



Research Paper

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

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ABSTRACT

In permeable soils, excess nitrate from agriculture is transported vertically and accumulates in aquifers. However, it can come back to the surface via groundwater movement and pollute watercourses. We hypothesized that vegetated ditches may mitigate significant amounts of nitrate from spring waters, and represent a buffer system to protect downstream water bodies from eutrophication. To test this hypothesis, nitrate removal was measured in ditches fed by nitrate-rich groundwater in presence and absence of emergent vegetation. Reach-scale methods (N₂ open-channel, N budgets) were coupled with laboratory incubations of sediment cores (benthic N fluxes, isotope pairing) and plant N uptake estimation. Studied ditches are representative of a wide hydrological network in Northern Italy, within the so-called “spring-belt” (Po River plain), a NO₃⁻-vulnerable area with high density of contaminated springs. Results indicated a greater reach-scale N removal in vegetated (38–84 mmol N m⁻² d⁻¹) as compared to unvegetated condition (12–45 mmol N m⁻² d⁻¹). Denitrification was the dominant N-removal pathway, while plant uptake represented a minor fraction of the net N abatement. Large development of interfaces for microbial growth provided by aquatic vegetation and more opportunities for biotic interactions are features that promote nitrate reduction in the ditch network. Despite the vegetated ditches were significant N-reactors, denitrification provided a little N-removal to in-stream high nitrate loads, with the exception of periods when plant coverage and water retention time peaked. Management of N-saturated ditches may consist in the enlargement of stretches to increase water retention and amplify the interfaces where biofilms develop, though preserving hydraulic efficiency. The maintenance of vegetation in the ditch networks would result in a significant N-abatement on a larger scale.

1. Introduction

In the last century, the intensification of agricultural activities and soil loss due to urbanization have deeply simplified the landscape in lowland areas, by removing natural habitats as wetlands and riparian vegetated zones (Groffman et al., 2003; Hefting et al., 2013). These human-impacted watersheds have lost their capacity to buffer excess nutrient loads, whose delivery to coastal waters is further accelerated by river morphological alterations such as channelization, burial, and water withdrawal, and by decreased connectivity between riverbeds and floodplains (Roley et al., 2012; Beaulieu et al., 2015). Modern agriculture has further modified the landscape through the implementation of extensive artificial canal networks, dug ex novo for wetland reclamation or resectioned in former natural river networks. In both

cases, they often constitute a capillary network arranged to maximize multiple water uses, as drainage and irrigation, and have become integral components and ubiquitous features of many productive agroecosystems (Pierce et al., 2012; Dollinger et al., 2015).

Small-size watercourses, no matter if natural or artificial, are the interfaces between agricultural lands and downstream aquatic ecosystems, such as rivers, estuaries or lagoons and coastal waters. Such canals are characterised by multiple interfaces among water, sediment and aquatic vegetation (Marion et al., 2014; Pinay et al., 2015). Here, nitrogen (N) removal takes place as a result of several plant and microbially-mediated N transformations, among which the dominant are assimilation and denitrification, the reduction of nitrate (NO₃⁻) to N gases under anaerobic conditions (Schaller et al., 2004; Bernot and Dodds, 2005; Mulholland et al., 2008). Small canals and ditches can

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contribute significantly to watershed N dynamics because of their capillary distribution and high metabolic capacity. The latter is sustained by the high ratio between bio-reactive surfaces, directly or indirectly ascribable to aquatic macrophytes, and water volumes (Marion et al., 2014; Srivastava et al., 2016).

Recent studies have suggested a new perspective on the role of canal networks in watershed N dynamics by proving that agricultural basins can generate large N excess but little export (Bartoli et al., 2012; Castaldelli et al., 2013; Romero et al., 2016). Interest has grown on the identification and parametrization of landscape elements that provide high rates of N removal. However, the debate is still open if intensively cultivated systems may maintain or not these relevant ecosystem functions, since aquatic vegetation is generally considered an impediment for water circulation and mechanically removed during routine management practices of ditches (Duncan et al., 2013; Pinay et al., 2015).

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

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

In fact, under N excess the competition between uptake by primary producers and microbial denitrification is smoothed and high rates of both processes can simultaneously occur (Soana et al., 2015; Racchetti et al., 2016).

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

2. Material and methods

2.1. Study area and experimental approach

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

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

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

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