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Physicochemical and microbiological indicators of surface water body contamination with different sources of digestate from biogas plants

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ABSTRACT

Transition from fossil energy sources to biogas production has resulted in a strong increase of leakage accidents from fermenters, but knowledge on the effects of fermentation product runoff into freshwater systems is currently restricted to direct toxicity due to oxygen depletion. This study provides first information about the influence of digestate runoff on the physicochemical habitat properties and the bacterial community composition of the hyporheic interstitial which is important in determining ecosystem functioning. We exposed natural stream beds to different concentrations of two different digestates from fermenters (corn and manure feedstock), hypothesizing that the digestate addition causes acute changes of the physicochemical parameters and has distinct effects on microbial community composition of the hyporheic interstitial depending on concentration and type of digestate. In line with the hypotheses, pH value, conductivity, redox potential and ammonium differed significantly from controls and among treatments after digestate addition, but only for a maximum of two days. pH values (controls: 7.8; corn: 7.9; manure: 7.9) and conductivity (controls: 813 μS/cm; corn: 969 μS/cm; manure: 1097 μS/cm) increased, the redox potential (controls: 153 mV; corn: 145 mV; manure: 144 mV) decreased the first two days. A high peak of ammonium-N was detected in the corn and manure treatments (controls: 5 mg/l, corn: 80 mg/l; manure: 60 mg/l) at day 1. In contrast, changes in bacterial community composition were detectable for longer periods of time (>5 days). Seventeen unique T-RF fingerprints of bacterial community response to each of the different digestate treatments (11 unique T-RFs in manure and 6 unique T-RFs in corn treatments) were found, suggesting that this approach provides a suitable ecological indicator for source tracking, e.g. in case of a biogas power plant leakage accident.

1. Introduction

Growing global energy demand, rapidly rising concentrations of greenhouse gases in the atmosphere, global warming and the security of energy supply are considered the most important global challenges of this century (Holm-Nielsen et al., 2009). In addition, the Fukushima accident has triggered intense discussion about safe and sustainable energy provision. In this context, biogas production is considered an important renewable energy source, particularly in central Europe. Biogas production can either use animal waste or crops for energy and heat generation, and is often referred to as a technology with limited environmental risk (Weiland, 2010).

Germany has become the largest biogas producing country in the world. By the end of 2014, nearly 8000 biogas plants were operated on German farms (Fachverband Biogas, 2014). With an increasing number of biogas plants, the probability of accidents like technical faults, handling errors and disturbances during the fermentation process increases. Given the high number of such facilities, such incidents were reported to happen on average every two minutes (FNR, 2010). During the fermentation process, the anaerobic digestion of biomass, induced by a special acetogenic and methanogenic microbial community under high oxygen consumption (Weiland, 2010), results in an anaerobic byproduct (digestate). Möller (2015) defined digestates as a complex mixture of organic and inorganic substances, nutrients, degradable organic matter and water. Digestates are stored in tanks until they can be applicated to fields as fertilizers (Weiland, 2010). Tank leakages are often the reason for uncontrolled runoffs of digestate into freshwaters. Depending on the composition and dilution of such pollution in receiving aquatic ecosystems, the effects can vary widely: At high concentrations of digestates in relation to the affected aquatic

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ecosystem, the resulting oxygen depletion can cause rapid die-offs of fish and other oxygen-dependent aquatic species which is well understood. At lower concentrations, the high nutrient loads from digestate runoffs can result in eutrophication and therefore a degradation of stream water quality (Carpenter et al., 1998). Depending on the dose, digestate runoffs into freshwater ecosystems also have the potential to alter habitat properties of the hyporheic interstitial and to cause sublethal effects within the aquatic food web, which is closely linked to hyporheic processes. To date, the influence of digestate input to the hyporheic interstitial is not well understood.

The hyporheic interstitial is the key zone between surface- and groundwater (Boulton et al., 1998) and is closely linked with ecosystem functions and services (Geist, 2011, 2014; Pander and Geist, 2013). It provides a great diversity of different microhabitats (Braun et al., 2012) serving as habitat for benthic macroinvertebrates (De Haas et al., 2005), mussel larvae (Geist, 2010), and fish eggs (Sternecker et al., 2013a,b). Additionally, water movement and temperature, the particle size of the substrate, the physicochemical properties of surface- and groundwater, and resident organisms influence important ecological processes of the interstitial zone (Boulton et al., 1998; Boeker et al., 2016; Pander et al., 2015). Microbes are considered the most important organisms of the hyporheic interstitial (Febria et al., 2012; Lowell et al., 2009). They provide crucial ecosystem services such as the mineralization of organic material, nutrient recycling and pollutant degradation (Boulton et al., 1998; Marmonier et al., 2012). Via the "microbial loop" they form an important component of aquatic food webs (Boulton et al., 1998; Findlay, 2010). In addition to physical and chemical parameters, microbial communities are important biological indicators for the functioning as well as for alterations in substratum conditions (Boeker and Geist, 2015; Boeker and Geist, 2016; Boeker et al., 2016; Mermillod-Blondin et al., 2001; Navel et al., 2012).

The objective of this study was to identify possible ecological indicators for biogas digestate contamination of freshwater ecosystems. We investigated the effects of different digestate types and concentrations on the physicochemical habitat properties (pH, oxygen concentration and saturation, conductivity, redox potential and ion composition) and the microbial community composition of the hyporheic interstitial. A standardized study design was set up applying the two most important types of digestates (corn and manure feedstock) in three different concentrations to experimental stream bed patches of a model stream system in comparison to a control without digestate. We hypothesized that the digestate treatment causes an acute reaction of the physicochemical habitat properties in the hyporheic interstitial depending on (i) the concentration of the digestate and on (ii) the type of digestate (corn or manure feedstock). Since the abiotic habitat properties are crucial for the colonization with bacteria we further hypothesized that (iii) the different types of digestate have long-term and distinct effects on the microbial community composition depending on the concentration.

2. Methods

2.1. Study design

The influence of digestates originating from two different biogas plants on abiotic habitat properties and microbial community composition was investigated in a 29-day experiment in a bypass channel of the River Moosach (12.0 m × 3.5 m × 0.50 m, a calcareous river, 48°23′37.8"N; 11°43′25.5"E), within the Danube catchment in Germany (Sternecker et al., 2013b).

To experimentally manipulate stream bed patches, plastic boxes (length, width, depth: 19.0 × 16.5 × 9 cm, Rotho Kunststoff AG, Würenlingen, Switzerland) filled with a pre-defined substrate were randomly buried (balanced latin square scheme) into the sediment of the Moosach bypass channel (Fig. 1). To ensure a constant water flow through the boxes during the experiment, vertical slits (4 × 4 slits; l = 1 cm, w = 2 mm) were cut into the sides perpendicular to the flow direction. This design was chosen based on results from a pre-test (data not shown) which indicated that this system allowed the most natural flow through the boxes compared to other cutouts (holes, crosses). The substrate consisted of a defined grain fraction mixture (2.0–6.3 mm) from the River Moosach and was washed and autoclaved to ensure identical starting conditions for all treatments. For the characterization of the two different digestates, physicochemical water parameters (oxygen concentration and saturation, pH, temperature, conductivity), BOD_5 (biochemical oxygen demand over five days) and dry matter contents (DMC) were analyzed.

The experiment was performed during June and July 2015 using three replicates per treatment (corn and manure digestate in three different concentrations each). To provide similar initial conditions (physicochemical parameters, bacterial community composition), the boxes stayed in the sediment for 14 days before the digestate treatments begun (starting on day −14). On day 0, all boxes were removed from the sediment. The boxes were drenched in different concentrations of "corn" digestate (biogas plant of 98% maize, 2% grass, DMC = 8%, BOD_5 = 6 g/l, Langengeisling, Germany) or "manure" digestate (biogas plant of 40% liquid manure, 60% maize/grass, DMC = 6%, BOD_5 = 7 g/l, Zötzelhofen, Germany) for two hours, simulating a typical peak contamination of streams following a leakage or an accident in a biogas plant. Three boxes per digestate type were drenched in undiluted digestate, three in 1:10 and three in 1:100 dilutions, to simulate different levels of severity of contamination. Nine control boxes were also handled in the same way but not treated with digestate. After the treatment, the boxes were placed back into the sediment of the Moosach and surveyed for another 15 days (Fig. 1).

2.2. Analysis of physical and chemical parameters

Physical and chemical water parameters (oxygen concentration and saturation, pH, temperature, conductivity and redox potential) were measured in the open and in the interstitial water using a WTW Multi 3430 Set G and a WTW pH 3110 Set-2 (Wissenschaftliche Technische Werkstätten, Weilheim, Germany) on days −14, −10, −5, 0, 1, 2, 5, 10 and 15 of the experiment in random order. Parameters in open water were measured 10 cm above the substrate surface. For the measurement in the interstitial water, water samples (30 ml) were collected from a depth of 5 cm in each box as described in Boeker and Geist (2016) After measurement of physicochemical variables, interstitial water samples of the days 0, 1, 5 and 15 were vacuum-filtrated (0.22 CME membrane filters, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and frozen at −20 °C for ion analysis, which is important for evaluating treatment-specific effects on water chemistry such as presence or absence of different forms of N (indicative of N-cycling and redox conditions). A mixture of 1.8 mM disodiumcarbonate and 1.7 mM sodiumhydrogencarbonate was used for eluting anions (i.e., Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}, F^-; AG-22 as guard column and AS-22 separation column) and 20 mM methanesulfonic acid for eluting cations (i.e., Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}; CG-12 as guard column in line with a CS-12 separation column). Ion chromatographs and columns were manufactured by Thermo Scientific, Dreieich, Germany.

2.3. Analysis of microbial diversity

Microbial community composition was analyzed with DNA-based terminal restriction fragment length polymorphism (T-RFLP)