al., 1996). However, the potential contribution of grass carp to those blooms has never been investigated.

We thus evaluated whether grass carp introduction could be at the root of historical changes in N loads exported from the the Po di Volano drainage basin, a network of artificial and semi-artificial canals in the Ferrara province (Northern Italy), to the Goro Lagoon. We focused on this area because grass carp were recently found to recruit naturally there after their introduction (Milardi et al., 2015), and because of the abundant historical information available on agricultural practices, nutrient loads (e.g. Castaldelli et al., 2013b), aquatic vegetation (e.g. Piccoli and Gerdol, 1981), fish introductions (e.g. Lanzoni et al., 2018) and their effects on biodiversity (e.g. Castaldelli et al., 2013a, Milardi et al., 2019a). We used this information to model aquatic vegetation production and standing crop, its denitrification potential, and its consumption by grass carp in the drainage network, with the aim to verify whether historical N loads were affected by grass carp or other changes in agricultural practices. We hypothesized that, if the denitrification function was lost due to grass carp overgrazing, a corresponding increase in N loads (in particular NO_3^-) would be observed during the irrigation period. Our study is intended to shed light on past broad-scale ecological dynamics, and to inform holistic management of these environments in the future.

Materials and Methods

Study area

The Po di Volano basin (~713 km^2) constitutes the terminal part of the Po River floodplain, the largest Italian alluvial plain (Fig. 1a and b). The Po River plain and the Po di Volano basin are heavily cultivated, with >70% of the Po di Volano basin area classified as utilized agricultural land (mainly cereals and industrial crops) and livestock farming is a minor component (Castaldelli et al. (2013b); Fig. 1c). Surface water movement in this basin is artificially controlled, regulated by a capillary network of open-earth canals and ditches (~1300 km of artificial waterways, Fig. 1d) serving for drainage and irrigation. The complex network of canals was implemented along the centuries, but the hydrological structure of the territory is the result of a long-term reclamation completed in the ‘60s. Bottom sediments are usually a combination of mud and sand or silt, and vegetated buffer zones are completely absent. The various drainage canals in the network contribute water to the Po di Volano, a large semi-artificial canal that carries water to the sea. The Po di Volano is the main contributor of freshwater to the Goro Lagoon (Fig. 1b), a shallow, eutrophic coastal lagoon where intensive mussel farming takes place. The Goro Lagoon is a sheltered coastal lagoon that has little connection with the Adriatic Sea. Although the lagoon can occasionally receive some freshwater inputs (and thus some nutrients) from the Po River plume, the main nutrient contribution comes from the Po di Volano.

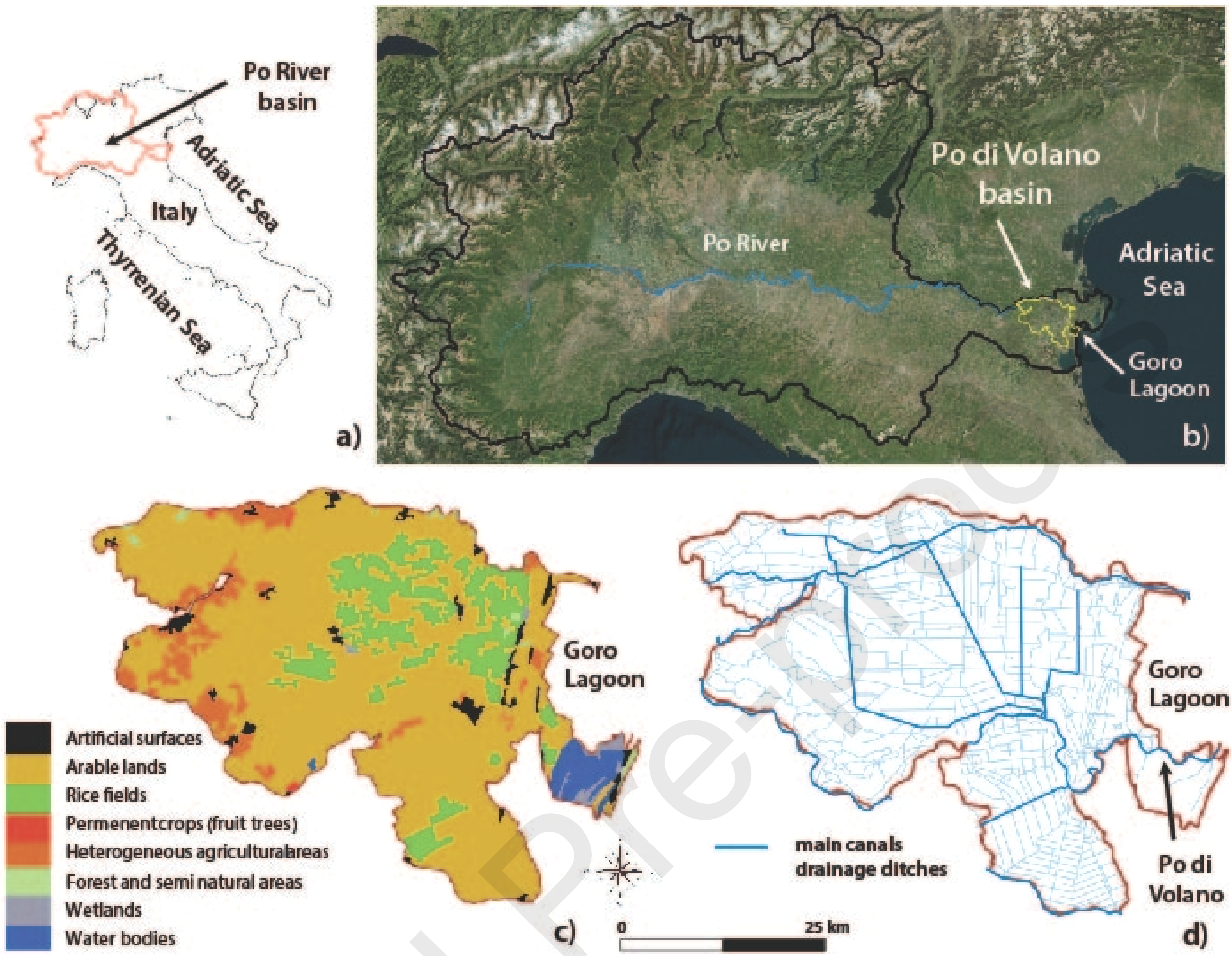


Figure 1 - Study area: a) location of the Po River basin in Italy; b) location of the Po di Volano basin within the Po River basin; c) land use of the Po di Volano between 1980s and 1990s (Corine Land Cover map 1990; https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-corine); and d) Po di Volano basin hydrographic network.

The irrigation season lasts from April to September, when the canals are flooded through a series of siphons that draw water from the Po River, which is a likely trigger for spawning by grass carp (Milardi et al., 2017). The water is delivered from irrigation canals to arable lands through a capillary ditch network; water level in the canals remains relatively stable through a complex management of the drainage system. The irrigation period overlaps with the vegetative phase and the warm season, when high water temperatures enhance N dissipation, supported by the action of macrophytes and microbial communities (Soana et al., 2015, Choudhury et al., 2018). In October, at the end of the irrigation season, little water is let through the siphons and large portions of the canal network are dry until the next irrigation season, occasionally serving as flood controls in case of intense rainfall. These hydraulic management practices have been in place, and have not significantly changed, over more than 50 years.

Aquatic vegetation overgrowth created hydraulic problems during the peak eutrophication period of the 1970s, with extensive effort by the local water management authority to mechanically remove it (Melotti et al., 1987). This led to experiments in biological control through introduction of grass carp in the mid-1980s, and to large-scale grass carp stocking in the whole area beginning in the early 1990s. Currently, submerged aquatic vegetation is almost nonexistent in the canals, and emergent vegetation has been actively managed since the mid-1990s (e.g. mechanical removal; Castaldelli et al., 2013b, Pierobon et al., 2013) and is now present only in stretches of the canal network at low flood risk, representing less than 5% of the total length (Soana et al., 2019).

General study setup

We designed a simple model to reconstruct changes in nutrient output from the Po di Volano drainage to the Goro Lagoon. We assumed that changes in nutrient loads in the lagoon would be mostly dependent on the Po di Volano system balance between inputs/outputs. We also assumed that the causes of changes in nutrient load could be inferred through their magnitude, by comparing it with different estimates of nutrient reduction/increase in load and denitrification processes. We focused on a period of two decades (1980-1999) that includes the time of grass carp introduction, and divided each year into two 6-month periods corresponding to the irrigation and non-irrigation seasons. We focused our investigation on N load, as it was identified as the most relevant component of algal blooms in the area. We used these models to explore changes in N balance, tallying the dynamics of N input in the system and transfer to the lagoon, with a pre/post approach (Fig. 2).

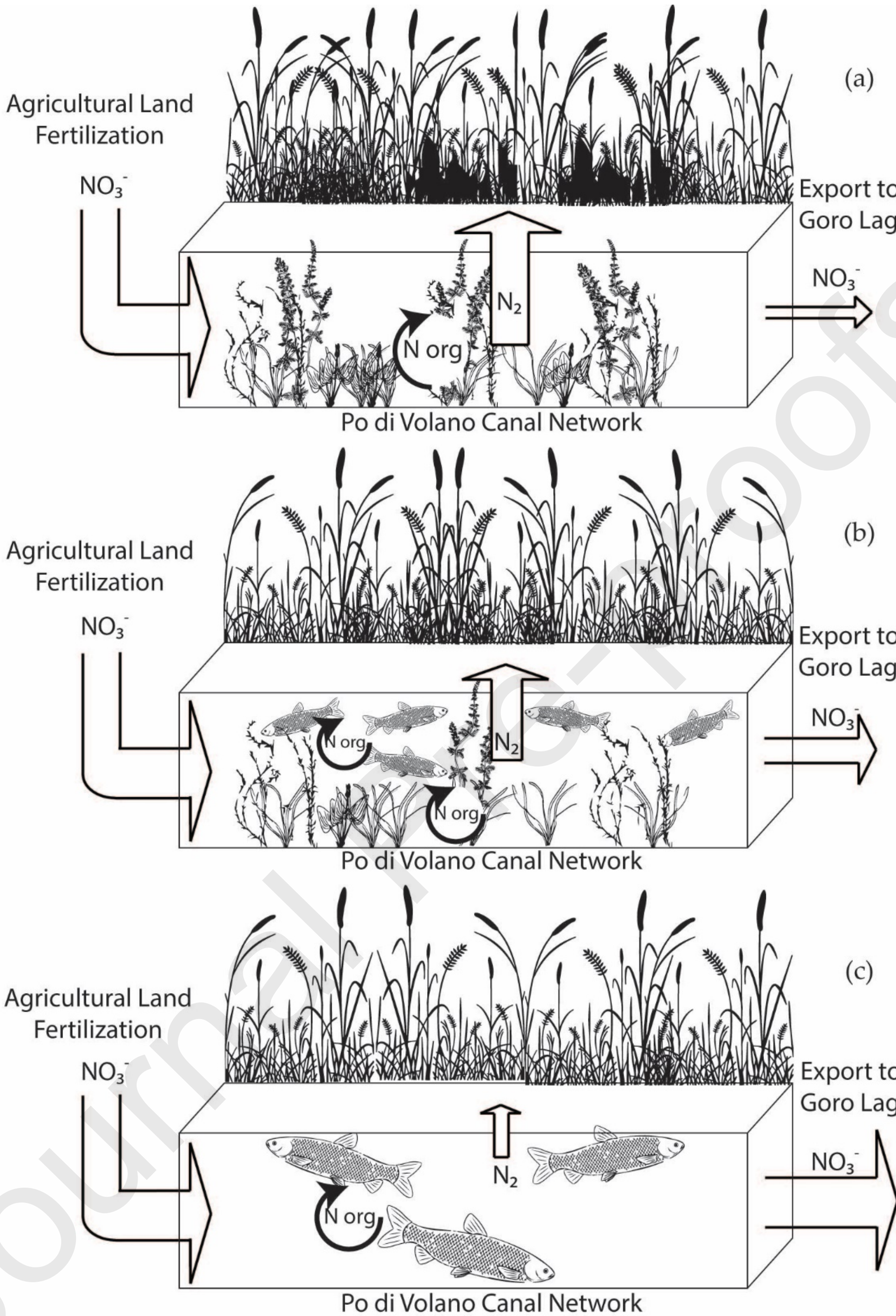


Figure 2 – A schematic representation of our model setup showing the system at theoretical baseline (a), initial invasion (b) and secondary invasion (c) conditions. White arrows represent the main input (fertilization) and permanent sinks (denitrification and downstream transport) of N, while black

arrows represent temporary sinks of N (in organic tissues), in the Po di Volano canal network. The relative size of the arrows in each figure shows the expected changes in fluxes within the system.

We modeled the effect of grass carp introduction through a bioenergetic model of their vegetation consumption, following a reconstructed historical timeline, to identify a precise time when grass carp consumption would collapse submerged vegetation and thus define a pre-post period. We used a plant growth and denitrification model to estimate the whole-basin vegetation nutrient sink, at baseline conditions (i.e. prior to grass carp introduction). We focused on submerged rather than emerged vegetation, because there was no historical data available on the abundance of the latter, and because grass carp tend to feed predominantly on this type of vegetation (Melotti et al., 1987, Filizadeh et al., 2004). We also reconstructed historical N loads from measured nutrient concentrations and water discharge rates and used agricultural surveys to reconstruct past changes in agricultural practices and to account for the possibility that changes in N load could stem from an increase in N release (i.e. from a switch to more intensive practices).

We used non-parametric tests (the Mann-Whitney U test and the Kolmogorov-Smirnov test on the mean and shape of the distributions, respectively) to verify changes in the N load before and after the modeled introduction effects (pre/post periods, 1980–1989/1990–1999). Statistical analyses were run with the PAST 3.06 software (Hammer et al., 2001).

Submerged vegetation growth and denitrification modeling

In the 1980s, submerged macrophyte communities in the Po di Volano basin were mostly represented by *Myriophyllum* spp., *Ceratophyllum* spp., *Potamogeton* spp., *Elodea* spp., and *Vallisneria spiralis*, which were reported to cover the entire area of the basin during the irrigation period (Piccoli and Gerdol, 1981, Piccoli and Gerdol, 1983, Melotti et al., 1987). The average density of submerged vegetation, prior to grass carp introduction, was 1 kg (wet weight) per square meter (0.2–1.95 kg per square meter; Melotti et al., 1987). Knowing canal surface areas, we used this value to estimate the total biomass of submerged aquatic vegetation in the basin at baseline conditions and the N pool of the standing stock by means of the vegetation N content, based on literature (i.e. a maximum 0.25% of wet biomass, see e.g. Pinardi et al., 2009, Nizzoli et al., 2014). A detailed map of the canal and ditch network was created in QGIS 2.18 by merging vector data obtained from the Emilia-Romagna Region (http://geoportale.regione.emilia-romagna.it/it) and Po River Basin Authority within the framework of the water management plan of the Po River Basin (http://pianoacque.adbpo.it/piano-di-gestione-2015/) geoportals.

We also used the reported density to model the growth of submerged macrophytes through the irrigation season, by fitting a Gompertz curve to the existing information (i.e. a daily average vegetation growth rate of around 2%; Larson, 2007, Nizzoli et al., 2014; Supplementary Fig. 1, Saunkaew et al., 2011). We estimated that, during winter when little water is present in the canals and temperatures are low, a 90% reduction to the standing crop of submerged vegetation would occur due to senescence and drying out of ample portions of the canals (Westlake, 1973). We also estimated that, when grass carp were present in the system, submerged macrophytes would also be grazed outside of the irrigation season, causing an additional 5% winter reduction.

We then modeled the denitrification function in the Po di Volano basin, taking into account the total surface of the canals and the seasonal evolution of denitrification rates for bare sediments and for sediments colonized by submerged macrophytes. Bare sediments were assigned denitrification rates based on experimental studies performed in the Po di Volano basin (average 53.5 (47–60) mg N per square meter per day; Castaldelli et al. 2015). Sediments colonized by submerged macrophytes were assigned rates measured in freshwater environments of the Po River plain similar to the Po di Volano canals for trophic conditions (in particular water NO_3^- availability) and substrate and accounting for seasonal variations (average 133 (98–168) mg N per square meter per day in April–May and average 259 (140–378) mg N per square meter per day in June–September; Nizzoli et al., 2014, Pinardi et al., 2009, Racchetti et al., 2017). We used this model to estimate the magnitude of the bottom sediment denitrification sink at baseline conditions during the 6-month period when the ditch network is active for irrigation (a period overlapping with the vegetative phase of submerged macrophytes) and for the 1986–1989 period. Because grass carp feed by pulling on the whole plant rather than nibbling at it, we assumed that a reduction in vegetation biomass would translate to a corresponding reduction in vegetated area and increase of bare sediment. As denitrification rates are higher in vegetated areas compared to bare sediments, this also corresponds to a reduction in the denitrification capacity of the system.

Grass carp consumption modeling

Grass carp is a specialized herbivorous fish, that can survive on alternative prey such as aquatic invertebrates or small fish (Shireman and Smith, 1983). We used the Wisconsin model (Hanson et al., 1997), a well-known and relatively simple bioenergetic model, to model grass carp consumption of submerged vegetation.

The model uses a set of species-specific parameters that define metabolic levels to determine consumption rates (Supplementary Table 1) as well as egestion (i.e. defecation) and excretion rates. Fish being poikilotherms, the model relies on water temperature to assess metabolic rates and on food energy content to model body mass gain. Water temperatures were not monitored on a regular basis in the area before 1992, so we used daily measures of water and air temperatures from 1992-2002 to derive a linear relationship between them (350 measures, $T_{water} = 0.9858 \times T_{air} + 2.404$, R-sq = 0.94). We then used this relationship to infer daily average water temperatures, from measures of daily average air temperatures recorded in the area between 1987 and 1989 (see Supplementary Fig. 2).

Both species-specific parameters and caloric content of submerged vegetation were taken from published values (see sources reported in Supplementary Table 1). Because no historical information was available on individual growth rates, we assumed that the individuals would grow at 89% of their maximum growth potential, which is a value commonly found for immature grass carp (e.g. van der Lee et al., 2017).

Official records on stocked grass carp total biomass and number of individuals are not available, but we were able to reconstruct the introductions prior to the '90s through interviews with the local administration and available grey literature (Melotti et al., 1987). In 1987, 5 tons of grass carp, with an average size of 250 g, were stocked in the Po di Volano drainage (a total of 20,000 individuals) and were subsequently able to spread throughout the system. A similar amount, with similar sizes, was stocked there in 1988. We used these values to model the growth and total submerged vegetation consumption of both cohorts between 1987 and 1989. The grass carp were not sexually mature in this timeframe, thus we did not account for reproduction dynamics (egg-release weight loss or population increase), but we assumed a conservative 10% yearly mortality in each cohort (native predators were few in the area and grass carp individuals would quickly reach a refuge size, Melotti et al., 1987). Some of the N temporarily stored in plant biomass would be transferred to fish biomass, as grass carp grow, so we also accounted for this (2.5% N in fish wet biomass Penczak et al., 1985).

N load calculation

We modeled the N loads from the Po di Volano to the Goro Lagoon through a reconstruction of historically measured dissolved inorganic N species concentrations (DIN=NH_4^+ + NO_3^-) and water discharges (monthly volumes, provided by the local water management authority). From 1980 to 1991, the Ferrara province administration measured N species concentrations fortnightly to monthly at the closing section of the Po di Volano basin (Ferrara, 1984, Ferrara, 1988 , Ferrara, 1991). From 1992 to

1999, these measurements were made by the Regional Environmental Protection Agency (ARPA, Emilia-Romagna Region, Provincial Department of Ferrara; https://www.arpae.it/index.asp?idlivello=112). N species concentrations were not available for 1985 and 1986, so these years were excluded from the analysis of N loads. N loads were calculated by interpolating linearly the N species concentrations between measurements (Kronvang and Bruhn, 1996; Letcher et al., 2002). This method, previously applied to several watercourses of this basin (Castaldelli et al., 2013a), was the most sensitive for small lowland waterways characterised by sudden changes in hydrological regime.

Reconstruction of historical changes in agricultural practices

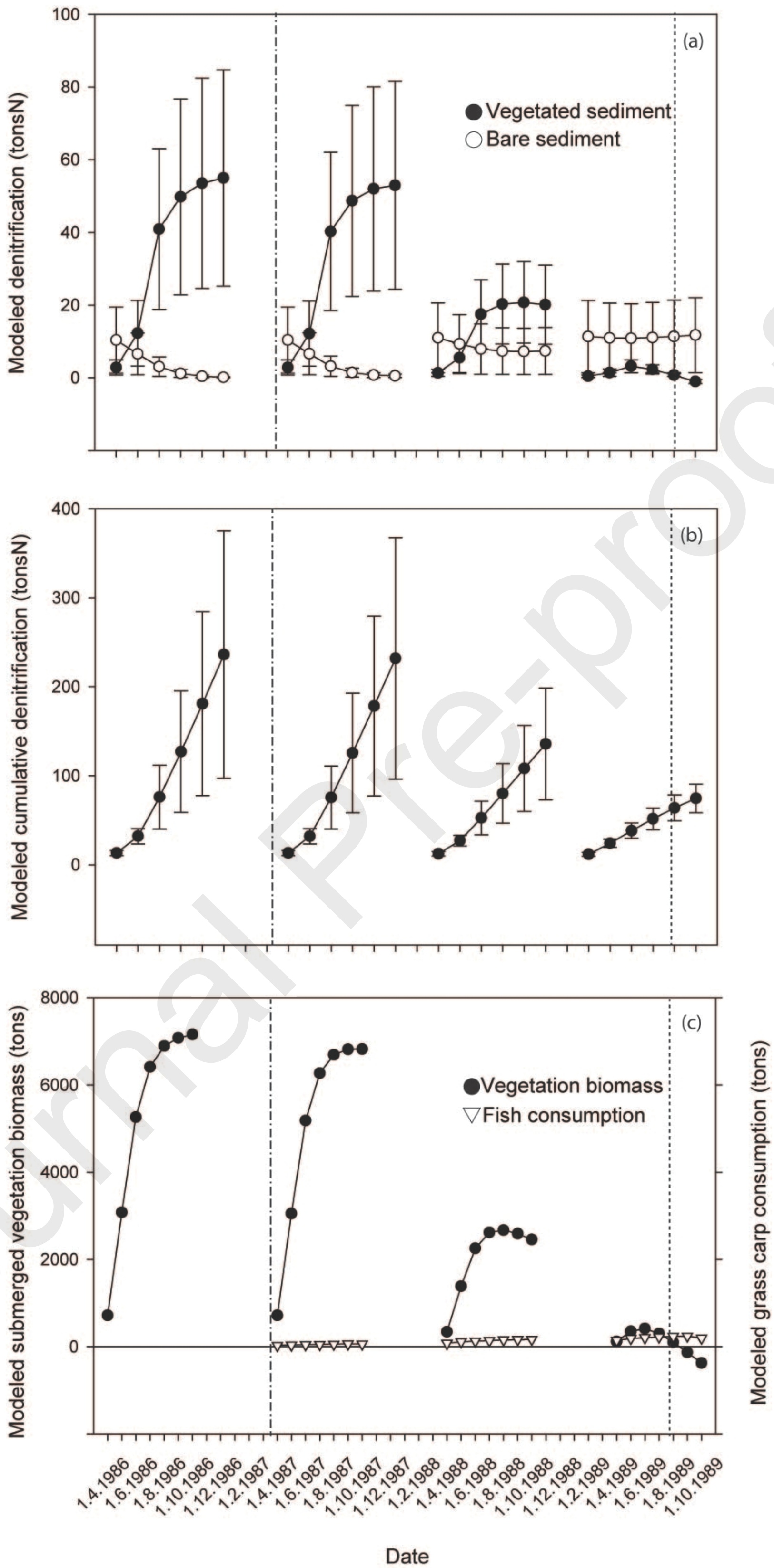
Because agriculture is the main land use in the studied area, changes in agricultural practices could result in possible changes in N loads in the Po di Volano drainage system. These changes were checked through agricultural survey data from 1987, 1990 and 1993 (National Institute of Statistics - ISTAT) at the basin scale. By using data at the provincial scale, we also verified possible changes in average N applications to utilized agricultural land over the same period. These data were retrieved from printed volumes of the Annals of Agrarian Statistics, published yearly by ISTAT for the whole national territory.

Results

Based on canal depth and total surface area and considering reported historical vegetation density we estimated that, at baseline conditions, the Po di Volano basin hosted a maximum of 7,156 tons of submerged macrophytes covering all the available sediment and sequestering 18 tons of N. In this vegetated environment, modelled denitrification reduced an average total of 220 tons of N (min-max 80-361 tons) during the irrigation phase of 1986 (Fig. 3a and b).

The grass carp population and its biomass were initially relatively small and consumed a modest amount of submerged macrophytes (a total of 393 tons during the irrigation phase of 1987), which was readily replaced by plant growth (Fig. 3b). However, as further introductions were made and fish biomass increased through natural growth, modelled total consumption nearly reached 1000 tons and exceeded 1400 tons during the irrigation seasons of 1988 and 1989, respectively (a maximum monthly consumption of 240.74 tons, Fig. 3c). Due to the combined effect of senescence and grazing, submerged macrophyte biomass at the beginning of the irrigation season had been progressively lower. The model indicated that, before the end of the 1989 irrigation season, grass carp consumption overtook the macrophyte standing crop, leading to a progressive loss of the denitrification function from submerged vegetation substrates, leaving only bare substrates denitrification (Fig. 3a). Less than 3 tons of N would be temporarily stored in fish biomass (Supplementary Table 2). At baseline conditions, the system denitrified an average total of 236 tons of N (min-max 138-334 tons of N). After the modelled submerged vegetation collapse, we modelled that the system bare sediments denitrified an average total of 69 tons of N (min-max 61-78 tons of N).

We thus estimated that grass carp introduction could have caused an increase in nutrient load of the system corresponding to the sum of lost sink of N in submerged vegetation tissues (18 tons) and lost denitrification function of submerged substrates i.e. an average of 185 tons of N (min-max 138-334 tons of N), after 1989. The results of this model are also reported in greater detail in our Supplementary Materials (Supplementary Table 2), in Microsoft Excel format.



(a)
Modeled denitrification (tonsN)
Vegetated sediment
Bare sediment
(b)
Modeled cumulative denitrification (tonsN)
(c)
Modeled submerged vegetation biomass (tons)
Modeled grass carp consumption (tons)
Vegetation biomass
Fish consumption
Date

295

Figure 3 –Modeled average denitrification operated on plant (black circles) and bare sediment (white circles) (a), modeled average cumulative denitrification over the same period (b) and modeled submerged vegetation biomass variations (black circles) and grass carp population vegetation consumption (white triangles) (c) over 1986–1989. Error bars indicate minimum and maximum denitrification values. Vertical dashed-and-dotted lines mark the date of grass carp introduction, the vertical dashed line and the horizontal solid line mark the limit below which submerged vegetation (and its denitrification function) was lost.

Annual discharge in the basin showed an increase after 1989 (pre/post averages 199/314 mega m^3, Fig. 4a), which was largely driven by a significant increase in the non-irrigation period (pre/post averages 54/88 mega m^3, Table 1), rather than in the irrigation period.

Overall, DIN loads showed a significant difference between pre/post periods (pre/post averages 515/977 tons, ~+190%, Fig. 4b, Table 1). This difference was largely driven by variations in NO_3^- loads (pre/post annual averages 215/658 tons, ~+306%, Fig. 4c), which were particularly evident in the irrigation season, but significantly different in both seasons (Table 1). NO_3^- concentrations in the non-irrigation season were similar throughout the series, but increased discharges in winter after 1989 also increased NO_3^- loads. Annual NH_4^+ loads did not show significant variations during the two decades of the study period (pre/post averages 300/319 tons, ~+6%, Fig. 4d), in either season (Table 1).

Interestingly, the average difference in NO_3^- loads in the irrigation season (pre/post averages 145/347 tons, a difference of 202 tons), roughly matches the modeled loss in the denitrification function (i.e. an average of 185 tons of N, min-max 138-334 tons of N, over the same period).

318

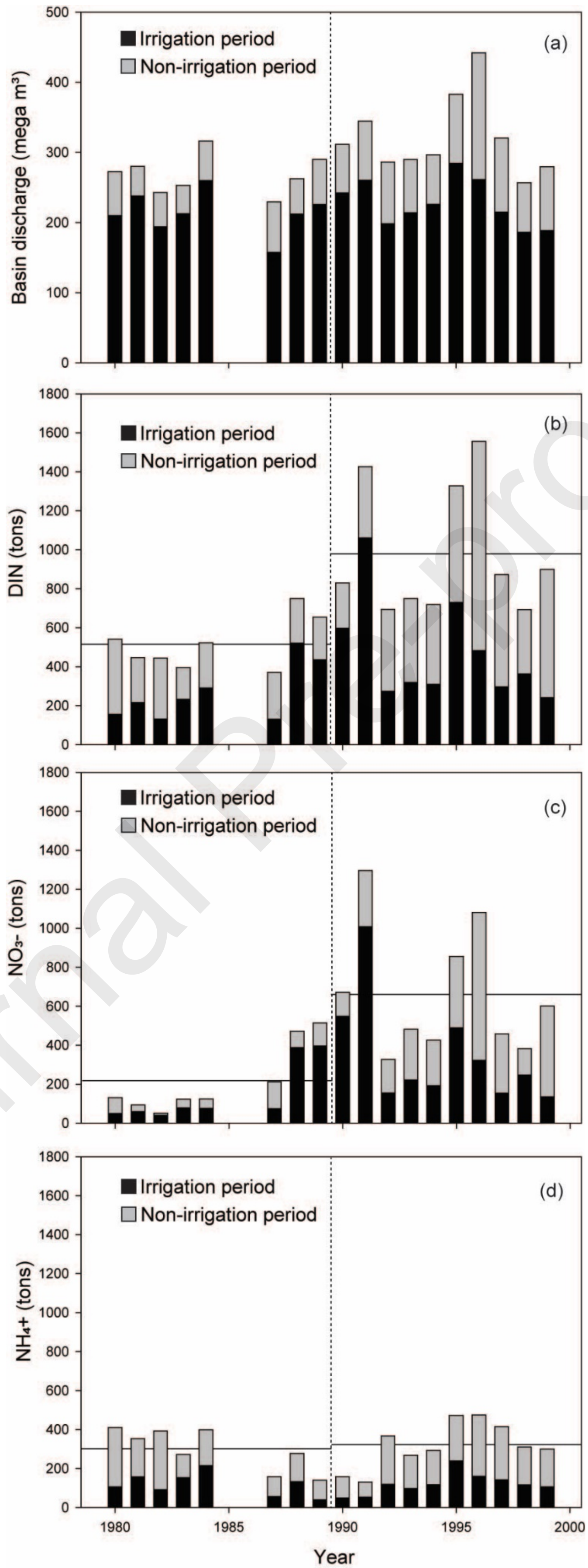

(a)
Irrigation period
Non-irrigation period
Basin discharge (mega m³)
0
100
200
300
400
500
(b)
DIN (tons)
0
200
400
600
800
1000
1200
1400
1600
1800
(c)
NO₃- (tons)
(d)
NH₄+ (tons)
1980
1985
1990
1995
2000
Year

Figure 4 – Historical annual discharge in the Po di Volano basin (a) and trends in N loads (dissolved inorganic nitrogen (DIN = NO_3^- + NH_4^+, b), NO_3^- (c) and NH_4^+ (d)) from the Po di Volano basin to the Goro Lagoon during the study period. Black and grey bars indicate discharges and loads during irrigation and non-irrigation periods, respectively. Dashed vertical lines mark the year when the denitrification function was lost according to our model, horizontal solid lines mark the average N load values pre/post the modeled loss of function.

Table 1 – Significance of differences between the periods pre/post the modeled denitrification function collapse (1980–1989/1990–1999) for discharge and N load variables in the Po di Volano basin.

Variable	Mann-Whitney P	Kolmogorov-Smirnov P
Discharge tot	**<0.05**	0.07
Discharge irrigation	0.36	0.67
Discharge non-irrigation	**<0.01**	**<0.01**
DIN tot	**<0.01**	**<0.01**
DIN irrigation	**<0.05**	**<0.05**
DIN non-irrigation	**<0.01**	**<0.01**
NO_3^- tot	**<0.01**	**<0.01**
NO_3^- irrigation	**<0.05**	**<0.01**
NO_3^- non-irrigation	**<0.01**	**<0.01**
NH_4^+ tot	0.75	0.73
NH_4^+ irrigation	0.96	0.98
NH_4^+ non-irrigation	0.62	0.73

According to our analysis of the potential changes in land use, the basin landscape was dominated by agriculture (50 – 53 *10^3 ha of utilized agricultural land) and a slight decline in its utilization was observed from 1987 to 1993 (-5% in surface area). Within utilized agricultural land, arable land dominated the agricultural landscape (85% of total utilized agricultural land) and the extension of major crop types did not show important changes over the years: industrial and vegetable crops (mostly soybean and sugar beet) had the biggest share (42.4±1.5%), followed by wheat (23.7±1.3%) and maize

(12.7±0.4%). The shares of woody crops, mostly fruit trees (7.8±0.19%), rice (6.8±0.3%), fodder crops (4.7±0.3%), other cereals (1.7±1.2) and permanent grasslands (0.08±0.002%) were all minor. Minor variations were detected between 1987 and 1993 in N supply as fertilizer to different crops at the provincial scale (average 91.7± 7.4 kgN ha^{-1}). N application slightly decreased over the period: 100 kg N ha^{-1} of arable land in 1987, 89 kg N ha^{-1} in 1990 and 86 kg N ha^{-1} in 1993 (a decrease of 11% and 14% kgN ha^{-1} in 1990 and 1993, respectively, compared to 1987). As arable land showed minor variations, this indicates that the N load from agricultural land was either stable or declining in the study period.

Discussion

Our results indicate that introduced grass carp could remove nearly all submerged vegetation of the Po di Volano canal network. This could lead to substantial loss in denitrification capacity of the system, causing an increase in downstream N load to the Goro Lagoon. A corresponding increase, matching both timing and magnitude, was detected in historical N loads (particularly NO_3^- in the irrigation season, with no significant variations in discharge), which were significantly different before and after the modeled collapse of the denitrification function. According to our data, the N loads increase could not be attributed to watershed use or agricultural practices (which did not change), and thus modeled grass carp introduction effects on submerged vegetation are a more likely explanation. We provide evidence that a freshwater fish introduction could have caused cascading and long-lasting changes in nutrient dynamics, with detrimental ecological consequences exported downstream, far from where grass carp were present.

Grass carp is a long-lived species (over 25 years, Clemens et al. (2016)) and our model indicates that effects on submerged macrophytes would persist, as individuals grow larger and increase consumption, even accounting for mortality losses (Osborne and Riddle, 1999), with a minimal effect on temporary N storage. Further grass carp stocking took place in the early 1990s (Melotti et al., 1987; Milardi, unpublished data) and could not be estimated during this study, but might have further contributed to these effects until modern times. When first introduced, grass carp were believed to be unable to reproduce in this area and thus were deemed an ideal candidate for biological control of vegetation, because densities could be controlled through a balance between stocking and mortality (Melotti et al., 1987). However, grass carp were recently found to recruit in this area (Milardi et al., 2015), which implies that population control might be already lost and that grass carp grazing pressure would not necessarily decrease through natural mortality.

Grass carp is considered to pose a substantial risk of invasion and detrimental effects elsewhere in its introduced range (Maceina et al., 1992, Scarnecchia, 2000, Cudmore and Mandrak, 2011), but the extent to which this risk applies to Italy is not yet clear (Milardi et al., 2015). In other invaded areas, detrimental effects on the environment have been reported through the overgrazing of submerged vegetation or bioturbation and nutrient resuspension (Cudmore and Mandrak, 2004, Cudmore and Mandrak, 2011, Dibble and Kovalenko, 2009). In Italy, nutrient and sediment resuspension and reduction of aquatic vegetation could have adversely influenced native species, which are mostly adapted to clearer water conditions and require a plant substrate for spawning. Increased turbidity would also favor exotic species that thrive in turbid environments (see Milardi and Castaldelli, 2018, Lanzoni et al., 2018, Milardi et al., 2018a, Gavioli et al., 2018, Milardi et al., 2018b). Our bioenergetic model suggested that egestion and excretion could contribute to the increase in N loads, but likely played a minor role (see Supplementary Table 2). However, challenges in modeling these effects for the entire time series and the entire fish community, as well as uncertainties on the biochemical pathways of the egested/excreted N, prevented us from tallying them in our N estimates.

Our model shows that the submerged macrophyte standing stock would be progressively diminished and ultimately eliminated, which is what was actually reported in this area (water management authority, personal communication) but also elsewhere (see e.g. Bonar et al., 2002), leading to a substantially diminished denitrification function of vegetated substrates and leaving only that of bare sediments. Stocking in the early 1990s would have sustained, or heightened, this effect. Mortality could have partly reduced this density, but was likely a minor factor in the study area, because fisheries for the species were not yet established and no native bird or fish predators could feed on grass carp that attained a refuge size (above 1 kg, reached during the first year according to our model). After the modeled submerged macrophyte collapse, the grass carp population of the Po di Volano could have survived on terrestrial vegetation and aquatic invertebrates (Terrell and Fox, 1974), which has also been observed in the study area (Mattia Lanzoni, personal communication) as well as for other carp species (Miller and Crowl, 2006). In narrow canals such as these, high ratios of water edge to surface area maximize terrestrial vegetation and terrestrial invertebrate resources, but terrestrial vegetation feeding could potentially cause bank degradation through the burrowing action of the carp (Kilgen and Smitherman, 1971).

Large-scale environmental changes can have multiple effects on components of the food web and on ecosystem functions (e.g. on organic material decomposition), making it hard to have a truly holistic view of the ultimate consequences of environmental disruptions. In this case, the increase in N load to the Goro Lagoon in the early 1990s caused dystrophic crises, which spurred research interest on eutrophication (see e.g. Pugnetti et al., 1992, Viaroli et al., 1995). The macroscopic effect of these dystrophic crises were massive algal blooms and, due to long water retention time in the Goro Lagoon, these effects persisted through the 1990s and continue today. It is probably not a coincidence that mussel (mainly the exotic *Tapes philippinarum*) farming and harvesting activities intensified in the Goro Lagoon during this period, taking advantage of higher productivity in the lagoon but also suffering from anoxic crises (Silvestri, 2013). Clam farming is now one of the main activities in the lagoon, with annual revenues estimated at 52 million euros in 2006 (~60 million USD, COPEGO, 2007). Surprisingly, nearly all past research was devoted to eutrophication consequences rather than causes. According to our data, an increased concentration of NO_3^- during the irrigation season and an increased discharge in the non-irrigation season led to an increased N transport from the Po di Volano system to the lagoon. However, a more complex estimation of external (e.g. the Po River plume) and internal (e.g. nutrient regeneration by clam farming) N loads to the lagoon would be needed to clearly attribute dystrophic crises to grass-carp-induced ecosystem shifts.

Some benefits have also accrued from grass carp introduction and the induced ecosystem shift. Grass carp has gained value in recreational fisheries in the area, becoming actively targeted by specialized catch-and-release anglers (see e.g. Rossetti, 2013). The reduction in submerged vegetation has reduced the costs of mechanical weed mowing, which was routinely performed to maintain hydraulic transport in the system (Melotti et al., 1987) and subsequently discontinued for submerged vegetation (water management authority, personal communication). At first glance, this might seem a classic situation where the outcomes of an introduction have been mostly beneficial. However, a more holistic perspective, considering loss of denitrification potential, might shift this perspective. Denitrification is currently achieved through water treatment plants, which have a major infrastructural and operational cost (Boerema et al., 2014). While fisheries for grass carp have been established, fishing license sales in the region have actually declined by approximately 90% over this period (from 240,000 units in early 1980s to 25,000 units in early 2010s, Emilia-Romagna Region, personal communication), perhaps due to the decline of preferred native fishes (Milardi et al., 2018a, Castaldelli et al., 2013a). Future studies should strive to use modern tools to evaluate costs and benefits in a holistic framework, which could be used by authorities to formulate improved management actions.

Grass carp have been introduced nearly worldwide, yet existing risk assessments (see e.g. Clayton and Wells, 1999, Cassani et al., 2008, Cudmore et al., 2017) have not considered the potential effect of denitrification function loss. This effect could be of substantial concern in North America's Laurentian Great Lakes, where hazardous algal blooms are becoming more frequent in Lake Erie's western basin

(Kane et al., 2014). The Maumee and Sandusky Rivers are primary nutrient sources for this basin and their associated estuaries host large vegetated areas. Grass carp spawning (Embke et al., 2016) and recruitment (Chapman et al., 2013) have recently been identified in those rivers. Grass carp grazing in these rivers might exacerbate eutrophication through denitrification function loss. No similar risk assessments have been conducted in Europe.

Conclusions

The importance of our study lies in the detection of potential downstream consequences of a fish introduction, reconstructed through modeling, supported by historical shifts in N loads. However, care should be given to considering the ultimate outcomes of this balance disruption, in order to formulate coherent restoration plans. Integrated basin management would be paramount in this case, where choices would have to be made between different ecosystem services, estimating the socio-economic impacts of these choices. The ecosystem services provided by aquatic vegetation (e.g. the denitrification function or the spawning substrate for native species) are clearly paramount in this case. Our results indicate that, in the Po di Volano system, a restored nutrient buffer capacity of inland waterways could substantially reduce the nutrient load from human activities. Controlling grass carp numbers could restore aquatic vegetation, but this in turn can cause hydraulic problems due to vegetation overgrowth. Coupling grass carp removal with widening the most critical canal sections to aid hydraulic flow would allow restoration of the N buffer capacity of the system without affecting its drainage properties.

Authors' Contributions

MM and GC conceived the idea and designed the methodology. ES, DC and MM collected and analyzed the data. MM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

We thank the Environmental Protection Agency of Ferrara (ARPA Emilia-Romagna) and the Emilia-Romagna Region Fisheries Bureau. We would also like to acknowledge Robert B. Jacobson and Mary A. Evans at USGS, as well as two anonymous referees, for their constructive review of our work. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Data accessibility

Data associated with this paper will be available as Supplementary Material upon acceptance

References

ALLDRED, M. & BAINES, S. B. 2016. Effects of wetland plants on denitrification rates: a meta-analysis. *Ecological applications,* 26**,** 676-685.

BASTVIKEN, S. K., WEISNER, S. E., THIERE, G., SVENSSON, J. M., EHDE, P. M. & TONDERSKI, K. S. 2009. Effects of vegetation and hydraulic load on seasonal nitrate removal in treatment wetlands. *Ecological Engineering,* 35**,** 946-952.

BEAULIEU, J. J., GOLDEN, H. E., KNIGHTES, C. D., MAYER, P. M., KAUSHAL, S. S., PENNINO, M. J., ARANGO, C. P., BALZ, D. A., ELONEN, C. M. & FRITZ, K. M. 2015. Urban stream burial increases watershed-scale nitrate export. *PLoS One,* 10**,** e0132256.

BILLEN, G., GARNIER, J. & LASSALETTA, L. 2013. The nitrogen cascade from agricultural soils to the sea: modelling nitrogen transfers at regional watershed and global scales. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 368**,** 20130123.

BOEREMA, A., SCHOELYNCK, J., BAL, K., VREBOS, D., JACOBS, S., STAES, J. & MEIRE, P. 2014. Economic valuation of ecosystem services, a case study for aquatic vegetation removal in the Nete catchment (Belgium). *Ecosystem Services,* 7**,** 46-56.

BONAR, S. A., BOLDING, B. & DIVENS, M. 2002. Effects of triploid grass carp on aquatic plants, water quality, and public satisfaction in Washington State. *North American Journal of Fisheries Management,* 22**,** 96-105.

CASSANI, J., HARDIN, S., MUDRAK, V. & ZAJICEK, P. 2008. A risk analysis pertaining to the use of triploid grass carp for the biological control of aquatic plants. Florida Department of Environmental Protection.

CASTALDELLI, G., PLUCHINOTTA, A., MILARDI, M., LANZONI, M., GIARI, L., ROSSI, R. & FANO, E. A. 2013a. Introduction of exotic fish species and decline of native species in the lower Po basin, north-eastern Italy. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 23**,** 405-417.

CASTALDELLI, G., SOANA, E., RACCHETTI, E., PIEROBON, E., MASTROCICCO, M., TESINI, E., FANO, E. A. & BARTOLI, M. 2013b. Nitrogen budget in a lowland coastal area within the Po river basin (Northern Italy): multiple evidences of equilibrium between sources and internal sinks. *Environmental management,* 52**,** 567-580.

CASTALDELLI, G., SOANA, E., RACCHETTI, E., VINCENZI, F., FANO, E. A. & BARTOLI, M. 2015. Vegetated canals mitigate nitrogen surplus in agricultural watersheds. *Agriculture, Ecosystems & Environment,* 212**,** 253-262.

CHAPMAN, D. C., DAVIS, J. J., JENKINS, J. A., KOCOVSKY, P. M., MINER, J. G., FARVER, J. & JACKSON, P. R. 2013. First evidence of grass carp recruitment in the Great Lakes Basin. *Journal of Great Lakes Research,* 39**,** 547-554.

CHOUDHURY, M. I., MCKIE, B. G., HALLIN, S. & ECKE, F. 2018. Mixtures of macrophyte growth forms promote nitrogen cycling in wetlands. *Science of The Total Environment,* 635**,** 1436-1443.

CLAYTON, J. S. & WELLS, R. D. S. 1999. Some issues in risk assessment reports on grass carp and silver carp. *Conservation Advisory Science Notes.* Wellington: Department of Conservation.

CLEMENS, B. J., SPANGLER, J. J., ROBERTSON, P. L., GALOVICH, G. M., BANNER, C. R., GUNCKEL, S. L., KETCHUM, L. L., BOWDEN, R. K., SPAGNOLI, S. T. & KENT, M. L. 2016. Persistence of triploid grass carp in Devils Lake, Oregon. *Journal of Fish and Wildlife Management,* 7**,** 153-161.

COPEGO 2007. Consorzio Pescatori di Goro. Largo Consumo.

CUDMORE, B. & MANDRAK, N. E. 2004. Biological synopsis of grass carp (*Ctenopharyngodon idella*). Canadian Manuscript Report of Fisheries and Aquatic Sciences.

CUDMORE, B. & MANDRAK, N. E. 2011. Assessing the biological risk of Asian carps to Canada. *In:* CHAPMAN, D. C. & HOFF, M. H. (eds.) *Invasive Asian Carps in North America.* Bethesda, Maryland: American Fisheries Society.

CUDMORE, B. C., JONES, L. A., MANDRAK, N. E., DETTMERS, J. M., CHAPMAN, D. C., KOLAR, C. S. & CONOVER, G. 2017. *Ecological risk assessment of Grass carp (Ctenopharyngodon idella) for the Great Lakes basin*, Canadian Science Advisory Secretariat.

DIBBLE, E. D. & KOVALENKO, K. 2009. Ecological impact of grass carp: a review of the available data. *Journal of Aquatic Plant Management,* 47**,** 1-15.

EMBKE, H. S., KOCOVSKY, P. M., RICHTER, C. A., PRITT, J. J., MAYER, C. M. & QIAN, S. S. 2016. First direct confirmation of grass carp spawning in a Great Lakes tributary. *Journal of Great Lakes Research,* 42**,** 899-903.

FAO 2016. *Fishery and Aquaculture Statistics yearbook,* Rome

FERRARA, P. D. 1984. Programma di controllo corpi idrici bacino Burana-Volano, anni 1980-1981-1982-1983-1984. Ferrara.

FERRARA, P. D. 1988 Considerazioni sullo stato di inquinamento dei corpi idrici del bacino Burana-Volano. Campagne di rilevamento 1985-1986-1987.

FERRARA, P. D. 1991. Indagine quali-quantitativa delle acque superficiali del bacino Burana-Volano.

FILIZADEH, Y., AHMADI, H. & ZOLFINEJAD, K. 2004. The feeding preferences of grass carp (*Ctenopharyngodon idella* Val.) for ten aquatic plants. *Fourth International Iran and Russia Conference in Agriculture and Natural Resources.* Shahrekord, Iran.

GARNIER, J., BEUSEN, A., THIEU, V., BILLEN, G. & BOUWMAN, L. 2010. N: P: Si nutrient export ratios and ecological consequences in coastal seas evaluated by the ICEP approach. *Global Biogeochemical Cycles,* 24.

GAVIOLI, A., MANCINI, M., MILARDI, M., ASCHONITIS, V., RACCHETTI, E., VIAROLI, P. & CASTALDELLI, G. 2018. Exotic species, rather than low flow, negatively affect native fish in the Oglio River, Northern Italy. *River Research and Applications***,** 1-11.

GLIBERT, P. M. 2017. Eutrophication, harmful algae and biodiversity—Challenging paradigms in a world of complex nutrient changes. *Marine pollution bulletin,* 124**,** 591-606.

HAMMER, Ø., HARPER, D. & RYAN, P. 2001. *PAST: Paleontological statistics software package for education and data analysis*.

HANSEN, A. T., DOLPH, C. L., FOUFOULA-GEORGIOU, E. & FINLAY, J. C. 2018. Contribution of wetlands to nitrate removal at the watershed scale. *Nature Geoscience,* 11**,** 127.

HANSON, P., JOHNSON, T., SCHINDLER, D. & KITCHELL, J. 1997. Fish bioenergetics 3.0 software and manual. *Sea grant institute*.

HILL, A. R. 2019. Groundwater nitrate removal in riparian buffer zones: a review of research progress in the past 20 years. *Biogeochemistry***,** 1-23.

KANE, D. D., CONROY, J. D., RICHARDS, R. P., BAKER, D. B. & CULVER, D. A. 2014. Re-eutrophication of Lake Erie: Correlations between tributary nutrient loads and phytoplankton biomass. *Journal of Great Lakes Research,* 40**,** 496-501.

KELLY, A. M., ENGLE, C. R., ARMSTRONG, M. L., FREEZE, M. & MITCHELL, A. J. 2011. History of introductions and governmental involvement in promoting the use of grass, silver, and bighead carps. *In:* CHAPMAN, D. C. & HOFF, M. H. (eds.) *Invasive Asian Carps in North America.* Bethesda, Maryland: American Fisheries Society.

KILGEN, R. H. & SMITHERMAN, R. O. 1971. Food habits of the white amur stocked in ponds alone and in combination with other species. *Progressive Fish-Culturist,* 33**,** 123-127.

LANZONI, M., MILARDI, M., ASCHONITIS, V., FANO, E. A. & CASTALDELLI, G. 2018. A regional fish inventory of inland waters in Northern Italy reveals the presence of fully exotic fish communities. *The European Zoological Journal,* 85**,** 1-7.

LARSON, D. 2007. Growth of three submerged plants below different densities of Nymphoides peltata (SG Gmel.) Kuntze. *Aquatic Botany,* 86**,** 280-284.

LEVAVASSEUR, F., BIARNÈS, A., BAILLY, J.-S. & LAGACHERIE, P. 2014. Time-varying impacts of different management regimes on vegetation cover in agricultural ditches. *Agricultural water management,* 140**,** 14-19.

LODGE, D. M. 1991. Herbivory on freshwater macrophytes. *Aquatic botany,* 41**,** 195-224.

MACEINA, M. J., CICHRA, M. F., BETSILL, R. K. & BETTOLI, P. W. 1992. Limnological changes in a large reservoir following vegetation removal by grass carp. *Journal of Freshwater Ecology,* 7**,** 81-95.

MELOTTI, P., RESTA, C. & CAVALLARI, A. 1987. La carpa erbivora in Emilia-Romagna aspetti biologici e gestionali. Ferrara: Regione Emilia-Romagna e Amministrazione Provinciale di Ferrara.

MILARDI, M., ASCHONITIS, V., GAVIOLI, A., LANZONI, M., FANO, E. A. & CASTALDELLI, G. 2018a. Run to the hills: exotic fish invasions and water quality degradation drive native fish to higher altitudes. *Science of The Total Environment,* 624**,** 1325-1335.

MILARDI, M. & CASTALDELLI, G. 2018. A novel approach to an ecofunctional fish index for Mediterranean countries. *Ecological Indicators,* 89**,** 376-385.

MILARDI, M., CHAPMAN, D., LANZONI, M., LONG, J. M. & CASTALDELLI, G. 2017. First evidence of bighead carp wild recruitment in Western Europe, and its relation to hydrology and temperature. *PloS one,* 12**,** e0189517.

MILARDI, M., GAVIOLI, A., SOANA, E., LANZONI, M., FANO, E. A. & CASTALDELLI, G. 2019a. The role of species introduction in modifying the functional diversity of native communities. *Science of The Total Environment*, 134364.

MILARDI, M., LANZONI, M., GAVIOLI, A., FANO, E. A. & CASTALDELLI, G. 2018b. Long-term fish monitoring underlines a rising tide of temperature tolerant, rheophilic, benthivore and generalist exotics, irrespective of hydrological conditions. *Journal of Limnology*.

MILARDI, M., LANZONI, M., KILJUNEN, M., TORNIAINEN, J. & CASTALDELLI, G. 2015. Natural recruitment contributes to high densities of grass carp Ctenopharyngodon idella (Valenciennes, 1844) in Western Europe. *Aquatic Invasions,* 10**,** 439-448.

MILARDI, M., LAPPALAINEN, J., MCGOWAN, S. & WECKSTROM, J. 2016. Can fish introductions alter nutrient cycles in previously fishless high-latitude lakes? *Journal of Limnology,* 76.

MILARDI, M., PETÄJÄ, T. & WECKSTRÖM, J. 2019b. Should we further investigate the cascading effects of introduced fish on insectivorous birds? *Boreal Environment Research,* in press.

MILLER, S. & CROWL, T. 2006. Effects of common carp (Cyprinus carpio) on macrophytes and invertebrate communities in a shallow lake. *Freshwater Biology,* 51**,** 85-94.

NIZZOLI, D., WELSH, D. T., LONGHI, D. & VIAROLI, P. 2014. Influence of Potamogeton pectinatus and microphytobenthos on benthic metabolism, nutrient fluxes and denitrification in a freshwater littoral sediment in an agricultural landscape: N assimilation versus N removal. *Hydrobiologia,* 737**,** 183-200.

OSBORNE, J. A. & RIDDLE, R. D. 1999. Feeding and growth rates for triploid grass carp as influenced by size and water temperature. *Journal of Freshwater Ecology,* 14**,** 41-45.

PAERL, H. W. 2009. Controlling eutrophication along the freshwater–marine continuum: dual nutrient (N and P) reductions are essential. *Estuaries and Coasts,* 32**,** 593-601.

PENCZAK, T., MOLINSKI, M., GALICKA, W. & PREJS, A. 1985. Factors Affecting Nutrient Budget in Lakes of the R. Jorka Watershed(Masurian Lakeland, Poland): VII. Input and Removal of Nutrients with Fish. *Ekologia Polska ELPLBS,* 33.

PICCOLI, F. & GERDOL, R. 1981. Rice-field weed communities in Ferrara Province (northern Italy). *Aquatic Botany,* 10**,** 317-328.

PICCOLI, F. & GERDOL, R. 1983. Correlation between macrophyte vegetation and some water properties in the irrigation system of the Lower river Po Plane. *Plant Biosystem,* 117**,** 261-270.

PIEROBON, E., CASTALDELLI, G., MANTOVANI, S., VINCENZI, F. & FANO, E. A. 2013. Nitrogen removal in vegetated and unvegetated drainage ditches impacted by diffuse and point sources of pollution. *CLEAN–Soil, Air, Water,* 41**,** 24-31.

PINARDI, M., BARTOLI, M., LONGHI, D., MARZOCCHI, U., LAINI, A., RIBAUDO, C. & VIAROLI, P. 2009. Benthic metabolism and denitrification in a river reach: a comparison between vegetated and bare sediments. *Journal of Limnology,* 68**,** 133-145.

PINAY, G., CLÉMENT, J. C. & NAIMAN, R. J. 2002. Basic principles and ecological consequences of changing water regimes on nitrogen cycling in fluvial systems. *Environmental Management,* 30**,** 481-491.

PUGNETTI, A., VIAROLI, P. & FERRARI, I. 1992. Processes leading to dystrophy in a Po River Delta lagoon (Sacca di Goro): phytoplankton–macroalgae interactions. *Marine Coastal Eutrophication.* Elsevier.

RABALAIS, N. N., TURNER, R. E. & WISEMAN JR, W. J. 2002. Gulf of Mexico hypoxia, aka “The dead zone”. *Annual Review of ecology and Systematics,* 33**,** 235-263.

RACCHETTI, E., LONGHI, D., RIBAUDO, C., SOANA, E. & BARTOLI, M. 2017. Nitrogen uptake and coupled nitrification–denitrification in riverine sediments with benthic microalgae and rooted macrophytes. *Aquatic Sciences,* 79**,** 487-505.

RÖNNBERG, C. & BONSDORFF, E. 2004. Baltic Sea eutrophication: area-specific ecological consequences. *Hydrobiologia,* 514**,** 227-241.

ROSSETTI, M. 2013. *A caccia di erbivore nel Ferrarese* [Online]. Available: https://www.carpmagazine.it/2013/09/02/a-caccia-di-erbivore-nel-ferrarese/ [Accessed June 2018].

SAUNKAEW, P., WANGPAKAPATTANAWONG, P. & JAMPEETONG, A. 2011. Growth, morphology, ammonium uptake and nutrient allocation of Myriophyllum brasiliense Cambess. under high NH 4+ concentrations. *Ecotoxicology,* 20.

SCARNECCHIA, D. L. 2000. An assessment of the environmental impact of introducing grass carp into Montana. Moscow, Idaho: Department of Fish and Wildlife Resources, University of Idaho.

SHIREMAN, J. V. & SMITH, C. R. 1983. *Synopsis of biological data on the grass carp, Ctenopharyngodon idella (Cuvier and Valenciennes, 1844)*, Food and Agriculture Organization of the United Nations Rome.

SILVESTRI, F. 2013. Il ruolo della conoscenza per lo sviluppo delle aree fragili: il caso di Goro. *Sviluppo locale XI (27), 15. Accessed via http://www.eco-eco.it/download/pubblicazioni/art_goro.pdf in June 2018.*

SOANA, E., BARTOLI, M., MILARDI, M., FANO, E. A. & CASTALDELLI, G. 2019. An ounce of prevention is worth a pound of cure: Managing macrophytes for nitrate mitigation in irrigated agricultural watersheds. *Science of the total environment,* 647**,** 301-312.

SOANA, E., NALDI, M., BONAGLIA, S., RACCHETTI, E., CASTALDELLI, G., BRÜCHERT, V., VIAROLI, P. & BARTOLI, M. 2015. Benthic nitrogen metabolism in a macrophyte meadow (Vallisneria spiralis L.) under increasing sedimentary organic matter loads. *Biogeochemistry,* 124**,** 387-404.

TERRELL, J. W. & FOX, A. C. 1974. Food habits, growth, and catchability of grass carp in the absence of aquatic vegetation. *Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies,* 28**,** 251-259.

VAN DER LEE, A. S., JOHNSON, T. B. & KOOPS, M. A. 2017. Bioenergetics modelling of grass carp: Estimated individual consumption and population impacts in Great Lakes wetlands. *Journal of Great Lakes Research,* 43**,** 308-318.

VIAROLI, P., BARTOLI, M., BONDAVALLI, C. & NALDI, M. 1995. Oxygen fluxes and dystrophy in a coastal lagoon colonized by Ulva rigida(Sacca di Goro, Po River Delta, northern Italy). *Fresenius Environmental Bulletin,* 4**,** 381-386.

VIAROLI, P., NALDI, M., BONDAVALLI, C. & BENCIVELLI, S. 1996. Growth of the seaweed Ulva rigida C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). *Hydrobiologia,* 329**,** 93-103.

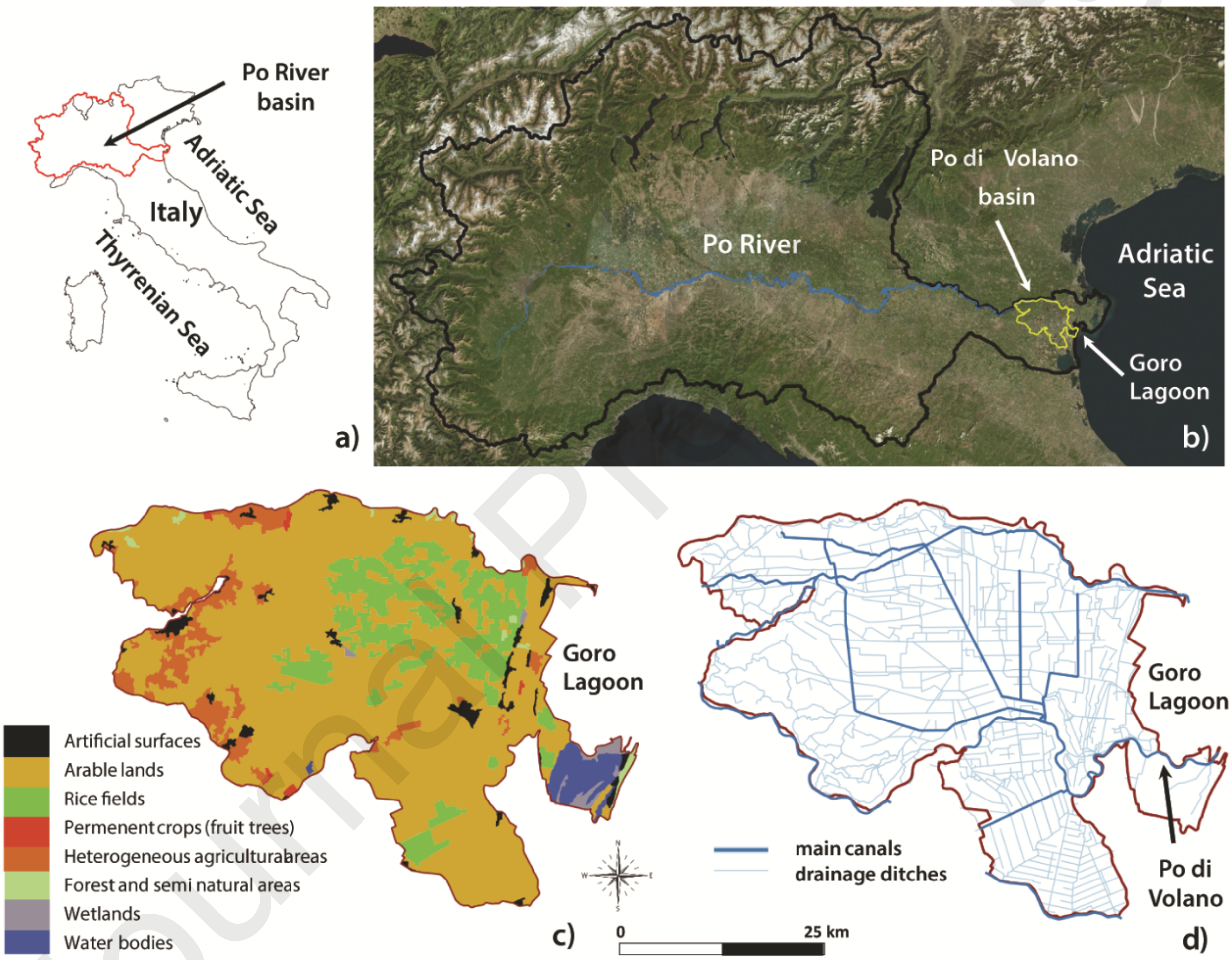
VYMAZAL, J. & BŘEZINOVÁ, T. D. 2018. Removal of nutrients, organics and suspended solids in vegetated agricultural drainage ditch. *Ecological Engineering,* 118**,** 97-103.

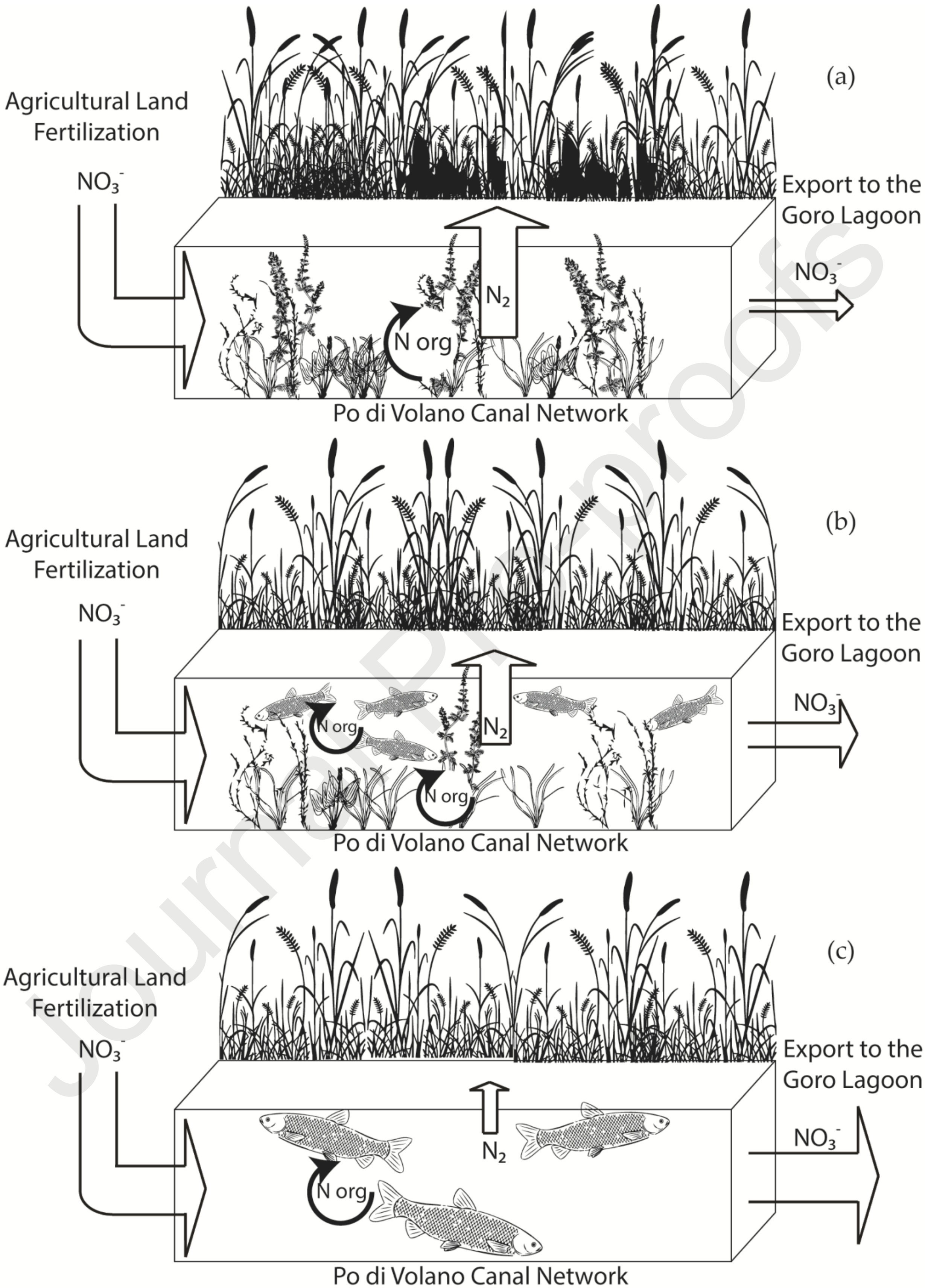
WESTLAKE, D. 1973. Aquatic macrophytes in rivers. A review. *Polskie Archiwum Hydrobiologii,* 20**,** 31-40.

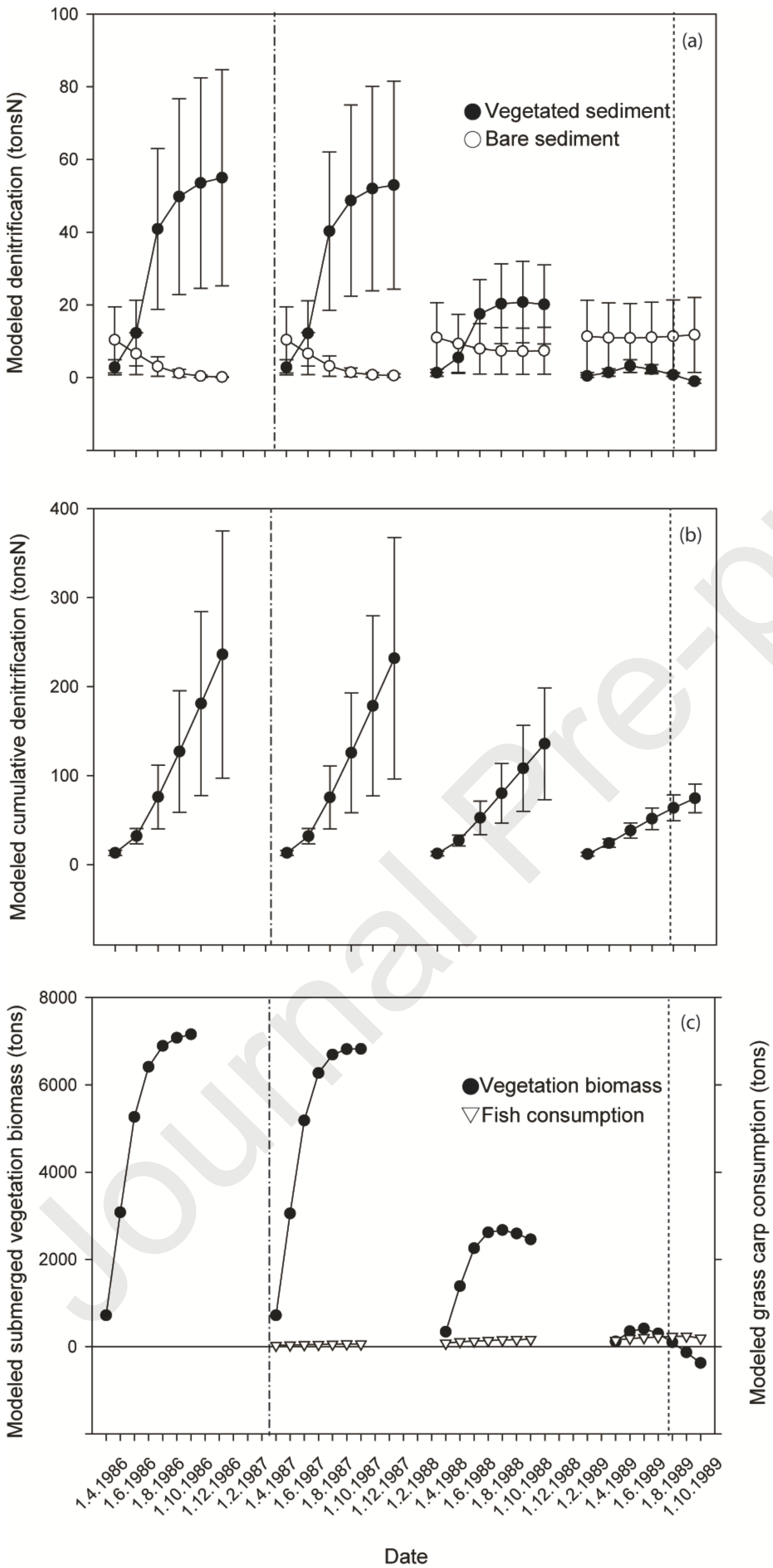
WITTMANN, M. E., JERDE, C. L., HOWETH, J. G., MAHER, S. P., DEINES, A. M., JENKINS, J. A., WHITLEDGE, G. W., BURBANK, S. R., CHADDERTON, W. L. & MAHON, A. R. 2014. Grass carp in the Great Lakes region: establishment potential, expert perceptions, and re-evaluation of experimental evidence of ecological impact. *Canadian Journal of Fisheries and Aquatic Sciences,* 71**,** 992-999.

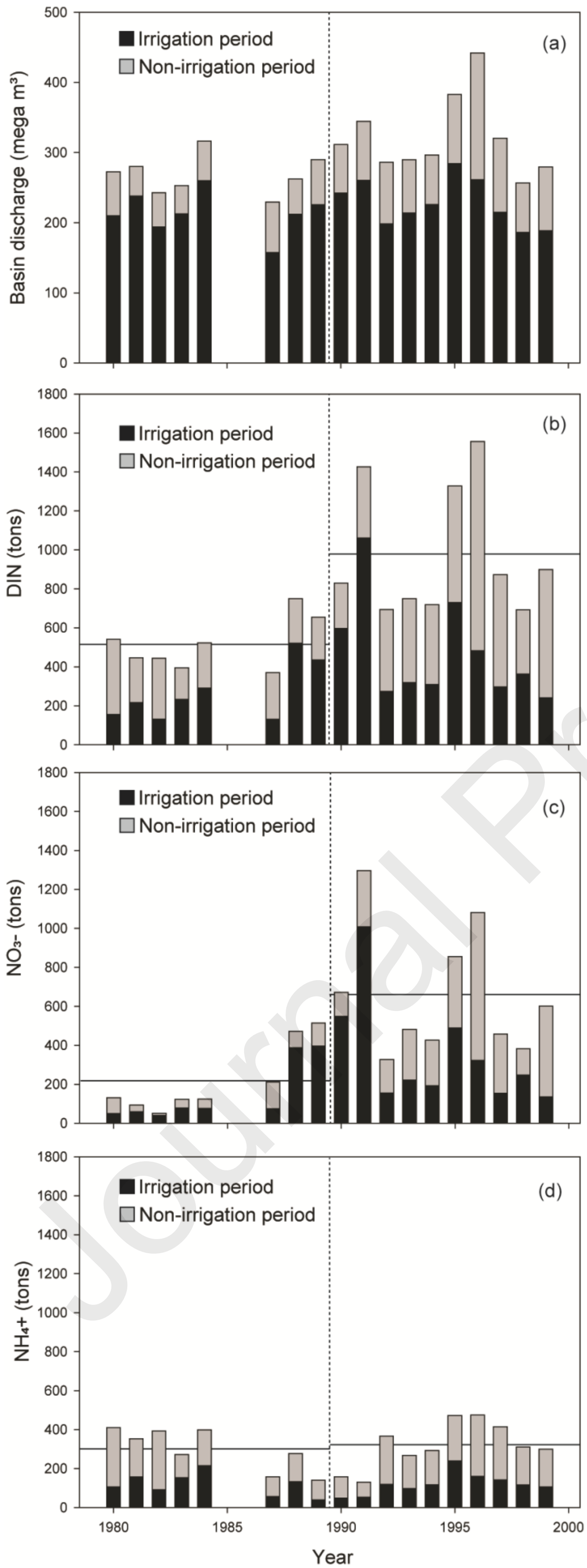
Highlights for the article "Could a freshwater fish be at the root of dystrophic crises in a coastal lagoon?" by Milardi et al.

- Introduced grass carp could remove submerged vegetation in a canal network
- Submerged vegetation loss leads to a major decrease of the denitrification function
- A following increase in downstream nitrogen loads to a coastal lagoon was detected
- Watershed use or agricultural practices could not explain this increase
- Processes resulting from grass carp introduction were a more likely explanation

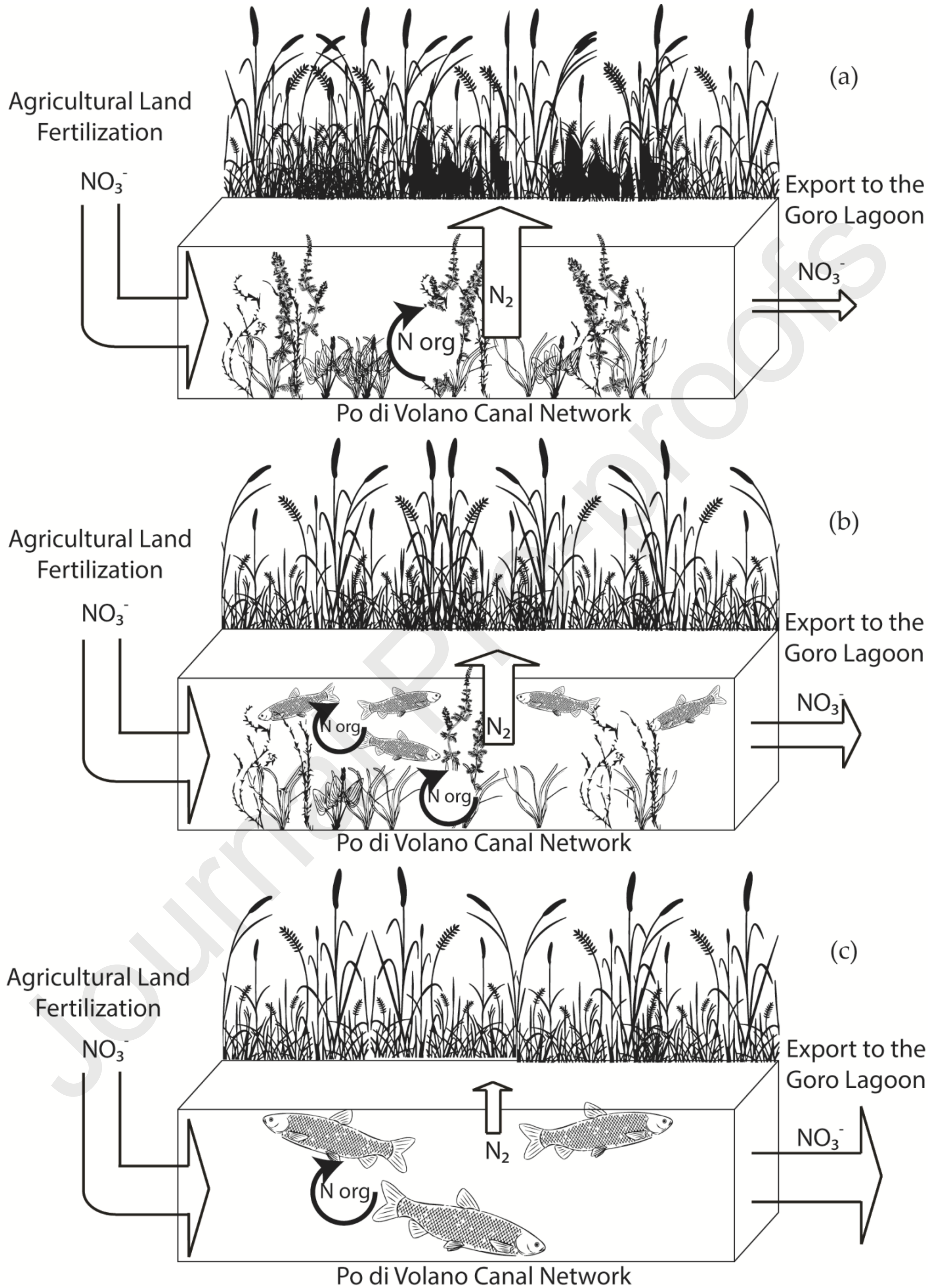


673 
(a)
Agricultural Land
Fertilization
NO_3^-
Export to the
Goro Lagoon
NO_3^-
N_2
N org
Po di Volano Canal Network
(b)
Agricultural Land
Fertilization
NO_3^-
Export to the
Goro Lagoon
NO_3^-
N org
N_2
N org
Po di Volano Canal Network
(c)
Agricultural Land
Fertilization
NO_3^-
Export to the
Goro Lagoon
NO_3^-
N_2
N org
Po di Volano Canal Network

674 
(a)
Vegetated sediment
Bare sediment
Modeled denitrification (tonsN)
100
80
60
40
20
0
(b)
Modeled cumulative denitrification (tonsN)
400
300
200
100
0
(c)
Vegetation biomass
Fish consumption
Modeled submerged vegetation biomass (tons)
Modeled grass carp consumption (tons)
8000
6000
4000
2000
0
1.4.1986
1.6.1986
1.8.1986
1.10.1986
1.12.1986
1.2.1987
1.4.1987
1.6.1987
1.8.1987
1.10.1987
1.12.1987
1.2.1988
1.4.1988
1.6.1988
1.8.1988
1.10.1988
1.12.1988
1.2.1989
1.4.1989
1.6.1989
1.8.1989
1.10.1989
Date


Irrigation period
Non-irrigation period
(a)
Basin discharge (mega m³)
(b)
DIN (tons)
(c)
NO3- (tons)
(d)
NH4+ (tons)
Year

675


(a)
Agricultural Land Fertilization
NO_3^-
Export to the Goro Lagoon
NO_3^-
N_2
N org
Po di Volano Canal Network
(b)
Agricultural Land Fertilization
NO_3^-
Export to the Goro Lagoon
NO_3^-
N_2
N org
N org
Po di Volano Canal Network
(c)
Agricultural Land Fertilization
NO_3^-
Export to the Goro Lagoon
NO_3^-
N_2
N org
Po di Volano Canal Network

676