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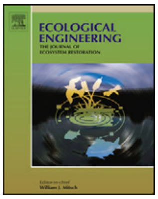

Wetland plants, micro-organisms and enzymatic activities interrelations in treating N polluted water

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ABSTRACT

This study compared the efficiency of five emergent plant species (*Carex elata* All., *Juncus effusus* L., *Typhoides arundinacea* (L.) Moench var. picta, *Phragmites australis* (Cav.) Trin. and *Typha latifolia* L.) used for the decontamination of nitrogen polluted water at mesoscale level. The correlations between N removal and microorganisms content and activity on root and gravel surfaces were also evaluated in order to broaden the knowledge about water purification processes.

The experiment was conducted in plastic tanks for two years (2008–2009), performing drying/wetting application cycles with NH_4NO_3 solution.

At the end of the experiment, root and gravel samples were collected from each tank for the measurement in aqueous extracts of total cultivable microbial population and *Pseudomonas* genus, and β-glucosidase, chitinase, phosphatase and leucine aminopeptidase activities; other base chemical analyses (electrical conductivity, water soluble carbon, nitrate and ammonia) were conducted.

The production of bio-available root exudates was evaluated though the quantification of water soluble carbon (WSC) in root and gravel washing water. WSC seems to support the plant denitrification activity because *T. arundinacea* and *Ph. australis* systems, which showed the highest WSC content, achieved the highest denitrification percentages (37% and 34%, respectively). In these plant systems the higher cultivable microbial population and rhizospheric hydrolytic activity found were probably induced by the high content of soluble organic substrates that derived from root turnover and root exudation, without addition of any other carbon source. The establishment of this active micro-environment (*rhizo-biospace*) has high ecological significance due to the role played by microbial activity in the nutrient cycles and is of great importance when choosing suitable plant species for wastewater treatment.

1. Introduction

Nitrogen is one of the most important nutrients for organisms. The excessive accumulation of nitrogen discharged into water due to urbanization and intensive farming can cause serious ecological problems. Ammonia, in particular, is an undesirable constituent of many wastewaters because of its toxicity to fish and its significant, microbial-induced, oxygen demand on the receiving water body (Camargo and Alonso, 2006). In addition, ammonia and nitrate stimulate biological productivity, which can ultimately lead to biomass decay and dissolved oxygen depletion (eutrophication process).

Constructed wetlands (CWs) are nowadays a low cost, efficient wastewater treatment and widely employed technology to treat agricultural, municipal and industrial wastewaters (Kadlec and Wallace, 2008).

Treatment behaviour in CWs is often considered to be a figurative black-box (Rousseau et al., 2004) where the interactions between vegetation, water and microorganisms are little known (Toscano et al., 2009).

The plants have many important functions in nitrogen removal in CWs, such as direct uptake of nitrogen for their growth, providing a surface and a carbon source for the growth of microbial communities, transferring oxygen from air to the medium, decreasing water

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speed and reducing its volume, stabilizing the bed, isolating the surface against frost in winter (Vymazal, 2002).

Different studies have shown that the nutrient removal capacities of plants differ, so plant selection is critical in the implementation of constructed wetlands (Brisson and Chazarenc, 2009; Vymazal and Kropfelova, 2009; Salvato and Borin, 2010). However, it remains unclear how these nutrient removal differences occur between plants with comparable size and growth form (Brisson and Chazarenc, 2009). Nutrient removal ability of wetland plants is reported to be correlated with biomass (Tanner, 1996), growth speed and growth rhythm (Cheng et al., 2009a), root morphology and distribution (Amon et al., 2007; Cheng et al., 2009b; Iannelli et al., 2011). Moreover, plants can excrete exogenous enzymes and release exudates and oxygen into the rhizosphere that affect the composition and diversity of the microbial population and thus, indirectly, enzyme activity (Kong et al., 2009; Brisson and Chazarenc, 2009; Faulwetter et al., 2009).

Measurements of enzyme activity can be used as indices of microbial biomass and microbial ability to study biogeochemical reactions (Sinsabaugh, 1994; Ajwa et al., 1999). Soil enzyme activity has been proposed as being an important aspect of soil quality (Alef and Sparling, 1995) and, in the context of CWs, of water quality improvement in wetland systems (Shackle et al., 2000). There is a general consensus that studying enzymes involved in decomposition may provide valuable information about the cycling of nutrients in ecosystems (Rejmankova and Sirova, 2007). It has also been suggested that root growth status and activity might have a more important effect than root biomass on enzyme activity in constructed wetlands; Kong et al. (2009) found many significant positive correlations between enzyme activity and root activity.

The aim of this work is to deepen the knowledge about water purification processes by evaluating the influence of interaction among microorganisms, enzyme activities and plant roots in N removal efficiency in CW systems with different emergent plant species. The studied macrophytes were managed with drying/wetting cycles. This management is important in increasing enzyme activities (Burns and Ryder, 2001; Corstanje and Reddy, 2004), and stimulating denitrification in wet cycles and increasing nitrification in dry cycles (Qiu and McComb, 1996; Tanner et al., 1999; Eaton, 2001; Venterink et al., 2002).

2. Materials and methods

2.1. Experimental layout

The experiment, at mesocosm scale, was conducted in the open air from May to November 2008 and from April to September 2009. It consisted of plastic tanks filled with gravel: grain size 0.1–10 mm (d_{10} = 3.9 mm; d_{60} = 5.2 mm) with porosity of 25%. The tanks, 50 cm × 40 cm × 29 cm in length, width and height respectively, had a vertical PVC pipe installed in the centre in order to control the water level, a tap at the outflow to collect the treated effluent and a lateral pipe and a graduated container to collect excess water in the case of heavy rainfall events (Fig. 1). Five plant species were tested in quadruple: *Carex elata* All. (*Cae*), *Juncus effusus* L. (*Jue*), *Phragmites australis* (Cav). Trin (*Phr*), *Typhoides arundinacea* (L.) Moench (syn. *Phalaris arundinacea* L.) var. picta (*Pha*), and *Typha latifolia* L. (*Ty*). Four mesocosms without plants were set up as controls. Planting was done in March 2008, at 30 plants/m^2 arranged in a completely randomized scheme. Each tank was filled once or twice a month with 60 L/m^2 of artificial wastewater so as to saturate the gravel bed.

One week after the wastewater application, each tank was drained by opening the tap. The treated effluent was collected, measured and analyzed for NO_3-N and NH_4-N concentrations. In the case of heavy rainfall events, the water collected in the graduated container under each tank was taken into account in the calculation of removal efficiency. Clean water was added to the tanks in order to replace the initial volume of 60 L/m^2 and maintained until the next application of artificial wastewater (for one or three weeks). The artificial wastewater was produced by dissolving ammonium-nitrate salts in fresh water at concentrations that did not cause any possible adverse effect to the selected plant species.

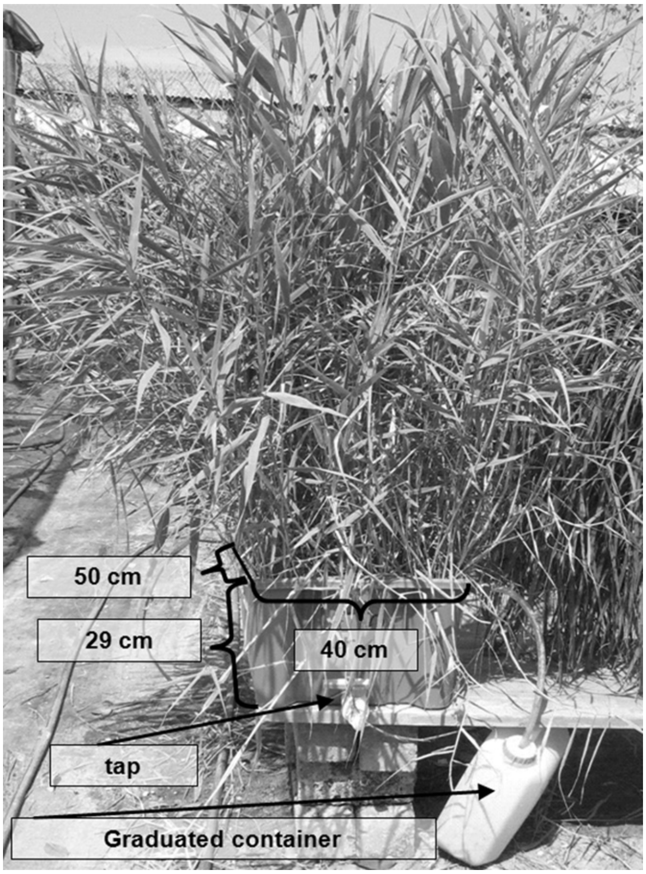

Fig. 1. Scheme of a vegetated mesocosm.

From May 2008 to September 2009, 17 weekly cycles were performed with an average input concentration of NH_4NO_3 ranging from 205 to 407 mg L^{-1}. In detail in 2008 seven monthly loading cycles with an average input concentration in NO_3-N of 105 mg L^{-1} of and in NH_4-N of 100 mg L^{-1} (cumulative load of 86 g N m^{-2}) were carried out. In 2009 two loads in April and May were of 104 mg L^{-1} of NO_3-N and 119 mg L^{-1} of NH_4-N, afterwards the input concentration was doubled (average NO_3-N 200 mg L^{-1} and 207 mg L^{-1} NH_4-N) for others 8 cycles (cumulative load of 222 g N m^{-2}). The total nitrogen load applied was of 308 g m^{-2}. Details of the nitrogen application are given in Table 1. The tanks

Table 1
Details of the artificial wastewater cycles and N input per cycle.

Period	Number of cycles	Length of cycle	N input per cycle (mg L^{-1})
09/05/08 06/11/08	7	Weekly	205
17/04/09 22/05/09	2	Weekly	223
29/05/09 17/09/09	8	Weekly	407