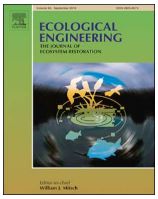
Ecological Engineering 113 (2018) 1–10

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



To mow or not to mow: reed biofilms as denitrification hotspots in drainage canals

Elisa Soana*, Anna Gavioli, Elena Tamburini, Elisa Anna Fano, Giuseppe Castaldelli

Department of Life Sciences and Biotechnology, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy

ARTICLE INFO

Keywords:
Biofilms
Nitrate removal
Denitrification
N_2 open-channel method
Water velocity
Canal network

ABSTRACT

In shallow-water systems with calm hydrodynamic, dense vegetation stands provide most of the available surface for periphyton development. The large ratio between biological active surfaces and water volume amplifies the influence of biofilm activity on water chemistry, resulting the key factor responsible for nitrogen removal performance of wetlands and waterways. However, the denitrification capacity of biofilms on emergent macrophytes remains understudied, especially if investigated on dead stems during the non-vegetative season.

The aims of the present study were: 1) to quantify the role of biofilms colonizing dead stems of *Phragmites australis* in NO_3^- mitigation via denitrification in winter (~11 °C) in a NO_3^--rich drainage canal; 2) to determine how the biofilm denitrifying capacity varies as a function of water velocity (0–6 $cm\,s^{-1}$). Denitrification was assessed by the concomitant measurements of NO_3^- consumption and N_2 production from analyses of N_2:Ar by Membrane Inlet Mass Spectrometry.

Sediments with biofilms were found more efficient in converting NO_3^- to N_2 (7–17 $mmol\,N\,m^{-2}\,d^{-1}$) than bare sediments (3–5 $mmol\,N\,m^{-2}\,d^{-1}$). Denitrification activity in biofilms responded positively to increasing water velocity that enhanced the rate of NO_3^- supply to the active surfaces.

Results of the present study showed that denitrification performed by biofilms on senescent stems proceeds beyond the vegetative season throughout the cold period and maintains the depuration capacity when drainage canals may still drive high NO_3^- loads leached from the agricultural fields. The development of a diversified and extended microbial community throughout the year together with water velocity should be taken into account as key elements in the management of the canal networks aimed at combining hydrological needs and water quality goals.

1. Introduction

Nitrogen (N) cycling in shallow aquatic environments is strongly regulated by the interactions between microbial communities and aquatic plants. It has commonly been observed that sediments colonised by emergent macrophytes mitigate nitrate (NO_3^-) loads more effectively than unvegetated ones, improving the N removal performances of natural and artificial aquatic ecosystems (Weisner et al., 1994; Faulwetter et al., 2009; Soana et al., 2017). Macrophytes control inorganic N availability both directly, by assimilation, and indirectly by affecting the coupling among ammonification, nitrification and denitrification, mainly due to oxygen injection into the rhizosphere via the aerenchyma and exudation of organic carbon from roots (Vila-Costa et al., 2016; Rehman et al., 2017). Aquatic plants may also drive N dynamics because submerged portions like stems and leaves provide large amounts of additional surfaces for the growth of periphyton, considered hot spots of N transformations (Toet et al., 2003; Pang et al., 2016). Periphyton (also "biofilms") is an ubiquitous complex matrix of living (algae, bacteria, and other micro-organisms, such as fungi and protozoa) and non-living components (organic detritus, deposited fine sediments, polymeric substances secreted to hold the biofilm together) attached to various submerged substrates in the photic zones of aquatic ecosystems (Wetzel, 1983; Roeselers et al., 2008). Referred as epiphyton when colonizing plant tissues, it is an ensemble of autotrophs and heterotrophs whose synergic metabolism results in the estabilishment of proximate oxic and anoxic micro-niches enabling the co-occurrence of contrasting redox processes at a small spatial scale, e.g. coupled nitrification/denitrification (Eriksson, 2001; Revsbech et al., 2005; Zhang et al., 2016).

Due to limited oxygen penetration, the benthic compartment is commonly considered the elective site for denitrification. However, a number of researchers have shown that biofilms attached to submerged plant surfaces, although highly variable in species composition and coverage, may sustain denitrification activity with rates comparable or

---

* Corresponding author.
E-mail address: elisa.soana@unife.it (E. Soana).

https://doi.org/10.1016/j.ecoleng.2017.12.029
Received 11 August 2017; Received in revised form 28 November 2017; Accepted 21 December 2017

even higher than in sediments, making a significant contribution to the overall NO_3^- removal at the ecosystem level in nutrient-rich environments. Eriksson and Weisner (1997) demonstrated that also old stems of submerged plants were sites as active as sediment for denitrification in a eutrophic wetland, due to leakage of nutrients from the senescent tissues and a shift from an autotrophic to a heterotrophic-dominated biofilm as it aged. Similarly, Toet et al. (2003) reported that on a daily basis the main contribution to the NO_3^- removal capacity of a wetland during winter was ascribed to biofilms on old stems of *Phragmites australis* that maintained denitrification rates comparable to those measured in periods with more favourable temperatures. Although rates are usually strongly affected by the intertwined action of several chemico-physical and biological drivers (e.g. nutrient level, hydraulic conditions, redox status, abundance of vegetation, periphyton biomass), the key elements promoting the biofilm denitrifying activity seem to be the supply of NO_3^- from the water column and of labile organic carbon produced by the periphytic algae, with a weak effect of temperature (Toet et al., 2003; Srivastava et al., 2017).

NO_3^- removal rates faster than diffusion from the surrounding waters may represent an important constraint for denitrification. Laboratory experiments aimed at investigating how variable velocities affect denitrification in benthic and epiphytic biofilms revealed that the process is regulated by the complex interplay between the transport of NO_3^- to anoxic niches and the overall redox status of the biological layers mediated by the water flow (Eriksson, 2001; Arnon et al., 2007a). Increasing velocity tends to enhance denitrification rates by the action of continuous mixing of NO_3^--poor water layers around uptake surfaces and overlying NO_3^--rich water, a condition that ensures a constant supply to the microbial community (Sirivedhin and Gray, 2006). However, being facultative anaerobes, denitrifiers shift to aerobic metabolism when oxygen is available, thus denitrification may be suppressed when biofilms become well flushed with oxygen as a conseguence of high hydrodynamic conditions (Arnon et al., 2007b; O'Connor and Hondzo, 2007).

In shallow-water systems with calm hydrodynamic, macrophytes generally provide most of the accessible surface for periphyton development, leading to a large ratio between the biologically active surfaces and the water volume and amplifying the influence of biofilm activity on water chemistry (Wetzel and Søndergaard, 1998). Although epiphytic biofilms are usually implied in the majority of organic matter processing and biogeochemical cycles, and they are thus responsible of the nutrient removal performance of wetlands and waterways, very few studies have provided quantitative estimations of the denitrification capacity in periphyton on emergent plants (Toet et al., 2003; Venterink et al., 2003; Yamamoto et al., 2005). In almost the totality of the cases, the process was evaluated in terms of potential rates obtained in non limitig conditions, whose extrapolation up to the ecosystem level is usually tricky and not reflecting the actual in field rates. The relationship between water velocity and denitrification has been scarcely investigated in biofilms on submerged macrophytes (Eriksson, 2001), and no studies are available for biofilms on emergent plants performed in intact freshwater sediments by the simultaneous measurements of NO_3^- consumption and N_2 production. Furthermore, very little attention has been paid to evaluate if NO_3^- mitigation by periplyton on dead stems of emergent vegetation is maintained also during the non-vegetative season when large loads of nutrients are carried by rivers and canals in agricultural landscapes.

The aims of the present study were: 1) to quantify the role of biofilms colonizing dead stems of *Phragmites australis* in NO_3^- dissipation via denitrification during winter season in a drainage canal affected by non-point source pollution; 2) to determine if biofilm denitrifying capacity varies as a function of water velocity. N removal was quantified in laboratory mesocosms simulating slow-flow waterways by the simultaneous measurement of N_2 production (N_2:Ar open-channel method) and NO_3^- consumption. Three velocities (1.5, 3, and 6 $cm\,s^{-1}$) and the condition with no flow were set to cover the typical range of flow variation in canals and ditches of lowland agricultural basins.

By working in nature, it is generally extremely difficult to isolate the effect of a single variable (e.g. water velocity) on denitrification, since other important regulating factors, such as NO_3^- concentration and water temperature, may vary at the same time. The mesocosm experimental design represents a reproducible alternative to *in situ* studies where elements of variability of the canal stretches are eliminated and the tested variable may be systematically manipulated.

2. Material and methods

2.1. Mesocosm construction and pre-incubation procedure

Mesocosms were built using water, bare sediment and sediment with dead stems of *P. australis* colonized by periphyton, sampled in winter in a slow-flow ($< 10\,cm\,s^{-1}$) canal (44°48′53.17“N; 11°43′23.14“E) where denitrification and NO_3^- removal rates were previously measured by N_2:Ar and N mass balance methods (Castaldelli et al., 2015). The study site belongs to the Po di Volano basin ($\sim 2600\,km^2$, Northern Italy), an intensively cropped deltaic territory characterized by a flat topography and an extensive network of drainage and irrigation ditches. Here, natural propagation and seasonal evolution of aquatic vegetation are strongly affected by yearly maintenance operations aimed at preserving hydraulic performance. Thus, stands of common reed (*Phragmites australis* Cav. Trin. ex Steud), cattail (*Typha latifolia* L.) and great manna grass (*Glyceria maxima* Hartm. Holmb.), usually monospecific, are maintained only in rare isolated stretches.

Mesocosms were designed to simulate vegetated waterways or wetlands with moving water and built as follows (Fig. 1): an external Plexiglass tube with internal diameter of 29 cm and an internal tube with external diameter of 12 cm were positioned concentrically on a Plexiglass circular base, to define an annulus of total surface 547 cm^2 (annular radius width 8.5 cm). Parcel of sediments were sampled using

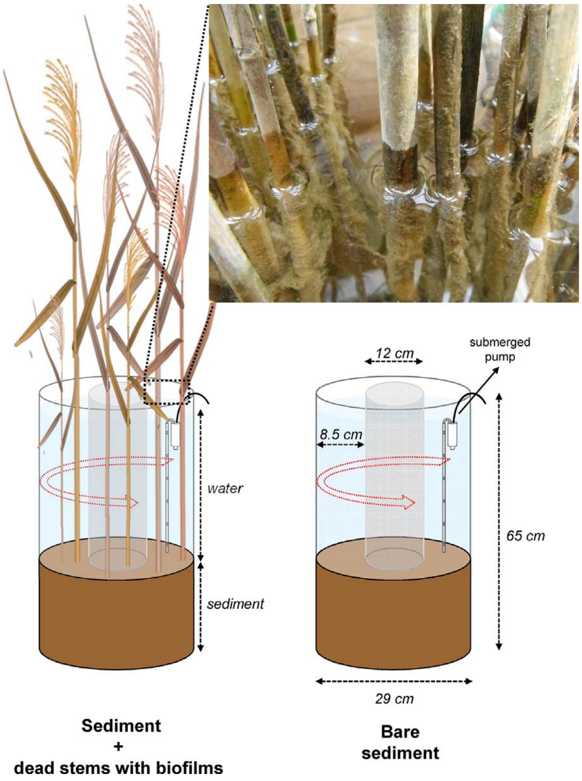


Fig. 1. Scheme of the experimental mesocosms.