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Research article

Role of plants in nitrogen and sulfur transformations in floating hydroponic root mats: A comparison of two helophytes

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ABSTRACT

Knowledge about the roles helophytes play in constructed wetlands (CWs) is limited, especially regarding their provision of organic rhizodeposits. Here, transformations of inorganic nitrogen and sulfur were monitored in a CW variety, floating hydroponic root mat (FHRM), treating synthetic wastewater containing low concentration of organic carbon. Two helophytes, *Phragmites australis* and *Juncus effusus*, were compared in duplicates. Striking differences were found between the FHRM of the two helophytes. Whereas ammonium was removed in all FHRMs to below detection level, total nitrogen of 1.15 ± 0.4 g m^{-2} d^{-1} was removed completely only in *P. australis* systems. The mats with *J. effusus* displayed effective nitrification but incomplete denitrification as 77% of the removed ammonium-nitrogen accumulated as nitrate. Furthermore, the *P. australis* treatment units showed on average 3 times higher sulfate-S removal rates (1.1 ± 0.45 g m^{-2} d^{-1}) than the systems planted with *J. effusus* (0.37 ± 0.29 g m^{-2} d^{-1}). Since the influent organic carbon was below the stoichiometric requirement for the observed N and S transformation processes, helophytes' organic rhizodeposits apparently contributed to these transformations, while *P. australis* provided about 6 times higher bioavailable organic rhizodeposits than *J. effusus*.

1. Introduction

The different roles helophytes play in wastewater treatment represent one of the vital questions in constructed wetlands (CWs) research. The most important benefits from helophytes in CWs were firstly thought to be the physical functions they provide, such as bed surface stabilization, conditions for physical filtration, clogging prevention, frost insulation and provision of surface for attached microbial growth (Brix, 1994). However, other benefits exist in addition to these physical effects, including the direct uptake of some contaminants, helophyte-mediated oxygen transfer to their rhizosphere and the provision of root exudates (Brix, 1997; Stottmeister et al., 2003). Microbial transformations are of the most important processes that contribute to pollutant removal in CWs (Kadlec and Wallace, 2008), yet the roles helophytes play in supporting the microbial communities are not sufficiently understood.

The rhizodeposits can constitute an important component of the total carbon balance in CWs, especially where organic carbon is limited in the contaminated waters intended for treatment. Firstly introduced by Whipps and Lynch (1985), the term rhizodeposition includes all organic materials originating from the roots. It is divided into five components, depending on their nature and method of formation: exudates (water soluble compounds, e.g. sugars and amino acids); secretions (e.g. low molecular weight compounds which are released metabolically); mucilages (polysaccharides); mucigel; and lysates (compounds released by lysis of cells). These five components were classified by Cheng and Gershenson (2007) into two main groups: water soluble exudates, including the first component; and water insoluble materials, comprising the last four components. Initially, the soluble portion of rhizodeposits, i.e. the soluble exudates, caught more attention since it encompasses substrate directly available to microorganisms. In recent years, an increased interest has been dedicated also to the insoluble organic materials produced by roots, which represent a potential substrate for rhizosphere

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microorganisms (Newman et al., 1985; Whipps, 2001).

Investigations on crop plants have shown that rhizodeposition differs between various plant species. In addition, the compounds released by an individual plant can vary significantly in quality and quantity over time and space (van Veen et al., 2007). Wetland plants are expected to vary in their rhizodeposition regimes analogously, yet precise information on the total extent as well as species-variations in rhizodeposition from helophytes is hard to obtain experimentally. So far, occasionally large differences in pollutant removal efficiencies of CWs planted with different species were noted (Brisson and Chazarenc, 2009). Hence, additional information is needed to explain these noted differences.

This investigation aimed at elucidating differences in the rhizodeposition capacities among helophytes via monitoring the pollutant transformations that may depend on organic carbon availability. Specifically, the turnover of inorganic nitrogen and sulfur compounds was monitored and the stoichiometric requirement of organic carbon for the processes of denitrification and dissimilatory sulfate reduction (DSR) was estimated. A special type of CWs, floating hydroponic root mats (FHRMs), was selected. FHRMs represent a variant of CWs in which helophytes grow as floating mats on the water surface, thus are not rooted in any media such as soil or sediment. They may be described as a hybrid between a pond and a wetland, as they share aspects of both systems (Chen et al., 2016; Headley and Tanner, 2008). The FHRMs allow better root development than soil-based systems as roots do not need to compete for space with the substratum and they allow a maximum contact between the roots and the contaminated water. This guarantees obtaining the highest benefit from the plants and their intensified root structures. FHRMs are being used for storm water management, treatment of combined sewer overflows, and numerous other types of contaminated waters (Chen et al., 2014; Headley and Tanner, 2008; Seeger et al., 2013; Richter et al. in press). (Smith and Kalin, 2001) applied FHRMs for the treatment of acid mine drainage (AMD) and found numerous benefits in selecting them over conventional soil-based CW systems for AMD treatment as well as for suspended solids elimination.

Two helophytes, *Phragmites australis* (common reed) and *Juncus effusus* (common or soft rush), were selected in this study since they fit the following criteria: they are commonly applied in CWs; they are frequently investigated; and they have different morphologies of both above-ground and below-ground components. FHRMs of these two species were set in a greenhouse, fed with synthetic wastewater loaded with low organic carbon and sampled weekly within a six-month period at inflow, outflow and internal points. The following questions were posed: firstly, how do the nitrogen and sulfur transformations vary; secondly, how does the organic carbon input from the two helophytes vary (estimated from the observed nitrogen and sulfur transformations); and thirdly, what does the observation imply for the selection of a helophyte for a given treatment process?

2. Material and methods

2.1. Experimental setup

Mature plants of *P. australis* or *J. effusus* were grown in duplicate in pilot-scale FHRMs inside a greenhouse in which the internal environment was not controlled. The 4 CW systems were made of metal containers with dimensions length × width × depth of: 100 × 15 × 35 cm. The water level was adjusted to 27 cm via an overflow control (Fig. 1). The systems were operated as FHRMs at the start of the experiment. However, the vigorous plant growth that took place within the experimental timeframe resulted in the roots of both species filling the whole submerged depth of the wetlands and eventually transforming the systems into dense root mat filters (RMFs) rather than FHRMs. For the purpose of presenting the output of this experiment, the systems will be referred to as FHRMs since it could not be precisely decided when the transformation into non-floating RMFs took place.

In order to insure a uniform distribution of the wastewater and to promote a plug-flow regime, sieves of perforated stainless steel were placed 3 cm in front of the inflow and outflow of each system to create plant-free zones. The plants were allowed to acclimatize in the containers for 6 months with tap water and nutrients and for further two and a half months with the synthetic wastewater prior the start of the sampling campaign. The synthetic wastewater was prepared essentially as described before (Wiessner et al., 2010) but omitting organic compounds. The resulting wastewater composition was (in mg/L, dissolved in deionized water): 118.0 NH_4Cl (corresponding to 30.9 NH_4^+-N; the actual measured NH_4^+-N concentration at the inflow tank was 26 ± 7, the variation was attributed to occasional non-ideal mixing of the inflow water); 36.7 $K_2HPO_4.3H_2O$ (corresponding to 5.0 $PO_4^{3-}-P$); 7.0 NaCl; 3.4 $MgCl_2.6H_2O$; 4.0 $CaCl_2.2H_2O$; 221.8 Na_2SO_4 (corresponding to 50.0 $SO_4^{2-}-S$) and a trace mineral solution after Kuschk (1991). Although no organic carbon compounds were added to the feed wastewater, limited concentrations of dissolved organic carbon (DOC) were detected in the feed tanks. This was recognized as resulting from impurities in the chemicals used for the preparation of the wastewater and partially coming from the inert plastic material of the tanks.

A buffer solution was added to the synthetic wastewater (420 mg/L $NaHCO_3$, corresponding to 5 mM HCO_3^-) to neutralize the acidification associated with nitrification which was observed in FHRMs of *J. effusus* in a previous experiment (data not shown) and estimated to affect the integrity of the plants' physiological status. The synthetic wastewater was freshly prepared twice a week and purged with a high-flow of oxygen-free N_2 gas for 15 min. The inflow tanks were kept closed at all times to minimize air intrusion. In addition, the inflow tanks were thoroughly washed every two weeks to limit microbial growth inside the tanks to hinder possible nitrification of the feed solution prior to entering the root mats.

The sampling of the well-established FHRMs started in June 2013 and was concluded early December 2013. The pore-volume was estimated at the start of April 2013 for all 4 systems and was found to be 25 ± 3 L for each wetland. The inflow rate was maintained between April and June at 5 L/d (hydraulic load of 33.3 L m^{-2} d^{-1}), corresponding to a nominal hydraulic retention time (nHRT) of about 5 days. As the season progressed, the water loss from the *P. australis* systems was so high that it led to zero-discharge; hence the flow rate was increased gradually in July and August to up to 8 L/d (53.3 L m^{-2} d^{-1}). This flow regime was applied to all 4 systems to assure equivalence of the operating conditions, even though the *J. effusus* root mats were not at zero-discharge at any time. The flow rate was then gradually reduced as the water loss from the *P. australis* plants decreased towards the end of the year (Fig. 2).

2.2. Sampling and analysis

Sampling was carried out on a weekly basis. For the quantification of the physical-chemical parameters, pore-water samples were collected from the 4 systems at distances 0, 25, 50, 75 and 100 cm from inflow and at depth 12.5 cm from the water surface. For the *P. australis* units, additional two depths of 5 and 22 cm were sampled (Fig. 1) to elaborate on the depth profiles of the reduced sulfur compounds. Pore-water was withdrawn using a peristaltic pump at a slow rate through stainless steel lances of 3.5 mm inner diameter which were inserted in each sampling point. The sample