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Bacterial communities in batch and continuous-flow wetlands treating the herbicide *S*-metolachlor

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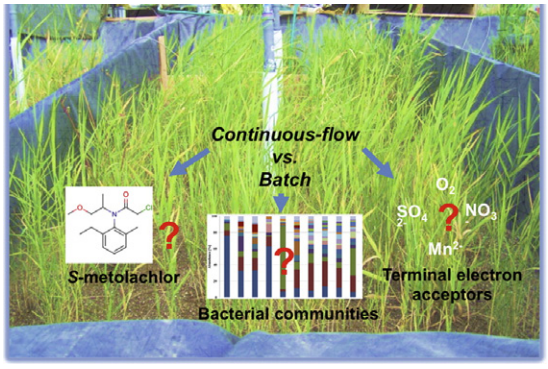
HIGHLIGHTS

- We evaluated the bacterial composition in wetlands treating *S*-metolachlor
- Hydraulic regime impacted biogeochemical processes and *S*-metolachlor removal
- Bacterial composition correlated with nitrate reduction and *S*-metolachlor removal
- T-RFLP and pyrosequencing analysis of bacterial diversity were in good agreement
- The bacterial composition reflects herbicide exposure and hydraulic regime

GRAPHICAL ABSTRACT



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ABSTRACT

Knowledge of wetland bacterial communities in the context of pesticide contamination and hydrological regime is scarce. We investigated the bacterial composition in constructed wetlands receiving Mercantor Gold® contaminated water (960 g L^{-1} of the herbicide *S*-metolachlor, >80% of the *S*-enantiomer) operated under continuous-flow or batch modes to evaluate the impact of the hydraulic regime. In the continuous-flow wetland, *S*-metolachlor mass removal was >40%, whereas in the batch wetland, almost complete removal of *S*-metolachlor (93–97%) was observed. Detection of ethanesulfonic and oxanilic acid degradation products further indicated *S*-metolachlor biodegradation in the two wetlands. The dominant bacterial populations were characterised by terminal restriction fragment length polymorphism (T-RFLP) and 454 pyrosequencing. The bacterial profiles evolved during the first 35 days of the experiment, starting from a composition similar to that of inlet water, with the use of nitrate and to a lesser extent sulphate and manganese as terminal electron acceptors for microbial metabolism. *Proteobacteria* were the most abundant phylum, with *Beta-*, *Alpha-* and *Gammaproteobacteria* representing 26%, 19% and 17% respectively of total bacterial abundance. Bacterial composition in wetland water changed gradually over time in continuous-flow wetland and more abruptly in the batch wetland. Differences in overall bacterial water structure in the two systems were modest but significant ($p = 0.008$), and *S*-metolachlor, nitrate, and total inorganic carbon concentrations correlated with changes in the bacterial profiles. Together, the results highlight that bacterial composition profiles and their dynamics may be used as bioindicators of herbicide exposure and hydraulic disturbances in wetland systems.

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1. Introduction

Wetlands are dynamic ecosystems with unique properties resulting from interactions between water, soil and biota. They provide essential ecological functions including flood control, wildlife habitat, biogeochemical cycling, and water quality improvement by pollution reduction (Hansson et al., 2005). Wetlands are thus finding increasing application for water treatment, particularly in the case of waters contaminated with pesticides from agricultural use (Grégoire et al., 2009).

Microbial communities are major drivers of wetland functions, supporting elemental cycling and biodegradation of organic contaminants such as pesticides. Microbial communities and pesticide biodegradation in wetlands are strongly impacted by hydraulic and hydrochemical conditions (Carleton et al., 2001; Truu et al., 2009). Fluctuations of the water table level shape wetland redox conditions by controlling oxygen transfer into wetland sediments (Williams and Oostrom, 2010; Rezanezhad et al., 2014). In turn, redox conditions influence availability of terminal electron acceptors for microbial metabolism, which may eventually affect pollutant removal (Borch et al., 2010). Several studies have documented the impact of hydraulic regime on biogeochemical cycling and organic contaminant degradation, including pharmaceuticals, in natural and constructed wetlands (Burgin et al., 2011; Zhang et al., 2012; Avila et al., 2013; Rezanezhad et al., 2014). In contrast, knowledge on the influence of hydrological conditions on microbial communities in wetlands degrading pesticides is still very scarce.

Exposure to pesticides, transient or chronic, may affect the composition and functioning of the microbial compartment of a given environment, both quantitatively and qualitatively (Imfeld and Vuilleumier, 2012). DNA fingerprinting and sequencing techniques have proven particularly useful in elucidating the relationship between degradation of organic contaminants, biogeochemical processes and bacterial community dynamics in wetland systems (Imfeld et al., 2009, 2010; Weber et al., 2011; Adrados et al., 2014). Ongoing advances in high-throughput sequencing techniques are boosting the exploration of microbial taxonomic diversity in wetlands (Hartman et al., 2008; Ligi et al., 2013; Serkebaeva et al., 2013). Characteristics of wetland microbial communities may represent potential indicators to assess their biological status (Imfeld and Vuilleumier, 2012), and aid in the design and operation of constructed wetlands for pollution treatment (Sims et al., 2013).

In this context, we embarked on a study to characterise wetland bacterial communities exposed to a commercial formulation of the chloroacetanilide herbicide *S*-metolachlor (i.e., Mercantor Gold®). *S*-metolachlor is widely applied to control annual weeds for a variety of crops including corn and sugar beet. The polar character of *S*-metolachlor and its application as a pre-emergence herbicide (i.e., before the development of a vegetal cover) favour its transport to non-target environmental compartments (Tran et al., 2007), which is reflected in its frequent detection in both ground and surface water (Hladik et al., 2005; Steele et al., 2008). Biological degradation of chloroacetanilides was repeatedly evidenced by detection of the corresponding ethanesulfonic and oxanilic acid degradation products (e.g., Baran and Gourcy, 2013), by enrichment cultures degrading other chloroacetanilide herbicides, such as propachlor, acetochlor and alachlor (Xu et al., 2006; Liu et al., 2012; Zheng et al., 2012) and by the isolation of bacterial strains degrading metolachlor (Wang et al., 2008; Zhang et al., 2011). However, the influence on wetland microbial communities of *S*-metolachlor exposure, and in particular its commercial formulation, remains largely unexplored.

The aim of the present study is to evaluate the influence of hydraulic and hydrochemical conditions on bacterial communities in two pilot subsurface horizontal flow wetlands treating *S*-metolachlor contaminated water. The two identical subsurface horizontal flow wetlands were operated with different hydraulic regimes to mimic conditions of either continuous low-level (continuous-flow wetland) or acute transient exposure to *S*-metolachlor (batch wetland). The continuous-flow wetland was continuously supplied with water, reproducing conditions occurring in flooded wetland systems that intercept water fluxes with background levels of pesticide contamination. The batch wetland was exposed to successive dry–wet cycles, as in wetlands that punctually received high loads of pesticides in runoff water. Hydrochemical analysis and quantification of *S*-metolachlor and its ethanesulfonic and oxanilic acid degradation products were used to evaluate prevailing wetland conditions as well as *S*-metolachlor degradation, and wetland bacterial communities were evaluated by terminal restriction fragment length polymorphism (T-RFLP) and 454 pyrosequencing.

2. Materials and methods

2.1. Experimental design

Two identical outdoor subsurface-flow constructed wetlands (48°4′54″N, 7°21′20″E, Colmar, Alsace, France) of 4 m length, 1.8 m width and 52 cm depth, filled with sand (grain size 0–4 mm) and a bottom layer of gravel (grain size 4–8 mm) were used (Fig. 1A). Sand had a porosity of 32 and 35%, and a saturated hydraulic conductivity of 6.9×10^{-5} m s^{-1} and 9.7×10^{-5} m s^{-1} for the continuous-flow and batch wetlands respectively. Three species of commonly used macrophytes, i.e. *Phragmites australis* (Cav.) Trin. ex Steud. (20 plants m^{-2}), *Phalaris arundinacea* L. (3 plants m^{-2}) and *Glyceria maxima* (Hartm.) Holmb. (2 plants m^{-2}) were planted. Three piezometers (1.05 m in length and 5.5 cm in diameter) embedded in the sand layer along the middle of each bed at successive one meter intervals from the inlet served to monitor water levels and for water sampling. The wetlands were filled with tap water three months and again one week before the start of the experiment.

In the continuous wetland, two 14-day periods of continuous supply of water contaminated with *S*-metolachlor were carried out (Fig. 1B). The mean inlet flow rate was 5.0 L h^{-1} and mean residence time was about 6.0 days. Initial continuous exposure with *S*-metolachlor contaminated water was performed from May 24 to June 7, 2012 (i.e. from day 0 to day 14), and the second continuous exposure was done from July 5 to July 19, 2012 (i.e. from day 42 to day 56). After each exposure, the wetland was continuously supplied with uncontaminated water using a screw pump to maintain flooded conditions throughout the experiment (Fig. 1B). In the batch wetland, 4 flood–drain cycles were carried out. Each cycle consisted of 14 days of saturated conditions (water residence time of 14 days) of water contaminated with *S*-metolachlor (prepared as described below), followed by a drained period of 7 days (Fig. 1B).

The two wetlands were exposed to the same pesticide load in each contamination cycle to facilitate comparison. Hydrochemistry of supplied tap water, water volumes, and associated *S*-metolachlor concentrations and masses supplied to the wetlands are described in Tables S1 and S2 of the Supplementary material. Mercantor Gold® (960 g L^{-1} of *S*-metolachlor, 87.4 ± 1.1% of the *S*-enantiomer, Syngenta, Basel, Switzerland) was dissolved in methanol (Chromasolv® Plus, analytical grade purity >99.9%; Sigma Aldrich, St. Louis, MO, USA) to obtain a stock solution of 30 g L^{-1} of *S*-metolachlor. 10 mL of this solution was added to 1 L of ultrapure water and stirred overnight to allow methanol evaporation. For the continuous-flow wetland, contaminated inlet water was prepared with tap water to yield a final concentration of 178 μg L^{-1}, thereby supplying 300 mg of *S*-metolachlor to the wetland over each two-week contamination phase. The same total amount of 300 mg *S*-metolachlor, dissolved in a tap water volume that varied from one batch operation to another depending on the initial volume of rainwater in the wetland prior to the batch operation, was provided to the batch wetland in each batch cycle. Initial concentration of *S*-metolachlor in the batch wetland was approximately 500 μg L^{-1}.