



## Research article

## The effect of water velocity on nitrate removal in vegetated waterways

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## ABSTRACT

The extended networks of canals and ditches in agricultural landscapes provide high buffer capacity towards nitrogen (N) excess. Their N mitigation potential depends on several biotic and abiotic factors, among which water velocity is poorly explored and generally omitted from the parameterization of this remarkable ecosystem service. The present work reports new insights on the role of flow velocity in regulating N removal via denitrification in sediments colonized by *Phragmites australis*. Denitrification was investigated in outdoor mesocosms in the presence and absence of *P. australis* and over a small range of flow velocity (0–6 cm s<sup>-1</sup>) typical of low-gradient water bodies. Simultaneous measurements of NO<sub>3</sub><sup>-</sup> consumption and N<sub>2</sub> production based on analyses of N<sub>2</sub>:Ar by Membrane Inlet Mass Spectrometry were undertaken. Vegetated sediments were found more efficient in converting NO<sub>3</sub><sup>-</sup> to N<sub>2</sub> via microbial-mediated denitrification (27–233 mmol N m<sup>-2</sup> d<sup>-1</sup>) than bare sediments (18–33 mmol N m<sup>-2</sup> d<sup>-1</sup>). Vegetation provides multiple interfaces, i.e. in the rhizosphere and on epiphytic biofilms, that support the development and activity of bacterial communities responsible for NO<sub>3</sub><sup>-</sup> dissipation. NO<sub>3</sub><sup>-</sup> removal and denitrification rates exhibited one order of magnitude raise when water velocity passed from 0 to 6 cm s<sup>-1</sup> in vegetated sediments. Indeed, in slow-flow vegetated waterways denitrification may be physically limited and the increase of water velocity enhances the rate of NO<sub>3</sub><sup>-</sup> supply through the diffusive boundary layer, thereby promoting its consumption and loss from the system. Water velocity should be taken into account as a key factor for management and restoration actions aimed at maximizing the NO<sub>3</sub><sup>-</sup> buffer capacity of low-flow drainage networks.

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## 1. Introduction

Budget simulations have highlighted that agricultural watersheds may maintain a high buffer capacity towards nitrogen (N) excess, by converting it from reactive forms (nitrate-NO<sub>3</sub><sup>-</sup> in particular) to gas (N<sub>2</sub>), thanks to the extended networks of drainage, irrigation ditches and higher order canals (Törnqvist et al., 2015; Romero et al., 2016). This potential is ascribed to some attributes that promote N processing and removal via denitrification, such as long water residence time, organic matter availability due to elevated primary production, hypoxic conditions at the bottom, and presence of multiple interfaces, provided mainly by vegetation, that favour differentiated microniches and rich microbial communities (Revsbech et al., 2005; Birgand et al., 2007; Veraart et al., 2016). Ditches and canals are generally extremely heterogeneous

in terms of hydraulic parameters (e.g. discharge, velocity), quality of water and benthic compartments (e.g. nutrient availability, quality and quantity of organic matter), and biological communities, due to differences in landscape position (e.g. proximity to N sources), functions and management. Thus, the regulation of denitrification in small watercourses has been extensively explored in relation to abiotic (e.g. inorganic N concentration, organic loading, redox conditions) and biotic features (e.g. communities of primary producers), and their multiple interactions (Pierobon et al., 2013; Veraart et al., 2016; Speir et al., 2017).

In slow-flow ditches and canals of intensively cultivated basins, NO<sub>3</sub><sup>-</sup> is generally not limited (Birgand et al., 2007; Garnier et al., 2014) and water velocity may become a regulatory factor of diffusive boundary layers and thus of NO<sub>3</sub><sup>-</sup> supply to bioactive surfaces characterised by rapid consumption, such as the rhizosphere and the epiphytic biofilms (Toet et al., 2003; Revsbech et al., 2005; Srivastava et al., 2016). Nonetheless, water velocity as a key driver controlling N removal remains poorly if not at all explored. Ecosystem-level metabolism is strongly affected by the solute mass transfer from the water column to the bioactive surfaces through

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the diffusive boundary layer, generally the rate-limiting process for nutrient and gas exchange (Silvester and Sleight, 1985; Larned et al., 2004). Increasing velocities decrease the thickness of the boundary layer adjacent to uptake surfaces resulting in shorter diffusive distances, thus enhancing solute mass transfer and stimulating metabolic processes (Madsen and Sand-Jensen, 1991; Eriksson, 2001; Arnon et al., 2013).  $\text{NO}_3^-$  loss by denitrification in water-courses is principally governed by its supply from the water column to anoxic sediments or other interfaces where denitrification occurs such as epiphytic biofilms on submersed vegetation. Since diffusion is generally extremely low, the most important process of  $\text{NO}_3^-$  supply to denitrifiers is usually the convective transfer, which depends on water velocity and presence of aquatic vegetation that increases turbulent flow and water column mixing (Silvester and Sleight, 1985; Nikora, 2010). Concurrently, increasing velocities promote more oxic conditions in the surface layer of sediment and biofilms, thereby stimulating aerobic metabolism and inhibiting denitrification (Arnon et al., 2007a), or forcing denitrification in deeper anoxic zones (O'Connor and Hondzo, 2007).

The effects of flow velocity on biogeochemical dynamics has been previously investigated in benthic biofilms (e.g., Madsen and Sand-Jensen, 1991; Larned et al., 2004; Arnon et al., 2013) and in biofilms growing on aquatic vegetation (Eriksson and Weisner, 1999; Eriksson, 2001), focusing mainly on photosynthesis, respiration and nutrient assimilation. With respect to N cycling, some studies have been performed on the relationship between flow conditions and denitrification. However, these studies were limited to laboratory cultivated periphyton mats (Eriksson, 2001; Arnon et al., 2007a; 2007b; Carleton and Mohamoud, 2013) and without the concomitant direct measurement of the process substrate ( $\text{NO}_3^-$ ) and end-product ( $\text{N}_2$ ), a condition necessary to validate the hypothesis that the reduced  $\text{NO}_3^-$  is actually denitrified and lost from the system. Therefore, this important issue still remains poorly explored and to the best of our knowledge, no studies have previously investigated the effects of flow velocity on N removal via denitrification in intact sediments colonized by emergent vegetation with naturally developed epiphytic communities.

This paper reports new insights on the role of flow velocity in regulating nitrogen removal via denitrification in presence of monospecific stands of *Phragmites australis* (Cav.) Trin. ex Steud. The present experiments were used to test the hypothesis that flow velocity is a fundamental physical property that regulates denitrification, although generally omitted in the parameterization of the  $\text{NO}_3^-$  mitigation capacity of slow-flow waterways and wetlands of agricultural landscapes.

## 2. Material and methods

### 2.1. Mesocosm construction and pre-incubation procedure

By working in field conditions, it is extremely difficult if not impossible, to isolate the effect of one parameter, such as water velocity, on denitrification, since other important parameters, e.g.  $\text{NO}_3^-$  availability and temperature, may vary at the same time. For instance, in shallow waterways, water temperature increases rapidly in summer daylight, affecting gas solubility and exchange with the atmosphere and making the estimation of reach-scale denitrification strenuous (Reisinger et al., 2016). To overcome this limitation, we utilized mesocosm simulations, that allow standardization of experimental conditions and systematic manipulation of a single or a limited suite of variables. Moreover, they represent a good compromise between the application of controlled conditions, typical of the laboratory approach, and realistic field measurements that allows the up-scaling of the results.

Experiments were performed using mesocosms representative of field conditions of slow-flow vegetated canals fed by  $\text{NO}_3^-$ -rich water (Pierobon et al., 2013; Castaldelli et al., 2015). Mesocosms were built using water, sediments colonised by *P. australis* and bare sediments collected, during the summer biomass peak of the plant stands, in a slow-flow ( $<10 \text{ cm s}^{-1}$ ) drainage canal ( $44^\circ 48' 53.17'' \text{ N}$ ;  $11^\circ 43' 23.14'' \text{ E}$ ) of the lower Po plain (Northern Italy). Here, denitrification and  $\text{NO}_3^-$  removal rates were previously measured by means of the  $\text{N}_2$  open-channel and N mass balance methods, both in the field, at the scale of entire reach and in laboratory mesocosms (Pierobon et al., 2013; Castaldelli et al., 2015; Soana et al., 2018). Mesocosms (Fig. 1a) were designed to simulate vegetated waterways or wetlands with moving water and were built as follows: an external Plexiglass liner with an internal diameter of 29 cm and an internal Plexiglass liner with an external diameter of 12 cm were positioned concentrically on a Plexiglass flat base. Only the volume between the two liners was filled with a sediment layer of  $\sim 35 \text{ cm}$  to define an annulus of total surface  $547 \text{ cm}^2$  (annular radius width  $8.5 \text{ cm}$ ). Sediment colonised by a dense stand of *P. australis* ( $1300\text{--}2200 \text{ g}$  of above-ground dry biomass per  $\text{m}^2$ ) and bare sediment of nearby positions were sampled using a steel shovel having a blade with a finely sharpened edge. The proposed mesocosm dimension allowed an operatively viable hand collection of the sediment by maintaining intact the rhizosphere without damaging the *P. australis* rhizomes and at the same time compatible for establishing an internal homogeneous water flow by using a 12 V submerged pump (as described below).

Three mesocosms with sediment and *P. australis* (18–30 plants in each one) and three mesocosms only with sediment were developed and transferred from the field to an outdoor, non-shaded area at the Department of Life Sciences and Biotechnology, University of Ferrara. Here, they were placed in two separate cylindrical tanks (87 cm diameter and 105 cm high polyethylene containers) (Fig. 1b), one for vegetated replicates and one for replicates devoid of vegetation, and maintained fully submerged with canal water. Water mixing among the mesocosms and between the tanks was supported by aquarium pumps. Water level in the tanks was checked every day and water from the canal was added to compensate for evapotranspiration loss. The mesocosms were allowed to equilibrate for about one month before the experiments. During pre-incubation and incubation procedures, performed according to standard protocols (Dalsgaard et al., 2000), each tank was connected with a thermostat to maintain a constant temperature at  $26\text{--}27^\circ \text{C}$ . These temperatures are typical summer values for slow-flow shallow waterways of the studied area.

### 2.2. Incubation procedure

N removal was quantified by the simultaneous measurement of  $\text{NO}_3^-$  consumption and  $\text{N}_2$  production based on analyses of  $\text{N}_2\text{:Ar}$  by Membrane Inlet Mass Spectrometry (MIMS). Three velocities ( $1.5$ ,  $3$ , and  $6 \text{ cm s}^{-1}$ ) along with a stagnant control condition were set to cover the typical range of variation in drainage canals and ditches in lowland agricultural basins, low-gradient waterways resulting from flat topography, low soil permeability and slopes of a few centimetres per kilometre. The experiment consisted in a total of eight incubations, i.e. four velocity levels ( $0$ ,  $1.5$ ,  $3$ , and  $6 \text{ cm s}^{-1}$ ) were tested in two light conditions (dark: from 3:00 a.m. to 6:00 a.m., light: from 10:30 a.m. to 01:30 p.m.) to discriminate the effect of photosynthetic processes on N removal. This procedure was repeated two times in the middle-late summer, i.e. during two periods of four consecutive days (from 25 July 2016 to 28 July 2016 and from 22 August 2016 to 25 August 2016), during stable meteorological conditions in order to minimize any unpredictable

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