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Microbial community metabolic function in constructed wetland mesocosms treating the pesticides imazalil and tebuconazole

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ABSTRACT

The objective of this research was to study the differences in microbial community metabolic function in planted and unplanted saturated constructed wetland mesocosm systems with five different wetland plant species, *Typha latifolia*, *Phragmites australis*, *Iris pseudacorus*, *Berula erecta* and *Juncus effusus* treating the pesticides imazalil and tebuconazole. Community level physiological profiling (CLPP) was used to determine the microbial community metabolic function from each mesocosm. Microbial activity and metabolic richness were in general not differentiated by pesticides presence. Significantly lower values in comparison with a non-pesticide control were only found in the *Iris* mesocosms exposed to imazalil and tebuconazole and in the *Berula* mesocosms exposed to tebuconazole during winter. Planted mesocosms had different microbial community carbon source utilization patterns in comparison to the unplanted mesocosms. In comparison to pesticide presence, plant species had a larger effect on differentiating the microbial community carbon source utilization patterns. Seasonal effects on carbon source utilization patterns were observed for both planted and unplanted mesocosms. In summer, microbial activity and metabolic richness were negatively correlated with TN, NH_4^+-N, TP concentration and pH in the control group, but not in the pesticide treatment groups, again suggesting a microbial community functional shift in the presence of pesticides. The main factors driving microbial community activity and functional diversity were plant species and season, while carbon source utilization revealed some pesticide adaptation in the summer period.

1. Introduction

Constructed wetland systems (CWs) are an economical, robust and sustainable technology for wastewater treatment, and emerging as a widely used technology for the treatment of a variety of pollutants, including pesticides (Vymazal and Březinová, 2015). Commonly used pesticides, such as imazalil and tebuconazole, are not only present in agricultural run-off due to their fungicide use applications, they are also used as biocides in different construction materials thus appearing in storm waters from urban settings. The discharge of polluted water to the environment has resulted in increasing concern due to the potential toxic effects on aquatic biota and human health (Bollmann et al., 2014; USEPA, 2000, 2002).

Regarding treatment, studies on imazalil and tebuconazole removal in CWs are limited and mainly focused on removal efficiency in flowing vegetated streams (Elsaesser et al., 2013; Lv et al., 2016a; Stang et al., 2013). More recently, removal of these compounds has been shown to also occur in saturated constructed wetlands, indicating the potential for subsurface flow systems for this application (Lv et al., 2016b). An important part of pesticide treatment in these systems was attributed to the uptake by plants and microbial activity/action. Phytoaccumulation has proven to be relatively low pointing to degradation (both microbial degradation and degradation within plant tissue) as the main probable pathway for removal. In addition to both plants and microbial communities likely being responsible for final degradation, the presence of plants can also promote microbial communities by releasing oxygen and low-molecular weight root exudates, and by providing surface area for attachment (Brix, 1997; Zhai et al., 2013; Zhang et al., 2016b).

Research on the influence of pesticides on microbial communities in CWs is limited. The bacterial community structure of the

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sediment from a stormwater basin (CW application) showed specific adaptations after being spiked with a cocktail of herbicides, diuron, 3, 4-dichloroaniline and glyphosate at the mg L^{-1} level (Bois et al., 2011). Additional results also suggested that the presence of plants may help to stabilise bacterial-driven pesticide mitigation in environments subject to variable conditions such as stormwater basins (Bois et al., 2011). For natural wetlands, Milenkovski et al. (2010) reported eight fungicides (benomyl, carbendazim, carboxin, captan, cycloheximide, fenpropimorph, propiconazole and thiram) to have toxic effects on two functions of natural wetland bacterial communities, specifically their activity (potential denitrification) and growth rate (leucine incorporation). However, the adverse effect (10% inhibitory effect on the microbial community) only occured at fungicide concentrations above 1 mg L^{-1}, not at the ng L^{-1} to μg L^{-1} levels which are more common for pesticides in agricultural runoff and stormwater. There is a large knowledge gap regarding the effect pesticides may have on established microbial communities in CWs, and the subsequent adaptations in community metabolic function.

Previous research found that microbial community activity and metabolic richness can be altered by plant species (*Phragmites australis* and *Phalaris arundinacea*) in horizontal subsurface flow CWs treating reconstituted wastewater from diluted fish farm sludge (Button et al., 2016). However, the effects of different wetland plant species on the microbial community when treating the pesticides imazalil and tebuconazole in CWs is unknown. Plant density was shown to have a strong positive correlation with tebuconazole removal in an *Elodea nuttallii* planted flow-through stream system (Elsaesser et al., 2013). Imazalil and tebuconazole removal efficiency and kinetics were different between non-planted and planted systems and also differet between five species of wetland plants in a saturated CW mesocosms study (Lv et al., 2016b). In Lv et al. (2016b), different plant species resulted in various pesticide efficiencies, but it is unknown if this was directly due to the plants themselves or by an indirect effect through stimulation and establishment of differing microbial communities. No direct relationships between pesticide presence and microbial communities were made in Lv et al. (2016b). Therefore the exact effect of microbial communities on pesticides or the effect of pesticides on microbial communities has not been systematically investigated in these types of systems. Additionally, it is widely known that season can affect the metabolic function (Chazarenc et al., 2010), composition (Morató et al., 2014) and structure (Zhang et al., 2016a) of the microbial communities when treating domestic wastewater in CWs. However, the effect of season on microbial communities subjected to the presence of the pesticides imazalil and tebucoanzole has not been previously investigated.

The CLPP method, using BIOLOG™ EcoPlates with multiple sole-carbon sources, has advantages over other techniques as it does not require specialised expertise and can be used to easily, accurately and rapidly determine differences in microbial community function, carbon utilization intensity and overall catabolic capability of non-isolated microbial community samples (Weber and Legge, 2010b). Recently, CLPP has become a tool in CW research to determine the time required for microbial community establishment during the start-up phase (Weber and Legge, 2011), evaluating the changes in microbial community with respect to plant identity and diversity (Bissegger et al., 2014), in understanding seasonal and spatial microbial community changes (Chazarenc et al., 2010), to spatially investigate microbial community function in different pilot scale designs (Button et al., 2015; Weber and Legge, 2013) and to evaluate the influence of various influent wastewater qualities on the inherent CW microbial communities (Zhao et al., 2010).

The objective of this study was to investigate the effects of season, plant presence, plant species, and pesticide presence on the microbial community metabolic function in saturated constructed wetland systems. Three parallel mesocosm groupings were fed with either simulated wastewater containing imazalil, simulated wastewater containing tebuconazole, or simulated wastewater with no added pesticide. The mesocosms were saturated CW systems, either unplanted or planted with one of five wetland plant species, *Typha latifolia*, *Phragmites australis, Iris pseudacorus, Berula erecta* or *Juncus effusus*.

2. Materials and methods

2.1. Mesocosm systems set-up and sampling

The study consisted of three CW mesocosm groups (imazalil exposed, tebuconazole exposed and a control with no pesticide), giving a total of 54 mesocosms. Each group consisted of triplicates of 6 types: unplanted, planted with *Typha latifolia (Typha)*, *Phragmites australis (Phragmites), Iris pseudacorus (Iris), Berula erecta (Berula), or Juncus effusus (Juncus)*. Samples from the influent tanks were also evaluated. The experiment ran at the Påskehøjgård greenhouse facilities of Aarhus University, Denmark. A detailed description of the experimental setup can be found in (Lv et al., 2016b). Briefly, each mescosom was set up in a 6 L plastic container (surface area 314 cm^2) filled with a 4 cm bottom layer of gravel (8–12 mm particle size), 10 cm layer of sand (0.5–1 mm particle size) and finally a 4 cm top layer of gravel, reaching a total depth of 18 cm. Synthetic wastewater was fed onto the mesocosm surface and the effluent was collected at the bottom by plastic tubes. The outlet height was set at 15 cm to keep the mesocosms constantly saturated. For each group, similar sized (120 ± 10 g fresh biomass) plant specimens were randomly selected and planted in the mesocosms.

The mesocosms were rain protected, but exposed to natural daily and seasonal temperature (minimum 14 ± 3 °C and maximum 30 ± 4 °C) and environmental light exposure variations. The systems were fed with "Pioner Grøn" (Brøste Group, Denmark) N:P:K full strength nutrient solution prepared with tap water having the following composition (mg L^{-1}): Total-N 8.3; P 2.0; Mg 3.0; K 15.4; S 3.9. A carbon feed using acetic acid was used to simulate a 12 mg L^{-1} TOC inflow concentration. The synthetic wastewater was prepared approximately every 5 days in 300 L doses and constantly mixed by a submerged pump placed at the bottom of the tank (light protected). Two different influent concentrations of imazalil and tebuconazole (10 and 100 μg L^{-1}) and five different hydraulic loading rates (HLR: 0.7, 1.8, 3.4, 6.9 and 13.8 cm d^{-1}) were tested in each season. The experimental period covered summer and winter seasons (from July 2014 to March 2015) after an initial 30 days of startup period (June 2014).

2.2. Sample collection

Microbial community interstitial water samples were collected at the end of the summer and winter period, when the systems were operated at a HLR of 3.4 cm d^{-1} and a pesticide concentration of 100 μg L^{-1}. The pH, dissolved oxygen (DO), water temperature and electrical conductivity (EC) were measured using portable meters (Multi-Parameter Meter HQ40d, and sensION + EC5, HACH, USA). Total nitrogen (TN) and total organic carbon (TOC) were measured by a TNM-1 unit of a TOC-V analyzer (Shimadzu, Japan). NH_4^+-N, NO_3^--N and PO_4^{3+}-P were analysed by QuikChem Methods® (10-107-06-3-D, 10-107-04-1-C, 10-115-01-1-A, respectively) on an automated flow injection analyzer (QuikChem FIA+ 8000 Series, Lachat instruments, Milwaukee, USA). Plant height and leaf chlorophyll (measured using a calibrated hand-held chlorophyll content meter, CCM-200, Opti-Science, USA) were monitored throughout the summer campaign. Pesticides were analysed by an HPLC (Thermo Scientific Ultimate 3000) equipped with a diode array