Use of two spatially separated plant species alters microbial community function in horizontal subsurface flow constructed wetlands

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ABSTRACT

Both the presence and diversity of plants are integral to the development and functional abilities of microbial communities within constructed wetlands (CWs). The aim of this study was to assess the impacts of different individual and paired plant species combinations on microbial community function in HSSF mesocosm CWs. Experimental systems were quadruplicated and operated as two mesocosms in series planted with *Phragmites australis* (X) or *Phalaris arundinacea* (O), giving four possible sequential combinations (XX, XO, OX, OO). Wastewater was loaded each day into position 1 mesocosm with the outflow entering position 2 mesocosm, and the corresponding position 2 mesocosm outflow representing an overall experimental system effluent. The metabolic function of the interstitial-based microbial communities within each of the 16 mesocosm pairs was assessed at a single time point in the spring (May) based on community-level physiological profiles (CLPPs) gathered using Biolog® EcoPlates. Microbial activity and metabolic richness (number of carbon sources utilised) were found to be higher in position 1 mesocosms compared to position two. Microbial community carbon source utilisation patterns (CSUPs), overall activity and metabolic richness were similar between all mesocosms from position 1—irrespective of plant species. When assessing microbial communities in position 2 of each pairing a greater variety of CSUPs, activities and metabolic richness' could be found suggesting the sequence and choice of plant can alter the microbial community function in the HSSF mesocosms. Of particular interest were the mesocosm/plant species combinations containing *Phalaris* in the position 2 which led to higher overall microbial activity and richness, distinct carbon source utilisation patterns (CSUPs) and an increased utilisation of specific carbon sources further from the inlet. More specifically the XO pairings (*Phragmites-Phalaris*) seemed to offer the most promising overall microbial function throughout both positions 1 and 2 in series suggesting plant diversity may help enhance microbial community function, and therefore microbial based water treatment capacity. The findings also suggest that the microbial communities associated with each plant species respond differently to factors such as nutrient availability, and although not yet clearly defined, further highlights the potential for improved or tailored water treatment in CWs through selection of specific plant species and combinations. Broader microbial community function or greater activity did not correlate with improved water treatment efficiency in this study. This may have been due to the relatively low contaminant loads utilised, or the limited amount of data collected. Further study across several seasons or with higher contaminant loads is recommended.

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1. Introduction

Constructed Wetlands (CWs) are an increasingly popular method for wastewater treatment due to their low cost, ease of use, provision of wildlife habitat and success in treating a wide range of water pollutants from a vast array of contaminated water sources (Faulwetter et al., 2009). One of the most widely employed designs is the Horizontal Sub-Surface Flow (HSSF) CW, typically consisting of a rectangular bed lined with an impermeable membrane and commonly planted with *Phragmites australis* or *Phalaris arundinacea* (Vymazal, 2005). The overall efficacy of CWs for wastewater treatment is determined by a combination of wetland design, vegetation type and microbial processes. Plants play a vital role within CWs mainly as a promoter of microbial community development by

providing structural support for microbial community attachment to root systems, oxygen transfer from aerial tissues into the rhizoshpere and through the secretion of root exudates containing a diverse assortment of enzymes and carbon-containing metabolites (Bais et al., 2006; Weber and Gagnon, 2014). These root exudates may be beneficially utilised by microorganisms for both growth and the development of a biofilm consisting of a microbial community bound together in a matrix of extracellular polymeric substances (EPS). The biofilm is where the majority of contaminant biodegradation takes place within CWs (Faulwetter et al., 2009; Iasur-Kruh et al., 2010; Weber and Gagnon, 2014) and provides a suitable habitat for the development of plant growth promoting rhizobacteria (PGPR) which enhance plant growth via the provision of inorganic nutrients and growth promoting substances (Iasur-Kruh et al., 2010). In this way plants and microbial communities in CWs are inherently interdependent with changes in one affecting the other. Until recently CW research dealt primarily with aspects of technological design with the main issue of concern being inlet and outlet loads and the internal active zones of microbial community activity viewed as a 'black box' (Stottmeister et al., 2003). However with recent advancements in our understanding of the central role of microorganisms to the biogeochemical processes in CWs it is now generally accepted that to improve the performance of constructed wetlands it is essential to have an understanding of the functional diversity and metabolic properties of the intrinsic microbial community (Deng et al., 2011).

Plant species differ in terms of growth rates, root morphology, production of root exudates, and oxygen transfer, opening the possibility of microbial community characteristics being specific to plant species. For example, in a study on bacterial dynamics in an HSSF CWs at Nucice, Czech Republic, Vymazal et al. (2001) reported significantly more bacteria on the roots of *Phragmites* by comparison to *Phalaris*. A more recent study on the influence macrophyte species on microbial density and activity in constructed wetlands found conversely that significantly elevated levels were associated with *Phalaris* in comparison to *Typha* and *Phragmites* (Gagnon et al., 2007). Distinct microbial communities were also correlated to *A. donax* and *Sarcocornia* sp. in an HSSF CWs used to treat high salinity industrial wastewater (Calheiros et al., 2010). Increased species (plant, animal, microbial) richness is known to lead to greater functional diversity and improved ecological stability (Hooper et al., 2005; Peterson et al., 1998; Tilman et al., 1996) providing for a more resilient and healthy ecosystem. In the context of treatment wetlands it is hypothesized that through capitalizing on the complimentary nature of different plant species, greater plant diversity can lead to greater microbial functional diversity, and possibly an enhancement in the microbial community function in terms of water treatment abilities. This theory has not yet been demonstrated conclusively in CWs and the few studies that have addressed the issue of plant diversity have produced mixed results with the recommendation that the effect of plant species diversity on microbial community functional diversity be studied further. Zhang et al. (2010) looked at the effects of plant diversity on microbial biomass and metabolic profiles, and found that increased plant species richness was correlated to increased microbial biomass but not diversity. A later study by the same authors reported that plant functional group richness, as opposed to plant species richness, exerted little effect on most microbial communities in the substrate of the studied VFCW with the exception of fungal abundance but did significantly impact several microbial enzyme activities (Zhang et al., 2011). When looking at four different free-floating plant species in mono, bi, and quadri-cultures Bissegger et al. (2014) found that increasing the number of plant species did not promote the development of microbial communities with a more active and diverse metabolic capability, but did find that plant/plant inter-

actions were important on defining the carbon source utilisation patterns CSUPs as collected with Biolog® EcoPlates.

Community-level physiological profiling (CLPP) has proven to be a useful tool for the assessment of microbial community function and functional diversity in both terrestrial and aquatic environments (Frac et al., 2012; Weber and Legge, 2013; Zhang et al., 2010). The method utilizes Biolog® EcoPlates containing 31 sole carbon sources plus a blank well (no C source) in triplicate. Carbon sources contained on the EcoPlate (Table 1) are designed to be ecologically relevant and diverse with many commonly found in plant root exudates (Campbell et al., 1997). An overall community-level physiological profile based on pattern and rate of utilisation of the 31 carbon sources is generated giving an indication of the community functional abilities. One of the strengths in using CLPP is the large amount of information which is obtained. Authors frequently discount the majority of information which is gained, focusing on the differentiation of microbial communities based on the CSUPs. Wastewater composition itself varies depending on origin but is most often a complex mixture of compounds, with many of those compounds containing carbon. The diverse set of carbon sources found on the BIOLOG® EcoPlate have varying compositions and can be separated into carbohydrates, polymers, carboxylic acids, amins/amides, and amino acids. Although not exhaustive the 31 different carbon sources on the BIOLOG® EcoPlate represents a fairly large range of compounds. By evaluating the degree with which specific compounds are utilised on the plate, a relative understanding of the water treatment potential of the microbial community being evaluated can be gained (Button et al., 2015b). In this way the CLPP method can be used to more closely evaluate and represent the water treatment potential of a specific microbial community (originating from a treatment wetland or other water treatment technology) for a range of compounds.

The objective of the present study was to assess the impacts of different individual and paired plant species combinations on microbial community function in HSSF CWs. Microbial community function, rather than structure, was chosen for study in order to more closely connect findings to CW water treatment potential. Mesocosm systems planted with *P. australis* (X) and/or *P. arundinacea* (O) were operated under four possible sequential combinations (i.e. XX, OO, OX, XO), and the function of the intrinsic microbial communities assessed via CLPP.

2. Methods

2.1. Experimental set-up and sampling

The experiment was carried out in a controlled greenhouse at the Montreal Botanical Garden, Québec (Canada). Sixteen mesocosm scale experimental constructed wetlands were set-up and operated for 12 months (Jul. 2012-Jul. 2013). The temperature of the greenhouse ranged from 35 °C in summer, 15 °C in autumn and spring, and 5 °C in winter. Each experimental unit consisted of two mesocosms connected in series (L 70 cm W 51 cm H 36 cm) filled with granitic river gravel (∅ = 10–15 mm) giving an interstitial/free water volume of 24L. To facilitate water distribution, each experimental unit had a 0.1 m section at the inlet with granitic coarse gravel (∅ = 30–40 mm). The mesocosms were planted according to each of the following four treatments: monocultures of *Phragmites* (XX) and *Phalaris* (OO) and the combination of the two plant species, *Phragmites* followed by *Phalaris* (XO), as well as *Phalaris* followed by *Phragmites* (OX). Each treatment was replicated four times following a randomized block design (Fig. 1). Following plant establishment (from spring 2009 to spring 2012), the mesocosms were fed from Apr. 2012 to Aug. 2013 with 15 L d^{-1} of reconstituted wastewater from diluted fish farm sludge, urea (20.2 mg L^{-1})