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Microbial community metabolic function in subsurface flow constructed wetlands of different designs

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ABSTRACT

Microorganisms are central to the biogeochemical processes in constructed treatment wetlands. In this study we explored the spatial dynamics of microbial community metabolic function in several different constructed treatment wetland designs at the pilot-scale constructed wetlands of the UFZ Ecotechnology Research Facility in Langenreichenbach, Germany. The constructed wetland designs differed in terms of flow direction, degree of saturation, depth, and intensification. Internal sampling was conducted in April 2013 at different locations along the flow path of each system. The microbial community metabolic function was characterized via community level physiological profiling (CLPP). Microbial community activity (substrate utilization rate) and metabolic richness (number of carbon sources utilize) decreased dramatically with increasing distance along the flow path for most systems. Variation in the metabolic function of microbial communities was observed based on carbon source utilization patterns (CSUPs) and was found to be strongly influenced by system design and sample location within the wetland but not by the presence of plants or depth of media. Analysis of specific carbon source guilds highlighted increased utilization of polymers and carbohydrates in the early stages of all systems, pointing to the establishment of microbial communities optimized to best utilize more easily degradable compounds close to the inlet. Correlations between measures of microbial functionality (activity, richness, and diversity) and turbidity, total organic carbon (TOC), total nitrogen (TN) were observed in horizontal-flow systems but less so in vertical-flow systems. The results presented provide useful insight into the spatial dynamics of microbial community function in constructed wetlands. Potential opportunities for system optimization, based on the results of this study, are provided.

1. Introduction

In recent decades constructed wetlands (CWs) have emerged as an economical, practical and ecologically appropriate technology for wastewater treatment. CWs can be used to treat a wide range of water pollutants from a vast array of contaminated waters (Kadlec and Wallace, 2009). As CW technology evolves, its potential applications are expanding. Current CW technology spans a diverse range of designs from completely passive horizontal surface or subsurface-flow systems to intermediate designs such as unsaturated vertical flow with pulse loading, and to intensified designs that utilize increased pumping, water level fluctuation or active aeration (Nivala et al., 2013a). Each constructed wetland design has distinct advantages and disadvantages, enabling engineers to tailor a system to a variety of wastewater treatment challenges.

Relatively little is known about factors that determine microbial community dynamics. Plants may act as a promoter of microbial community development by providing structural support for attachment to root systems, oxygen transfer from aerial tissues to the rhizosphere, or through the secretion of root exudates which contain a diverse assortment of enzymes and carbon-containing metabolites (Bais et al., 2006). The influence of bed depth, flow direction and degree of intensification are less clear. Constructed wetlands are often viewed as a "black box" technology, with design approaches commonly based on regression equations or simplified first-order decay models (Langergraber, 2007; Rousseau et al., 2004). However, it is generally recognized that many pollutant removal mechanisms in constructed wetlands are driven by microbial processes (Faulwetter et al., 2009). It is clear that in order to improve or optimize treatment wetland technologies, it is essential to have a better understanding of the microbial communities within (Deng et al., 2011).

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Microbial function is arguably a more relevant measure of potential treatment processes within a CW than genetic diversity. As such, several techniques are now available that can provide understanding of the functional diversity and catabolic potential of intrinsic microbial communities. This is often achieved using culture-based methods, assessments of microbial biomass and enzyme activity, and more recently, community level physiological profiling (CLPP) (Liu et al., 2013; Truu et al., 2009; Weber and Legge, 2010b). Despite recent advances in microbial community characterization techniques, several important knowledge gaps remain in our understanding of microbial community spatial dynamics in constructed wetlands. An improved understanding of the spatial patterns of microbial community activity and genetic diversity will help in ongoing optimization and future design of constructed treatment wetlands (Faulwetter et al., 2009; Weber and Legge, 2009). There is a need for detail-oriented, fundamental microbial process investigations in order to provide a better understanding of system dynamics, which will allow for new knowledge and ideas to be applied to the design and optimization of treatment wetland systems.

One of the more recently documented limitations of many published microbial community studies in the field of CWs is the widespread use of outlet water samples to represent microbial communities within CW systems (Weber and Gagnon, 2014). It is the fixed-film microbial communities inside the CW which are responsible for the majority of transformation and degradation processes. Representative biofilm samples taken from gravel or sediment are difficult to obtain, and in most cases are impractical to extract from CWs due to the destructive nature of such sampling techniques. Only a few studies have investigated spatial microbial community functional dynamics in CW systems through use of destructive sampling methods (Salomo et al., 2009; Weber and Legge, 2013). A more practical method of microbial community sampling, employed in the present study, can be conducted with the use of internal sampling wells where interstitial water and biofilm can be extracted from the bed.

With these points in mind, the main objective of this study was to explore the spatial dynamics of microbial community function in several different constructed wetlands designed for the treatment of domestic wastewater. Within this aim, the following sub-objectives were:

- to determine how microbial community function differs both between and within different constructed wetland designs according to the factors of flow direction, bed depth, presence of plants, and active aeration.
- to investigate the microbial community profiles in constructed wetlands used for secondary treatment of domestic wastewater compared to those used for tertiary treatment of domestic wastewater.
- to compare microbial community profiles of internal samples versus effluent water samples.
- to correlate microbial community metabolic function to conventional water quality data and identify factors of most importance to microbial community function.

2. Materials and methods

2.1. Experimental site

The pilot-scale constructed wetlands at the research facility in Langenreichenbach, Germany differ in terms of flow direction, presence of plants, media saturation, media type, loading regime and intensification. All systems treat domestic wastewater. A detailed description of the research facility can be found in Nivala et al. (2013a). Table 1 provides a summary of the systems surveyed in the present study, which at the time had been in steady-state operation for approximately three years. Briefly, the following systems in the study provide secondary treatment of domestic wastewater. The systems receive effluent from a common septic tank. The systems include: one pair of planted and unplanted 25-cm deep HF wetlands (H25p and H25); one pair of planted and unplanted 50-cm deep HF wetlands (H50p and H50), one planted horizontal flow wetland with aeration (HAp), and one planted vertical flow wetland with aeration (VAp). The aerated wetlands were designed and operated under conditions of continuous (24 h per day) aeration according to Wallace (2001) and explained in detail in Nivala et al. (2013a). One pair of tertiary treatment beds is also included in this study; one planted and one unplanted unsaturated vertical flow sand bed (VSp and VS, respectively). The VSp and VS beds each received effluent from an upstream unsaturated vertical flow gravel bed (VGp and VG, which were not included in the current study).

2.2. Sample collection

Samples were collected on April 8, 2013. Interstitial samples from H25, H25p, H50, H50p, and HAp were collected from 25 mm diameter PVC pipe tees with holes drilled through the horizontal part of the tee to enable sample collection at the mid-depth of the wetland at four locations equating to 12.5, 25, 50, and 75% of the distance along the flow path (Fig. 1a). Water samples from VAp

Table 1
Design and operational details of the studied constructed wetland systems.

System abbreviation[a]	System type	Treatment step	Effective depth[b] (cm)	Saturation status	Main media	Surface area (m^2)	Design flow (L/d)	Hydraulic loading rate (L/m^2d)
Horizontal flow								
H25, H25p	HF	Secondary	25	Saturated	8–16 mm gravel	5.6	100	18
H50, H50p	HF	Secondary	50	Saturated	8–16 mm gravel	5.6	200	36
Vertical flow								
VS, VSp	VF	Tertiary	85	Unsaturated	1–3 mm sand	6.2	600	95
Aerated								
VAp	VF + aeration	Secondary	85	Saturated	8–16 mm gravel	6.2	600	95
HAp	HF + aeration	Secondary	100	Saturated	8–16 mm gravel	5.6	730	130

[a] Systems planted exclusively with *Phragmites australis* are denoted with "p" in the system abbreviation.
[b] Effective depth refers to the depth of the media involved in treatment. In saturated systems, it is equal to the wetted depth of the bed.