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# Effects of municipal wastewater on aquatic ecosystem structure and function in the receiving stream

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#### HIGHLIGHTS

• Wastewater affects structural and functional endpoints in ecosystem.

• Adverse effects occur some hundred meters below the effluent if dilution potential is low.

· Powdered activated carbon effectively reduces ecotoxicity of wastewater.

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#### ABSTRACT

During recent years, increasing incidences of summer droughts - likely driven by climate change - reduced the dilution potential of low-order streams for secondary treated wastewater also in temperate Europe. Despite the potential risks to ecosystem integrity, there is a paucity of knowledge regarding the effects of different wastewater dilution potentials on ecosystem functions. The present study investigated the implications of secondary treated wastewater released into a third-order stream (Queich, southwest Germany) during a season with low dilution potential (summer; ~90% wastewater) as compared to a season with high dilution potential (winter; ~35% wastewater) in terms of leaf litter decomposition and macroinvertebrate communities. Adverse effects in macroinvertebrate mediated leaf mass loss (~65%), gammarids' feeding rate (~80%), leaf associated fungal biomass (>40%) and shifts in macroinvertebrate community structure were apparent up to 100 and 300 m (partially 500 m) downstream of the wastewater treatment plant effluent during winter and summer, respectively. In addition, a Gammarus fossarum laboratory feeding trial demonstrated the potential of powdered activated carbon to reduce the ecotoxicity of released wastewater. These results urge the development and evaluation of adequate management strategies, e.g. the application of advanced wastewater treatment technologies, to protect the integrity of freshwater ecosystems, which is required by the European Water Framework Directive - also considering decreasing dilution potential of streams as projected by climate change scenarios.

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#### 1. Introduction

Many chemicals of anthropogenic origin are only partly retained or degraded in municipal wastewater treatment plants (WWTPs) by conventional secondary treatment (i.e. mechanical and biological). Therefore, WWTP effluents are considered as one of the major sources of nutrients (e.g. Martí et al., 2010) as well as inorganic (Dyer and Wang, 2002) and organic (micro)pollutants (Bueno et al., 2012; Daughton and Ternes, 1999) for aquatic ecosystems (Schwarzenbach et al., 2006). The structure of aquatic communities inhabiting these ecosystems may suffer from this continuous contamination directly (Bundschuh et al., 2011a) or indirectly (Bundschuh et al., 2011b; Duddridge and Wainwright, 1980). In addition, ecosystem functions including the decomposition of leaf litter, which provides energy in the form of fine particulate and dissolved organic matter for local as well as downstream communities (Cummins and Klug, 1979), may be affected by conventionally treated wastewater (Bundschuh et al., 2011a).

Implications of wastewater on both ecosystem structure and function were, however, until now, mainly assessed in effluent dominated streams of semi-arid regions (e.g. Canobbio et al., 2009; Ortiz et al., 2005; Rueda et al., 2002; Ruggiero et al., 2006). Although previous studies indicated either no or only negligible impairments of secondary treated wastewater in the ecosystem function of leaf litter decomposition in temperate

Abbreviations: CI, confidence interval; NMDS, non-metric multidimensional scaling; PAC, powdered activated carbon; PERMANOVA, permutational multivariate analysis of variance; SIMPER, similarity percentage analysis; WWTP, wastewater treatment plant.

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regions (Gücker et al., 2006; Ladewig et al., 2006; Spänhoff et al., 2007; Suberkropp et al., 1988), the increasing frequency and intensity of summer droughts, which are potentially due to climate change, have resulted in decreased water levels, particularly in low-order streams, thereby additionally reducing their dilution potential (Prellberg, 2010). Therefore, it may be hypothesized that Central European regions with low precipitation (i.e. Palatinate, Germany; Ramm, 2005), already suffer from a low dilution rate of secondary treated wastewater released into streams, especially during the dry summer months. However, there is a scarcity of knowledge regarding the implications on ecosystem functions, such as the leaf litter decomposition, during seasons with low (summer) compared to high (winter) dilution potential within the receiving stream (cp. Bundschuh et al., 2011c). Knowledge in this field may help to predict future implications of water scarcity and to develop appropriate management strategies.

To address this gap in scientific knowledge, the present study assessed the potential adverse effects of secondary treated wastewater released from the WWTP Landau-Mörlheim, Germany, on leaf litter decomposition. The study was conducted within a third-order stream, the Queich, during both winter and summer seasons assuming that effects are more pronounced during low flow conditions (=summer). Alterations in the macroinvertebrate community were assessed by surber sampling as well as by collecting organisms associated with coarse-mesh leaf litter bags. Moreover, bacterial cell numbers and fungal biomass were assessed to enable the determination of changes in the activity of leaf-associated microbial decomposers, bacteria and fungi, which may trigger the changes seen in leaf litter decomposition process, such as leaf mass loss. In addition, in situ bioassays investigating the feeding rate of the leaf-shredding amphipod Gammarus fossarum were performed. Such in situ bioassays are supposed to display exclusively direct - not food quality related implications as discussed by Bundschuh and Schulz (2011) for a comparable laboratory-based experimental set-up. In order to achieve regulatory requirements such as the European Water Framework Directive (European Commission, 2000) also during seasons with low dilution potential, advanced treatment technologies may be useful in the medium term (Stalter et al., 2010), which is, however, insufficiently underpinned from an ecotoxicological viewpoint (Bundschuh et al., 2011d). Powdered activated carbon (PAC) is one technology with the potential to reduce the load of (in)organic micropollutants in wastewater. Therefore, the present study bolstered the (semi)field experiments by a laboratory feeding trial – also using G. fossarum – investigating the potential of PAC for the reduction of wastewaters' ecotoxicity.

#### 2. Materials & methods

#### 2.1. Study site

The present study was performed in a third-order stream (the Queich) within the region of Palatinate, Germany near the city of Landau. The municipal WWTP Landau-Mörlheim (49°12′N; 8°10′E) releases wastewater of a population equivalent of 80,000, 30,000 of which comes from industry (Raisin, EWL, personal communication), into the Queich. The secondary treated wastewater (200-300 L/s; EWL, 2012) is initially discharged through a sewage channel without aquatic macrophytes, which enters after approximately 2 km into a branch of the Queich. Since no dilution takes place within the sewer, this inflow point is referred to as "WWTP effluent" in the remainder of the manuscript. Approximately 350 m downstream of the WWTP effluent, the branch enters the Queichs' main stream, further diluting the introduced wastewater. The sandy streambed of the investigated Queich section had a width of 3-4 m and a water depth between 0.5 to 0.7 m (up to 1.5 m in pools that were scattered along the area), which varied among sites and seasons. Trees of the genera Alnus, Acer, Prunus and Sambucus dominated the riparian vegetation along the investigated stream section.

#### 2.2. Experimental design

For the present study, the control site (LD1w/s) was located in the Queich's main stream 250 m upstream of the WWTP effluent (Fig. 1). This site is not affected by WWTPs as no effluent was located up to 15 km further upstream. To assess the ecotoxicological implications of secondary treated wastewater released into the Queich, macroinvertebrate- and microorganism-mediated leaf mass loss, leaf associated microbial endpoints (bacterial cell number and fungal biomass) as well as shifts in the macroinvertebrate community structure were assessed 100 m (LD2w/s) downstream during winter (w; 2010/11 for six weeks after 14, 28 and 42 d of exposure) by using the methods described below. As substantial effects in all endpoints were observed during winter (see results section), additional sampling sites (up to 1000 m further downstream) were assessed during the experiments conducted in the following summer season to determine the area downstream that was affected by the wastewater. Hence, during the summer (s; 2011 only three weeks due to a faster decomposition as detailed below) three additional sampling sites located 300 (LD3s), 500 (LD4s) and 1000 m (LD5s) downstream of the WWTP effluent were investigated (Fig. 1).

#### 2.3. Wastewater dilution and water parameters

Water quality parameters were measured in situ weekly at all sites during winter and summer. Oxygen saturation, temperature, pH and conductivity were measured with a WTW Multi 340i/SET (Wissenschaftlich Technische Werkstätten GmbH, Weilheim, Germany). Current velocity was measured with a Höntzsch instrumentals flow meter (type µO-TAD; Waiblingen, Germany) respectively. Additional parameters including ammonium, nitrate, nitrite, phosphate, chloride, sulfate concentrations and hardness were quantified using Macherey-Nagel visocolor<sup>®</sup> kits (Macherey-Nagel, Düren, Germany) in the laboratory. Moreover, the seasonal contribution of wastewater to the branch's discharge was calculated during both seasons. For this, the volumetric flow rate (w: 467.0 L/s; s: 28.8 L/s) of the Queich's branch was calculated by multiplying the stream cross-sectional area (upstream of the WWTP effluent; 2.44 and 0.24 m<sup>2</sup>) with the mean flow velocity (of the crosssection; 0.20 and 0.12 m/s) both measured during winter and summer, respectively. The average contribution of the wastewater to the discharge of the Queich's branch was calculated by considering 250 L/s as the average WWTP discharge (EWL, 2012) for both seasons.

#### 2.4. Macroinvertebrate sampling

A Surber Sampler (500-µm mesh-size; surface: 1/8 m<sup>2</sup>; Surber, 1970) was used to obtain a representative assessment of the macroinvertebrate community composition (Storey et al., 1991). Three samples were taken at the beginning as well as at the termination of the experiments conducted in the winter season. Since, during the winter, no significant differences were observed regarding the community composition at both sampling sites between sampling dates (see Supplemental data Fig. A1), macroinvertebrates were sampled only once in summer; this occurred after ten days of the beginning of the study (i.e. in the middle of the experiment). Organisms were fixed in 70% ethanol, counted and identified to the lowest possible taxonomic level (genus for the majority of taxa; Table 1).

#### 2.5. Leaf litter bags

Leaves of *Alnus glutinosa* (L.) Gaertn. (black alder), a common species in riparian zones of temperate Europe (Hewitt, 1999) and present at the investigated stream section, were utilized to assess leaf litter decomposition in terms of leaf mass loss at each sampling site. Leaves were collected shortly before defoliation in October 2008 from trees near Landau, Germany (49°11'N; 8°05'E), and were stored

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