Contaminant *Transport*
in a Highly Dynamic Riverbank Filtration System

Vienna Doctoral Programme on Water Resource Systems

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Contaminant Transport in a Highly Dynamic Riverbank Filtration System

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by

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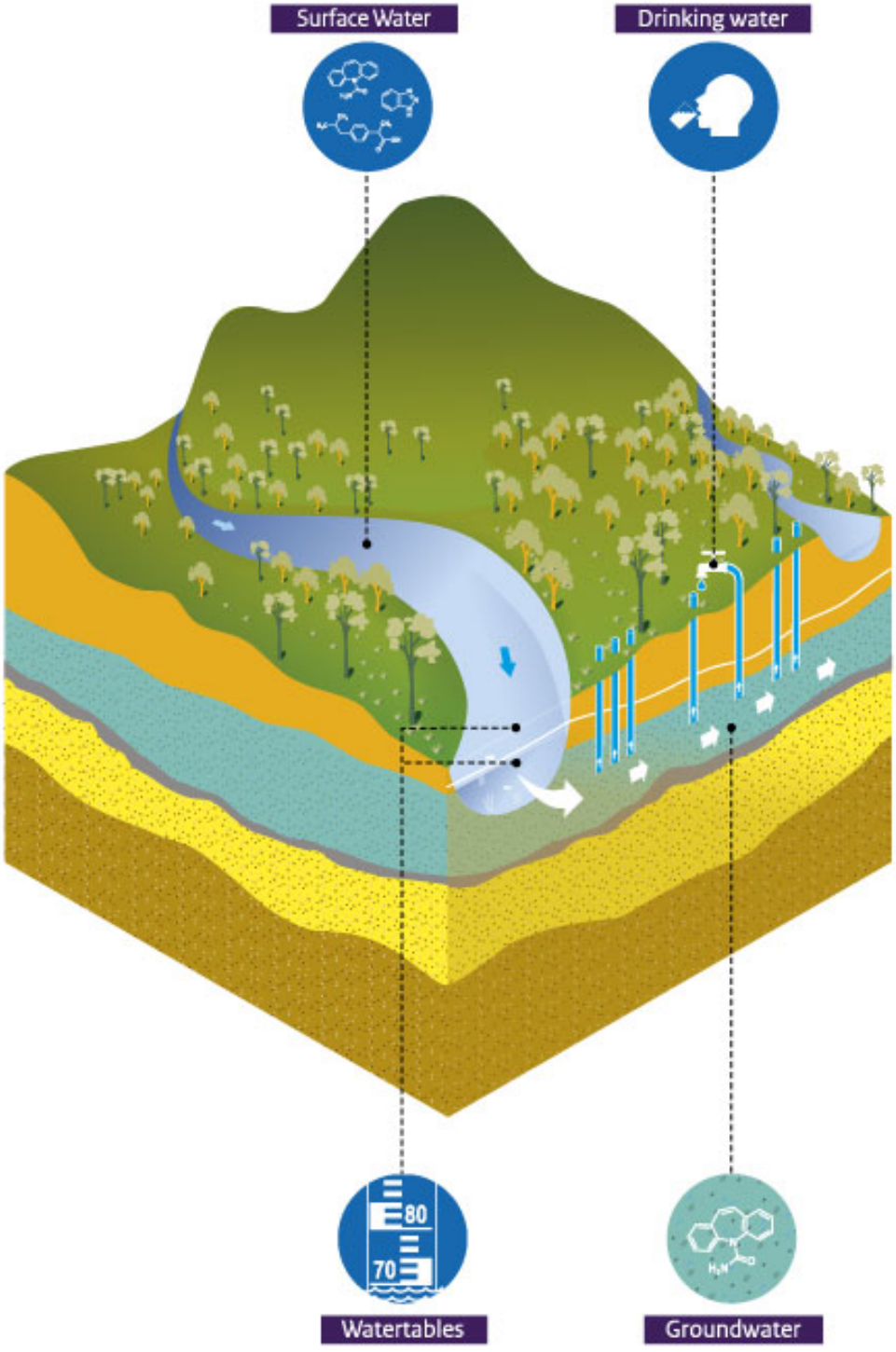
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Author's Statement

I hereby declare that I independently drafted this manuscript, that all sources and references are correctly cited, and that the respective parts of this manuscript – including tables, maps, and figures – which were included from other manuscripts of the internet either semantically or syntactically are made clearly evident in the text and all respective sources are correctly cited.

Inge van Driezum

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Summary

As can be seen by the amount of actions taken by several international organizations, the need for safe drinking water is becoming more and more evident. Since the quality of drinking water sources is decreasing, old and new methodologies are being used to improve the quality of the source water. One of these old methods is riverbank filtration (RBF). RBF systems are relatively inexpensive and are able to produce water which is relatively consistent in quality and usually easier treatable. Processes like adsorption, biodegradation and physicochemical filtration are responsible for the increase in water quality during aquifer passage. Not only these processes, but also the quality of the infiltrating surface water is of importance for the eventual quality of the groundwater. Therefore, the focus of this doctoral thesis was on the influence of surface water on both the chemical and microbial quality of the aquifer. The combination of chemical and microbial parameters is crucial due to the increasing global contamination of surface waters with these contaminants. Many RBF systems are situated along rivers with a high dynamics in water levels and in chemical and microbial water quality. It is of paramount importance to get an insight in this dynamics, since processes taking place in the aquifer can be influenced by it.

RBF systems along large rivers like the Danube provide drinking water for millions of people. The dynamics in RBF systems along large rivers can be much higher than in RBF systems connected to for example lakes. Together with the increasing anthropogenic activities in many of the (sub-)catchments, the stress on the groundwater is increasing. Organic micropollutants (OMPs) and microbial contaminants are introduced to the environment through for example wastewater treatment plant (WWTP) effluents and can have serious health effects. Their behaviour during aquifer passage is different, just as their analysis. The aim of this doctoral thesis is therefore to elucidate the influences of surface water infiltrating into the aquifer, on one hand on the microbial community naturally found in the groundwater and on the other hand on the behavior of OMPs during aquifer passage. The highest biological activity, and therefore the fastest removal of contaminants, can be found in the first few meters of the aquifer. It is therefore crucial to obtain samples representative for the surrounding aquifer, especially close to the river. Along a highly dynamic river like the Danube, this can be a challenge since stabilization of parameters might not be as quick as the changes in water levels.

Therefore, chapter 2 discusses the effect of pumping volume on the concentration of OMPs and microbial contaminants amongst others in a highly dynamic RBF system along the river Danube. Samples were taken after different pumping volumes, both close to the river as well as further into the aquifer. It was found that both the fluctuations in groundwater table and the fluctuations in contaminant concentrations did not affect the stability of the obtained chemical samples. Microbial parameters such as leucine incorporation (which is a measure for the biological activity of the microbial community) however did show a significant relation between stabilization and pumping volume.

With this information at hand, chapter 3 discusses the influence of surface water on the microbial characteristics of the aquifer. The response of the microbial community on seasonal dynamics, nutrient stimuli and hydrological fluctuations was studied during a 20 months period including 2 flood events which were sampled more extensively. The results showed that bacterial abundance, biomass and carbon production decreased significantly from the river towards the drinking water abstraction well. This was not influenced by the availability of nutrients or by seasonal dynamics, but mainly by fluctuations in groundwater flow velocity. During the flood events, this correlation was even more apparent and it could be seen that the rivers influence extended further into the aquifer, as was shown by a much higher proportion of larger cells in the groundwater during flood events than under normal conditions.

In chapter 4, the behavior of OMPs during RBF was studied. The samples were drawn over a slightly longer period than described in chapter 3 and also included the 2 flood events. The OMPs showed a likewise extended influence of the river during the two flood events. Some highly degradable OMPs were not found in the groundwater, whereas concentrations of common wastewater markers benzotriazole (BTri), carbamazepine (CBZ) and sulfamethoxazole (SMZ) were higher than under normal conditions. It was shown that in this oxic aquifer, BTri was almost fully removed under normal conditions. CBZ and SMZ, which were assumed to have a rather conservative behavior during aquifer passage, were attenuated to a certain extent. Mixing with groundwater of a better quality could not solely explain this decrease in concentration.

This thesis showed that obtaining samples for a combination of chemical and microbial parameters was not an easy task. Furthermore, wells especially close to the river and situated in oxic aquifers with high hydraulic conductivities can react quickly on changing hydrological conditions. One of the most important parameters for the extent of the surface water –

groundwater interaction was shown to be the potential difference between the river water and groundwater level. Not only the presence of OMPs can be influenced, also the microbial community can be altered by the infiltrating river. Since microbiological characteristics and the potential difference can be measured (near) real-time, this could be a very effective way for drinking water utilities to manage their abstraction strategies during periods of high discharge and rapidly changing hydrological conditions.

Kurzfassung

Wie die vielen Maßnahmen von Organisationen wie die Europäische Union und die World Health Organisation zeigen, wird die Notwendigkeit für sicheres Trinkwasser immer deutlicher. Seit der Abnahme der Qualität der Trinkwasserresourcen werden alte und neue Methoden verwendet um die Qualität zu verbessern. Eine dieser alten Methoden ist Uferfiltration. Uferfiltrations-Systeme (UFS) sind relativ preisgünstig und können einfacher Wasser von einer stabilen Qualität herstellen. Prozesse wie Adsorption, biologischer Abbau und physisch-chemische Filtration sind verantwortlich für die Zuhname der Wasserqualität während Uferpassage. Nicht nur diese Prozesse, sondern auch die Qualität von infiltrierenden Oberflächengewässern sind wichtig für die endgültige Qualität des Grundwassers. Der Schwerpunkt dieser Doktorarbeit war deswegen der Einfluss von Oberflächengewässern auf die chemische und mikrobiologische Qualität vom Grundwasserleiter. Weil die weltweite chemische und mikrobiologische Verunreinigungen von Oberflächengewässern zunehmen, ist die Kombination dieser Parameter sehr kritisch. Viele UFS liegen neben Flüssen mit einer hohen Dynamik in Wasserstand und chemischer und mikrobiologischer Qualität. Es ist sehr wichtig um diese Dynamik zu verstehen, weil Fluss- und Transportprozesse im Grundwasserleiter davon beeinflusst werden können.

UFS entlang großen Flüssen wie die Donau liefern Trinkwasser an Millionen von Menschen. Die Dynamik bei UFS an größen Flüssen kann viel höher sein als bei UFS an z.B. Seeen. Zusammen mit zunehmenden antropogenen Aktivitäten in vielen Einzugsgebieten, nimmt der Stress auf Grundwasser zu. Organische Spurenstoffe und mikrobiologische Verunreinigungen werden in die Umwelt introduziert durch z.B. Kläranlagen und können große Gesundheitsschäden verursachen. Deren Verhalten während Uferfiltration ist unterschiedlich, sowie auch deren Analytik. Das Ziel dieser Doktorarbeit ist deswegen, den Einfluss von infiltrierenden Oberflächengewässern auf das Grundwasser zu erforschen, einerseits auf die natürliche mikrobielle Gemeinschaft im Grundwasser und andererseits auf das Verhalten von organischen Spurenstoffen bei Uferfiltration. Die höchste biologische Aktivität und deswegen der schnellste Abbau der Verunreinigungen wird in den ersten paar Metern des Grundwasserleiters gefunden. Es ist darum äußerst wichtig, Proben zu nehmen, die repräsentativ sind für den umliegenden Grundwasserleiter, vor allem in der Nähe von einem Fluss. Entlang einem hoch dynamischen Fluss

wie der Donau kann das eine Herausforderung sein, weil die Stabilisation der Parameter vielleicht nicht so schnell ist wie die Veränderungen im Wasserspiegel.

Kapittel 2 diskuttiert deswegen den Effekt von Pumpvolumen auf die Konzentration von u.a. organischen Spurenstoffen und mikrobiologischen Verunreinigungen im hoch dynamischen UFS an der Donau. Die Proben wurden nach unterschiedlichen Pumpvolumen entnommen, sowohl in direkter Nähe vom Fluss als auch weiter im Grundwasserleiter. Die Schwankungen des Grundwasserspiegels und der Konzentrationen von Verunreinigungen haben keinen Effekt gehabt auf die Stabilität der chemischen Parameter. Mikrobielle Parameter wie Leucin-Inkorporation (ein Maß für die biologische Aktivität der mikrobiellen Gemeinschaft) zeigten jedoch einen signifikanten Zusammenhang zwischen Stabilität und Pumpvolumen.

Mit diesen Informationen behandelt Kapittel 3 den Einfluß von Oberflächengewässern auf die mikrobielle Charakteristika des Grundwasserleiters. Die Reaktion der mikrobiellen Gemeinschaft auf saisonbedingte Dynamik, Nährstoffe und hydrologische Schwankungen wurde während einer Periode von 20 Monaten erforscht, wobei 2 Hochwässer öfter beprobt wurden. Die Ergebnisse zeigten, dass bakterieller Reichtum, Biomasse und Bakterienproduktion vom Fluss in Richtung Trinkwasserquelle signifikant abgenommen haben. Das wurde nicht beeinflusst durch die Anwesentheit von Nährstoffen oder die saisonbedingte Dynamik, sondern hauptsächlich durch Schwankungen in der Strömungsgeschwindigkeit des Grundwassers. Während der Hochwässer war diese Korrelation noch deutlicher, und man hat sehen können, dass die Donau mehr Einfluss auf den Grundwasserleiter hatte als unter normalen Umständen. Das wurde deutlich durch einen höheren Anteil großer Zellen im Grundwasser während Hochwässern.

In Kapittel 4 ist das Verhalten von organischen Spurenstoffen während der Uferfiltration erforscht worden. Die Proben wurden während einer längeren Periode entnommen als die Proben die in Kapittel 3 beschrieben wurden und enthielten auch die beiden Hochwässer. Die organischen Spurenstoffe haben auch einen verlängerten Einfluß vom Fluss gezeigt während der 2 Hochwässer. Die einfach abbaubaren Spurenstoffe sind nicht im Grundwasser gefunden, während die Konzentrationen der Abwassermarker Benzotriazole (BTri), Carbamazepine (CBZ) und Sulfamethoxazole (SMZ) höher waren als unter normalen Bedingungen. In diesem oxyschen Grundwasserleiter war BTri fast völlig verschwunden. CBZ und SMZ, die normalerweise ein eher konservatives Verhalten haben während

Uferfiltration, haben zu einem gewissen Punkt abgenommen. Vermischung mit Grundwasser einer höheren Qualität war nicht ausreichend um den Konzentrationsabbau zu erklären.

Diese Dissertation hat gezeigt, dass die Probenentnahme für eine Kombination von chemischen und mikrobiologischen Parametern nicht einfach war. Grundwassersonden in der Nähe vom Fluss in oxyschen Grundwasserleitern mit hoher hydraulischer Leitfähigkeit können schnell reagieren auf Veränderungen der hydrologischen Bedingungen. Einer der wichtigsten Parameter für den Ausmaß der Interaktion von Oberflächengewässer auf Grundwasser war der Potentialunterschied zwischen Wasserspiegel vom Fluss und vom Grundwasser. Nicht nur das Vorhandensein von organischen Spurenstoffen kann beeinflusst werden durch den infiltrierenden Fluss, auch die mikrobielle Gemeinschaft kann dadurch verändert werden. Weil mikrobielle Eigenschaften des Grundwassers und der Potentialunterschied fast Realtime gemessen werden können, könnte das eine effektive Möglichkeit für Trinkwasserbetriebe sein um ihre Wasserentnahme zu steuern während Perioden mit höheren Durchflüssen und schnellen Veränderungen in den hydrologischen Bedingungen.

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1. Introduction

Globally, more than 2 billion people rely on groundwater for their primary source of drinking water (Alley et al., 2002). In countries like Austria, Denmark and Hungary, the World Health Organization (WHO, 2006) estimated over 90% of potable water originates from groundwater. It has been shown that the quality of surface water and groundwater is decreasing all over the world. Therefore, many international actions and efforts were defined in the last decades with the need for safe drinking water as a key point. One of them, **the 2030 Agenda for Sustainable Development**, was adopted by Member States of the United Nations in September 2015 and stressed, amongst others, the importance of drinking water (United Nations, 2015). This was defined as sustainable development goal 6: ensuring safe access to water and sanitation for all. In response to this Agenda, the UN member states adopted a resolution (71/222) on an International Decade for Action on "Water for Sustainable Development" (United Nations, 2017). The Water Action Decade aims to accelerate efforts towards meeting water-related challenges, including limited access to safe water and the increasing pressure on water resources and ecosystems, amongst others.
The European commission installed the **Water Framework Directive** (WFD) in 2000 (European Parliament & Council, 2000), with the goal to increase water quality in the EU and ensure sustainable usage of the water sources. It doesn't only comprise surface water, but also groundwater. To ensure drinking water of good quality, the **EU Drinking Water Directive** (DWD) was installed in 1998 (EC (European Community), 1998). The DWD obliges Member States to take all measures necessary to ensure clean drinking water and describes chemical and microbiological minimum requirements necessary for clean drinking water.

When using groundwater as a potable resource, several factors can have an influence on its quality. The age of groundwater for example can vary significantly depending on its source and might be a quality indicator. Deep groundwater (which is sometimes referred to as fossil water) can have ages of hundreds and even up to thousands of years (Oki and Kanae, 2006) and are usually of good quality, whereas groundwater fed by rivers (for example through riverbank filtration) is usually much younger and might be contaminated (Baillieux et al., 2014; Boano et al., 2014; Diem et al., 2014). Variations in groundwater quality can not only be influenced by for example the surrounding geology (e.g. contamination with arsenic, Winkel et al., 2008), but also by redox conditions in the aquifer (e.g. Borch et al., 2010)

and the activity of the microbial community (e.g. Peralta-Maraver et al., 2018; Tran et al., 2013). Furthermore, groundwater residence times, as a result of hydrogeological parameters like hydraulic conductivity and porosity, can also have an influence on groundwater quality (J Derx et al., 2013). Another highly important aspect is the quality of the source water. An aquifer fed by merely rain water in an uncontaminated area is likely to have a better quality than an aquifer fed by for example wastewater impacted surface water.

Aquifers fed by surface waters through riverbank filtration (RBF) are becoming more and more popular as a drinking water resource in many countries (Ray et al., 2002). Drinking water abstraction wells in these systems are located close to the river and exert a constant head difference between the river and the aquifer. Surface water is therefore infiltrating into the aquifer and moves towards the drinking water abstraction wells (Tufenkji et al., 2002). In countries along the river Danube, RBF is an important technique for obtaining drinking water. The river supplies approximately 10 million people with drinking water in this region (Kirschner et al., 2017).

A disadvantage of using surface water as a source is the (increasing) global contamination of these waters with chemical (Schwarzenbach et al., 2006) and microbial contaminants (Fenwick, 2006). Although an increasing amount of chemicals is being detected due to advancements in many analytical procedures, more than 10.000 substances are submitted every year to the European Chemicals Agency for registration under REACH (EU regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals). Many of the organic micropollutants (OMPs) responsible for the chemical contamination of surface waters originate from WWTPs and comprise substances like industrial chemicals and pharmaceuticals and personal care products (PPCPs). WWTPs can to a certain extent remove these substances, but some OMPs are persistent and can therefore enter the aquatic environment. Not only OMPs, but also the use of pesticides (Fenner et al., 2013) and fertilizers like nitrogen (Gruber and Galloway, 2008), are of great concern. Their presence is not only of concern to the environment due to the possible ecological impact to biota, but also to the quality of drinking water originating from RBF systems.

As estimated by the WHO, more than 500.000 people die every year due to diarrheal infections caused by the consumption of unsafe drinking water. These infections are caused by microbial contaminants, which can be of both human and animal faecal origin (Farnleitner et al., 2011). Human faecal pollution can therefore also often be related to municipal WWTP discharges.

The pathogens present in wastewater are only partly removed and are released into the environment (Wen et al., 2009). The health risks of these pathogens to humans are therefore highly dependent on their transport and survival in water (Bradford et al., 2013).

RBF has the capacity to reduce or eliminate OMPs and pathogens, but various processes are of influence on this removal. The removal of OMPs for example depends on the redox conditions in the aquifer and the ability of the microbial community to degrade these contaminants (Farnsworth and Hering, 2011; Hoppe-Jones et al., 2012). The highest biological activity, which depends particularly on bacteria, can be found in the first few meters of the RBF systems, the hyporheic zone (Peralta-Maraver et al., 2018). Next to the redox conditions and the biological activity, groundwater residence times can also play an important role in the removal of OMPs. This is also the case for the removal of microbial contaminants, where groundwater flow velocity influences the attachment and detachment of for example viruses (Schijven and Hassanizadeh, 2000).

RBF systems with high hydraulic conductivities (and most likely short groundwater residence times) are under more anthropogenic stress than RBF systems with lower hydraulic conductivities. Some RBF systems along the Danube are situated in former floodplain areas and consist of coarse materials with a high hydraulic conductivity. Groundwater residence times are not only dependent on the hydraulic conductivities of the aquifer material, but also on the potential difference between the river water- and groundwater level. Some RBF systems are situated along lakes (like the RBF systems in Berlin, Massmann and Sültenfuß (2007)) or consist of aquifers with regulated groundwater tables (like in the Netherlands along the river Lek, Hamann et al. (2016)). In these systems, varieties in groundwater flow velocities are limited and flow conditions are (mostly) assumed to be steady state. The microbial community is therefore expected not to change dramatically due to groundwater flow conditions and attenuation processes in the aquifer can be fairly stable. Along large rivers like the Danube however, flow conditions are far from steady state due to the high dynamics in river water levels. This could not only influence the removal of contaminants, but also the microbial community.

Since the health risk of drinking water obtained from RBF systems depends on a combination of parameters as described previously, it is of paramount importance to quantify the influence of both chemical and microbial contaminants originating in the river on the groundwater quality of these systems. The overall objective of this doctoral thesis was therefore to identify the effect of riverbank filtration on the chemical and microbial

groundwater quality of a highly dynamic RBF system along the river Danube.

Both groups of parameters behave differently and are analyzed in a different way. This makes it difficult to get a good overview of contaminants that might be a risk for environmental or human health. In order to quantify the effect of RBF on the groundwater quality for the combined parameter set, the samples taken in the RBF system need to be representative for both the aquifer and the set of parameters. Due to the highly dynamic nature of the investigated RBF system, the influence of the variability in the potential difference between river water and groundwater level, which influences the groundwater flow velocities, was also an important asset of this thesis. The following research questions were therefore formulated:

1. Does pumping volume affect the concentration of various contaminants in a highly dynamic RBF environment?
2. Is the microbial water quality in the RBF environment vulnerable to surface water infiltration?
3. What is the behavior of several organic micropollutants in a highly dynamic RBF environment?

In order to quantify the effect of surface water infiltration, an extended period of time was taken into account to be able to capture the dynamics of the river.

Chapter 2 of this thesis describes the development of an adequate sampling technique for both chemical and microbial contaminants in an RBF system along the Danube with a high variability in surface water and groundwater levels. During two sampling campaigns, which were performed at wells situated close to the Danube and in a transect along the groundwater flow path further away from the river, several well volumes were analyzed for a combination of OMPs, microbial contaminants and bacterial abundance and activity. It was investigated whether samples for the analysis of previously mentioned parameters could be taken simultaneously with those collected for standard chemical parameters.

Chapter 3 uses the sampling technique described in chapter 2 as a basis for the analysis of the microbial characteristics of the aquifer. These characteristics comprise total bacterial abundance, biomass and bacterial activity, which are important features of groundwater used as drinking water. They are likely to be influenced by the interaction with surface water. The relation between these characteristics and the hydrological

dynamics was studied by several groups, but they were either limited to the distance between the river and the groundwater wells or to water table changes. Important hydrogeological factors like aquifer characteristics and the potential difference between river water and groundwater level were not taken into account. Therefore, during a period of 20 months, samples were taken both very close to the Danube as well as further away along a transect towards a drinking water abstraction well. Due to the high variability in surface water levels, not only the seasonal dynamics was taken into account, but also the dynamics during two separate flood events. The results were than correlated to both seasonal responses and responses to nutrient stimuli, and to hydrological fluctuations.

Chapter 4 addresses the question what the influence of these fluctuations is on the transport and removal of a total of 7 OMPs. Amongst them were benzotriazole (BTri), carbamazepine (CBZ) and sulfamethoxazole (SMZ), which were identified as WWTP marker parameters. During a period of 27 months, samples were taken from both surface water and groundwater locations. To be able to identify the difference between near steady state conditions and conditions with a higher dynamics, two flood events were sampled with a higher sampling frequency. The analyzed OMPs were correlated to hydrological parameters such as the potential difference between river water and groundwater level and mixing with other sources of water in order to quantify their removal.

2. Does Pumping Volume Affect the Concentration of Micropollutants in Groundwater Samples?

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Key messages

- Samples taken for the analysis of micropollutants and standard chemical parameters were stable during pumping
- Samples drawn directly after the onset of pumping were not representative for the microbiological water quality

Abstract

Information on concentrations of micropollutants (such as pharmaceuticals, pesticides and industrial chemicals) in most highly dynamic riverbank filtration (RBF) systems is lacking, in contrast to data on standard chemical parameters. Sampling protocols have thus far been based on the stabilization of standard chemical parameters in relatively pristine environments. To determine whether groundwater samples for micropollutant analysis can be taken at a similar pumping volume as samples for testing standard chemical parameters in both environments, three groundwater monitoring wells in an RBF system were sampled at two points in time (after pumping of 3 well volumes and after pumping of 15 well volumes). Micropollutant concentrations were not significantly different between the two sampling points; therefore, appropriate samples can be drawn after pumping 3 well volumes. For certain microbiological parameters, a statistically significant difference in concentration was found.

2.1 Introduction

Particularly in aquifers that are used for drinking water, such as riverbank filtration (RBF) systems (J Derx et al., 2013; Hiscock and Grischek, 2002), groundwater quality is of great importance, and appropriate water-protection measures should be applied (European Parliament, 2006). An important task for hydrogeologists and water hygienists is to obtain representative groundwater samples when exploring the groundwater quality. A key environmental problem expected in the near future is the increasing contamination of surface- and groundwater bodies with thousands of chemical compounds. Many long-term effects of, for example, micropollutants on aquatic life and on human health remain unknown (Schwarzenbach et al., 2006). Groundwater originating from aquifers which are influenced by surface waters, such as RBF systems frequently contain micropollutants (Heberer 2002; Heberer et al. 2004; Kreuzinger et al. 2004; Hoppe-Jones et al. 2010; Huntscha et al. 2013). It is therefore of paramount importance that groundwater samples are representative of the part of the aquifer surrounding the monitoring well. To adequately determine micropollutant concentrations, adequate sampling procedures that can tackle changes caused in the RBF system by high variability in river water levels are needed. The current standard procedure for sampling groundwater is to pump the monitoring well for 3-5 well volumes (DVWK,

1992; Nielsen and Nielsen, 2007; USEPA, 1986) or until various physico-chemical parameters, such as pH, temperature, electrical conductivity (EC), and dissolved oxygen, stabilize (BMLFUW, 2015; Robin and Gillham, 1987). In several studies, field tests for obtaining representative groundwater samples were performed (Robin and Gillham 1987; Gibs and Imbrigiotta 1990; Barcelona et al. 1994; Puls and Paul 1995; Novak and Watts 1998; Barcelona et al. 2005; Kwon et al. 2008; Kozuskanich et al. 2011; Shani et al. 2012; Harter et al. 2014; Roudnew et al. 2014). Most focused on standard chemical parameters or microbiological constituents. The study by Novak and Watts (1998) tested pesticide concentrations in shallow coastal plain aquifers which were not influenced by large water table fluctuations. In aquifers with low hydraulic conductivities (ranging from 2.1×10^{-4} to 2.9×10^{-5} m/s), pesticides were shown to stabilize after pumping two well volumes. Gibs and Imbrigiotta (1990) showed that organic compounds such as benzene stabilized in 55% of the cases after purging three well volumes in unconfined sand and gravel aquifers. Barcelona et al. (1994), on the other hand, showed that pumping only a fraction of a bore volume (<50%) was sufficient to achieve stabilization of volatile organic compounds such as trichloroethylene. These studies showed the amount of well volumes pumped before stabilization was reached differ between the type of compound and the type of aquifer. These studies had in common that the studied aquifers were little permeable with hydraulic conductivity values of less than 1×10^{-4} m/s, and were not under direct influence of surface water. Due to the high variability in water levels and input concentrations in the river, it was expected that a low number of pumping volumes as used by previous mentioned studies would not be sufficient to test whether micropollutants stabilize similarly to standard chemical parameters.

We therefore investigated whether pumping time affects micropollutant concentrations in a highly dynamic RBF environment and whether samples can be obtained simultaneously with those for testing standard chemical parameters. To test the hypothesis that micropollutant concentrations after 3 well volumes are not statistically different than those found after 15 well volumes, three representative groundwater monitoring wells were sampled. Several other chemical and microbiological parameters were measured to allow cross-comparison and to support interpretation of results.

2.2 Research method

The site investigated was the Porous Groundwater Aquifer (PGA) study site

(see Figure 1). It is an alluvial backwater and floodplain area, extending on the left bank of a river downstream of the city of Vienna. A total of five groundwater abstraction wells are located in the PGA. When water levels in the river rise, water flows from the river into the backwater river of the floodplain, causing regular flooding events. The main river always infiltrates into the aquifer, which is part of one of the main groundwater bodies in Austria. Groundwater quality in the area is therefore potentially influenced by a combination of anthropogenic activities, industry, wastewater treatment plants further upstream, and flooding events. Surface waters in the PGA have been extensively studied since they are regularly situated in the well capture zones of the groundwater abstraction wells. The PGA was monitored with a high temporal and spatial resolution with more than 200 hydraulic pressure data loggers distributed over the study area. Furthermore, a calibrated 3D groundwater flow and transport model was available which was used to study the transient well capture zones and the impact of river water level fluctuations on the microbiological groundwater quality (Farnleitner et al. 2014). However, groundwater data related to the behavior of chemical contaminants has been scarce. The riverbank in this

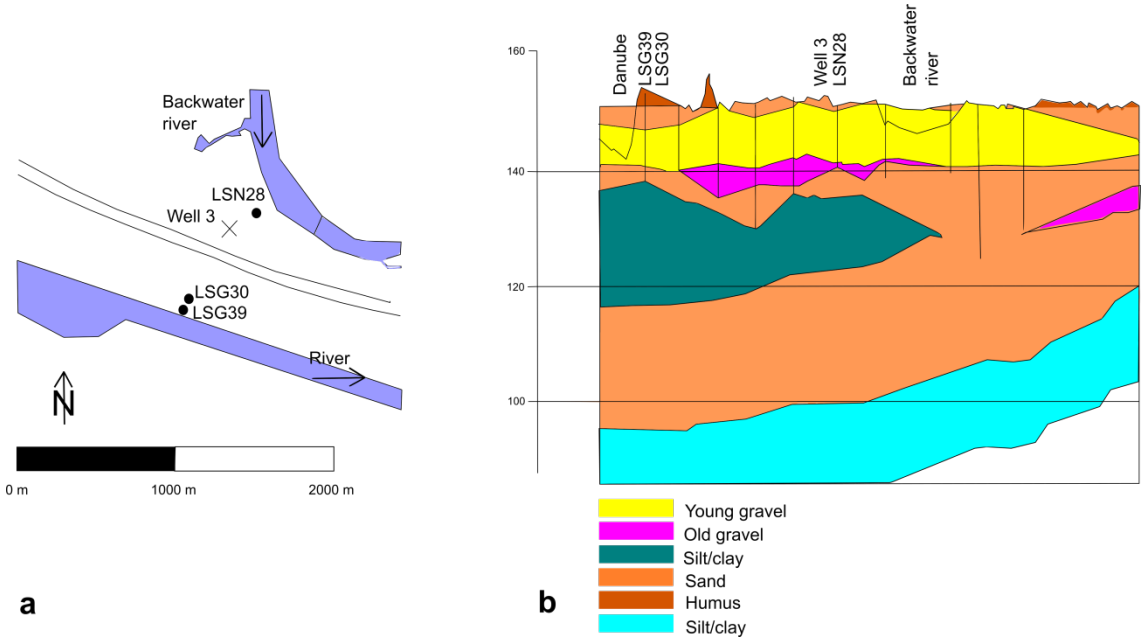

Figure 1 **a)** Situation of the three sampled groundwater monitoring wells LSG39, LSG30 and LSN28. The groundwater abstraction well is depicted as well 3 and **b)** schematic cross section of the transect with the hydrogeological layers

area consists of riprap. Due to clogging between these boulders, no or almost no infiltration directly through the riverbank occurred. River water can infiltrate into the groundwater only through the riverbed (Blaschke et al., 2003). The upper layer of the PGA consists of silt and has a thickness from 1 to 10 meters. The underlying confined aquifer consists of sand and gravel and has a thickness in between 3 and 15 meters. Hydraulic conductivities of

the PGA range from 5 x 10^{-2} to 5 x 10^{-4} m/s as depicted by a 3D groundwater flow and transport model after calibration to both steady flow conditions during high pumping rates of the well and to transient flow conditions during a flood event (Farnleitner et al. 2014).These values were also confirmed by pumping tests conducted in the area. Underneath the aquifer are alternating sand and clay/silt layers. Conditions in the PGA are predominantly oxic. Dissolved organic carbon concentrations of the aquifer ranged from 0.5 to 4.0 mg/L, with most of the concentrations below 2 mg/L (data from 2005 to 2013, Mayr et al. 2014).

Two sampling campaigns were performed at wells which were situated in a transect along the groundwater flow direction toward groundwater abstraction well 3 (PGAW3) and the backwater river as described below. The monitoring wells, with a diameter of 2-5 inches, extended from the surface till the clay layer, which was at a maximum depth of 14 m. The construction details of one of the wells are given in the supporting information (Appendix A Figure 12). The wells were situated in an area with high variation in groundwater levels, which represented the high dynamics of the system. Two wells were situated close to the river, one (LSG39) 10 m from the river and one (LSG30) 19 m from the river. The other well (LSN28) was located 705 m from the river, between PGAW3 and the backwater river. Particle tracking simulations performed with the calibrated 3D model revealed that the travel times from the Danube towards LSG39 ranged from 1.5 days to 18.5 days during mean flow conditions and to a maximum of 1 day to 10 days during high flow conditions (Derx et al., 2013). Travel times towards LSG30 were in a similar range as LSG39. Travel times from LSN28 towards PGAW3 were influenced by both the backwater and the pumping rate of PGAW3. Travel times increased from 62 days to >100 days during low flow conditions. As a measure for groundwater level dynamics, the sum of the absolute differences of hourly groundwater levels over the course of a year was calculated (the higher the sum, the higher the dynamics). In order to allow for comparability, we chose a time period in which continuous data was available for all wells. Therefore, the period between April 2015 and December 2015 was chosen.

Samples were taken in August and September 2014, where sampling was performed at an abstraction rate of 0.77 L/s for a total of 15 well volumes. Groundwater levels were measured with pressure transducers during sampling in order to quantify whether a drawdown occurred in the monitoring wells. Water levels in the river and groundwater fluctuated considerably between the two campaigns. During the second sampling

campaign, PGAW3 was pumping, which caused a difference in groundwater travel time from either the river or the backwater river toward PGAW3.

After pumping of 3 well volumes and again after pumping of 15 well volumes, 1-L samples were obtained and stored in glass bottles in the dark until analyses for a set of eight micropollutants. Directly afterwards, 250-mL samples were obtained and stored in plastic bottles for analysis of several organic parameters. In addition, 4-L samples were obtained at 15-min intervals and stored in sterilized plastic containers for bacteriological analysis per ISO standards and published protocols (Simon and Azam 1989; Kirschner and Velimirov 1999; Farnleitner et al. 2010; Riepl et al. 2011).

Temperature, pH, and EC were measured in the field using a portable Sension+ MM150 sensor system (Hach-Lange, Austria). To show whether there was a contamination due to the river, carbamazepine (CBZ; Drewes et al. 2003; Clara et al. 2004; Huntscha et al. 2013), benzotriazole (BTri; Kahle et al. 2009; Huntscha et al. 2013), and sulfamethoxazole (SMZ; Kolpin et al. 2002; Miao et al. 2004) were analysed. All micropollutants were determined using solid phase extraction (SPE) combined with HPLC-MS/MS (see Appendix A for a detailed chemical and microbial analysis description and definition of parameters). Tested microbiological parameters included *Escherichia coli*, intestinal enterococci, bacterial spores from aerobic spore formers representing microorganisms in their permanent stage, total bacterial abundance (including presence or absence of biofilm particles), and bacterial 3H-leucine incorporation (LI; Kirschner and Velimirov 1997; Cole 1999; Eiler et al. 2003; Kirschner et al. 2009).

A normalization procedure (z-transformation) was performed to enable pooling and comparison of parameters between 3 and 15 well volumes for all wells. This normalized deviate was calculated using

$$C_{st}^{n} = \frac{c^{i} - m_c}{s_c} \qquad (1)$$

where C_{st}^{n} is the normalized deviate, c^{i} is the original concentration, m_c is the sample mean of c, and s_c is the sample standard deviation of *c*. Using these standardized numbers, a Mann-Whitney test (Sokal and Rohlf, 1997) was performed to assess potential differences between samples taken after 3 and 15 well volumes.

2.3 Results

As seen in Table 1, the technical duplicates deviated by less than 20% from the mean. Supported by high recovery values of the solid phase extraction method (48%-92%, Ternes and Joss 2007), the analytical method was appropriate for the purpose of this investigation. A Mann-Whitney test performed using normalized concentrations revealed no statistically significant difference in micropollutant concentration after 3 versus 15 pumping volumes (see Appendix A Table 14).

Table 1 Micropollutants found in duplicate samples after pumping 3 well volumes and 15 well volumes

Parameter	LSG39 3 vol[1]	LSG39 15 vol[1]	LSN28 3 vol[1]	LSN28 15 vol[1]	LSN28 3 vol[2]	LSN28 15 vol[2]	LSG30 3 vol[2]	LSG30 15 vol[2]
BTri	60.0	62.5	<LOQ	<LOQ	<LOQ	<LOQ	95.2	84.8
ng/L	52.8	82.0	<LOQ	<LOQ	<LOQ	4.50	66.0	69.3
	56.4 (6%)	**72.3 (13%)**					**80.6 (18%)**	**77.1 (10%)**
CBZ	14.6	11.4	3.85	3.28	4.36	3.70	18.4	17.6
ng/L	13.2	16.4	3.40	2.83	3.51	3.72	15.0	11.7
	13.9 (5%)	**13.9 (18%)**	**3.63 (6%)**	**3.06 (8%)**	**3.93 (11%)**	**3.71 (1%)**	**16.7 (10%)**	**14.6 (20%)**
SMZ	3.27	3.08	LOD	LOD	LOD	LOD	6.00	3.78
ng/L	3.17	2.11	LOD	LOD	LOD	LOD	5.00	4.23
	3.22 (2%)	**2.59 (19%)**					**5.50 (9%)**	**4.00 (5%)**

Note: Values in bold are arithmetic means; values in parentheses are deviations from the mean.
LOQ = limit of quantification; LOD = limit of detection.
[1]August 2014 sampling campaign.
[2]September 2014 sampling campaign.

The standard chemical parameters and the physicochemical parameters also showed no statistically significant difference in concentrations (see Table 2).

The micropollutant with the highest groundwater concentration was BTri. It was below the limit of quantification (<LOQ) in well LSN28 but averaged 80.6 ng/L in LSG30. On the other hand, SMZ was below the limit of detection (LOD) in LSN28 but was found in all other wells, averaging up to 6.00 ng/L. CBZ was found in all wells, averaging as little as 3.34 ng/L in LSN28 and as much as 15.7 ng/L in LSG30. All other micropollutants were

below the LOD (see Table 1). Micropollutant concentrations were higher closer to the river and decreased significantly toward the backwater river.

Table 2 Standard chemical and microbiological parameters found after pumping 3 and 15 well volumes

Parameter	LSG39 3 vol[1]	LSG39 15 vol[1]	LSN28 3 vol[1]	LSN28 15 vol[1]	LSN28 3 vol[2]	LSN28 15 vol[2]	LSG30 3 vol[2]	LSG30 15 vol[2]
pH	7.3	7.44	7.47	7.44	7.44	7.49	7.71	7.66
EC (µS/cm)	457	433	555	559	558	547	367	374
Temp (°C)	14.4	15.3	14	13.5	14.6	15.8	17.7	16.7
$CaCO_3$ (mg/L)	138	136	175	177	175	177	112	113
TOC (mg/L)	0.85	0.84	0.88	0.88	0.80	0.80	0.90	0.90
NH_4^+ (mg/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
NO_2^- (mg/L)	0.01	0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
NO_3^- (mg/L)	1.7	1.80	<LOQ	<LOQ	<LOQ	<LOQ	3.30	3.20
Ca^{2+} (mg/L)	71.0	69.0	82.0	82.0	82.0	82.0	55.0	56.0
Mg^{2+} (mg/L)	13.0	13.0	22.0	22.0	22.0	22.0	12.0	12.0
Cl^- (mg/L)	12.0	12.0	16.0	16.0	16.0	17.0	12.0	12.0
SO_4^{2-} (mg/L)	26.0	26.0	36.0	37.0	35.0	35.0	23.0	23.0
Na^{2+} (mg/L)	9.20	9.20	12.0	12.0	12.0	12.0	8.20	8.20
K^+ (mg/L)	1.90	1.90	2.10	2.10	2.20	2.20	1.90	1.90
Bacterial spores (#/L)	180	70	1100	530	900	1400	500	140
Bacterial abundance (cells/mL)	2.54E+5	2.79E+5	2.96E+5	3.28E+5	2.03E+5	2.22E+5	1.51E+5	1.30E+5
Leucin incorporation (pmol/L/h)[3]	0.275 (0.049)	0.181 (0.013)	0.062 (0.002)	0.041 (0.012)	0.065 (0.003)	0.369 (0.150)	0.064 (0.009)	0.056 (0.003)

Note: Values in parentheses are deviations from the mean.
LOQ = limit of quantification.
[1]August 2014 sampling campaign.
[2]September 2014 sampling campaign.
[3]Average (standard deviation) of technical replicates.

Physicochemical and standard chemical parameters were used to describe general water quality. Temperature, pH, and EC stabilized after pumping

only one well volume (approximately 4 minutes, see Appendix A Figure 11). Of the standard chemical parameters, none were significantly different at any point in time (see Table 2). Temperature and EC varied through the system. *E. coli* was not detected in any of the wells after pumping of 3 to 15 well volumes. Enterococci were not detected in LSG39 or LSG30, but were detected in low numbers after pumping of 15 well volumes in LSN28 in September (data not shown). In addition, bacterial spores, LI, and total bacterial abundance were used to describe general microbiological characteristics. Bacterial spores were found in all samples and ranged from 20 cfu/L in LSG39 in August to 1500 cfu/L in LSN28 in September. A Mann-Whitney test performed with normalized concentrations revealed statistically significant evidence that pumping time influences LI (see Appendix A Table 14). The concentration of bacterial spores was generally highest in LSN28. Bacterial LI was different between LSG39 and wells further from the river, falling as distance from the river increased. Samples taken after pumping of 3 well volumes and sometimes up to 6 well volumes contained biofilm (see Appendix A), especially in wells closer to the river.

The dynamics of the system was shown by the calculation of the absolute differences in groundwater levels. LSN28 had the lowest dynamics, of 24.6m in a 9-month period. Further towards the river, the dynamics increased to 30.70 m for LSG30 and 31.07 m for LSG39. Measurements of groundwater levels taken during sampling showed the groundwater level decreased only 5 cm during pumping.

2.4 Discussion

It has been shown that human pharmaceuticals in rivers can vary on a daily basis due to the fluctuations in WWTP effluent concentrations (Kreuzinger, 2007; Weigelhofer et al., 2015; Zoboli et al., 2015). Furthermore, the discharge fluctuations in the river can also have an impact on micropollutant concentrations in the river. Despite of these fluctuations, the concentrations in the PGA were demonstrated to be the same whether a monitoring well was pumped for 3 or 15 well volumes. No detectable difference between stabilization of micropollutant concentrations and standard chemical parameter concentrations was found, as suggested by Gibs and Imbrigiotta (1990). Hydraulic conductivities from the study of Gibs and Imbrigiotta (1990) were lower than in the PGA (a maximum value of 1 x 10^{-3} m/s versus 5 x 10^{-2} m/s in this study). Furthermore, no change in stabilization of micropollutant concentrations between the monitoring wells

was found, although a difference in stabilization between monitoring wells was suggested by Novak and Watts (1998). Because hydraulic conductivities at the study site were high, the standard chemical parameters stabilized earlier than in a chalk environment, studied by Sorensen et al. (2013) and by Kwon et al. (2008). Differences in micropollutant concentrations from well to well can be explained by different travel times, dilution of the infiltrating river water and the effectiveness of removing contaminants in an RBF system by for example biodegradation or sorption (Hamann et al., 2016; Henzler et al., 2014). Therefore, concentrations in wells LSG39 and LSG30 were higher than those in LSN28.

Microbiological parameters were more variable, particularly LI, as was shown by Kwon et al. (2008) and Roudnew et al. (2014). In this paper, the maximum increase in bacterial population was much lower than was found by Kwon et al. (2008). This can be explained by the high hydraulic conductivity values of the PGA and the presence of major external influences, as opposed to a relatively pristine environment lacking such influences. Although Roudnew et al. (2014) suggested more stability of microbial parameters due to a constant recharge by a river, this can only partly be concluded from this study. Harter et al. (2014) suggested that field water quality parameters were sufficient indicators to screen wellbore and near-well microbiological contamination. However, we found a large amount of biofilm present once the micropollutants and physicochemical parameters stabilized. Because *E. coli* could be present and could detach from biofilm (LeChevallier et al. 1987; Banning et al. 2003), these samples are unlikely representative for the aquifer, even if *E. coli* concentrations were below the LOD. The high number of biofilm particles was most likely caused by the location of the river and the great amounts of nutrients in the area.

The pumping rate caused a minor drawdown of 5 cm during sampling. Vandenberg and Varljen (2000) and Barcelona et al. (2005) showed that stabilization (and not the degree of drawdown) was important in collecting representative samples. Pumping rates, however, could influence the sampled microbiological community. In coarse gravel, like in the PGA, variations in the microbiological community induced by pumping could not be distinguished from natural temporal variations (Shani et al., 2012). Because of the high conductivity of the PGA aquifer and the low drawdown, we do not propose use of a low-flow pumping procedure.

2.5 Conclusion

Results of the studied alluvial porous groundwater aquifer clearly demonstrate that samples for determining micropollutant concentrations, can be taken at the same time as those taken for determining standard chemical parameters (e.g., after 3 well volumes). This might also apply to similar sites where the aquifer is strongly influenced by surface waters and where the hydraulic conductivity of the aquifer is in a similar range. No statistical significant evidence was present that suggested micropollutant concentrations were not stable during pumping, neither was there for standard chemical parameters. The fluctuations of the watertable and the fluctuation of contaminant concentrations in the river did not affect the stabilization of the chemical parameters. Leucine incorporation however did show a statistically significant difference between the samples taken at the two different points in time. Furthermore, samples taken after pumping of 3 well volumes from wells close to the river do not represent the microbiological quality of the study site due to the presence of biofilm. Stabilization of standard chemical parameters and micropollutants is insufficient for measuring microbiological parameters.

2.6 Acknowledgements

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3. Spatiotemporal analysis of bacterial biomass and activity to understand surface and groundwater interactions in a highly dynamic riverbank filtration system

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Key messages

- Bacteria in groundwater in RBF systems are influenced by the infiltrating river
- Fluctuations in bacterial variables are linked to the hydrological dynamics
- An increased influence was observed during flood events
- During flood events the infiltration extends further into the aquifer
- Increases in bacterial numbers and activity are not caused by a nutrient input

Abstract

Characterization of surface water – groundwater interaction in riverbank filtration (RBF) systems is of decisive importance to drinking water utilities due to the increasing microbial and chemical contamination of surface waters. These interactions are commonly assessed by monitoring changes in chemical water quality, but this might not be indicative for microbial contamination. The hydrological dynamics of the infiltrating river can influence these interactions, but seasonal temperature fluctuations and the supply of oxygen and nutrients from the surface water can also play a role. In order to understand the interaction between surface water and groundwater in a highly dynamic RBF system of a large river, bacterial abundance, biomass and carbon production as well as standard chemical parameters were analyzed during a 20 month period under different hydrological conditions. In the investigated RBF system, groundwater table changes exhibited striking dynamics even though flow velocities were rather low under regular discharge conditions. Bacterial abundance, biomass, and bacterial carbon production decreased significantly from the river towards the drinking water abstraction well. The cell size distribution changed from a higher proportion of large cells in the river, towards a higher proportion of small cells in the groundwater. Although biomass and bacterial abundance were correlated to water temperatures and several other chemical parameters in the river, such correlations were not present in the groundwater. In contrast, the dynamics of the bacterial groundwater community was predominantly governed by the hydrogeological dynamics. Especially during flood events, large riverine bacteria infiltrated further into the aquifer compared to average discharge conditions. With such information at hand, drinking water utilities are able to improve their water abstraction strategies and react quicker to changing hydrological conditions in the RBF system.

3.1 Introduction

Riverbank filtration (RBF) systems are important sources for drinking water abstraction in many countries (Henzler et al., 2014; Hoppe-Jones et al., 2010; Ray et al., 2002; Tufenkji et al., 2002) due to their effective removal of contaminants like bacteria (Pang et al., 2005), viruses (Schijven and Hassanizadeh, 2000) and organic micropollutants (Huntscha et al., 2013; Massmann et al., 2008). During RBF, surface water interacts with the

aquifer and may pose a threat for the microbial and chemical water quality. These interactions and their exchange processes and pathways are of vital importance for the protection of water resources used by drinking water utilities. Although surface water infiltration into the aquifer can be indicated by changes in physical and chemical water quality characteristics, these may not be necessarily indicative for the transport of microorganisms and pathogens (Taylor et al., 2004). All these processes are dependent on hydrogeological, biochemical and biological factors (Hiscock and Grischek, 2002) and take place mostly in the transition zone (Kalbus et al., 2006). In this zone, hydrogeological characteristics affect flow velocity, infiltration rates and mixing proportions of river water with groundwater and impact the efficacy of the reduction or elimination of contaminants. Although the transition zone usually extends not more than a few meters away from the river bank, it can extend up to several kilometers inland in large alluvial river systems with highly porous aquifers (Boulton et al., 1998; Stanford and Ward, 1988). Due to the infiltration of oxygen-rich river water high in particulate (POC) and dissolved organic carbon (DOC), the highest biological activity, which depends particularly on bacteria (Craft et al., 2002; Findlay et al., 1993; Pusch, 1996), can be found in the hyporheic zone (Gibert and Mathieu, 1997).

The nature and extent of surface water-groundwater interaction can be determined by assessing the changes in the microbial characteristics of both water bodies, such as total bacterial abundance, biomass and activities. Changes of these parameters in the groundwater are likely to be influenced by the interaction with surface water and can be affected by the composition of the aquifer material, the hydraulic gradient, temperature fluctuations in the surface water, and the supply of oxygen and inorganic nutrients (Bott and Kaplan, 1985; Vanek, 1997). Bacterial abundance, biomass and activities are also important features of groundwater or spring water used as drinking water (Farnleitner et al., 2005). Due to their importance, several studies (Brugger et al., 2001; Ellis et al., 1998; Lin et al., 2012; Stegen et al., 2016; Velasco Ayuso et al., 2009b; Zhou et al., 2012) examined the changes in the microbial characteristics in relation to the hydrological dynamics. In addition to hydrological dynamics, groundwater quality and seasonal temperature fluctuations were also shown to have an influence on the microbial characteristics. These fluctuations impacted the microbial characteristics to the greatest extent where river water and groundwater mixing was greatest (Lin et al., 2012). It could even be that less frequent and large increases in river water levels may enhance the microbial activity due to the transport of larger quantities of labile organic carbon into the hyporheic zone (Stegen et al., 2016).

An approach to study changes in microbial groundwater characteristics is the analysis of spatiotemporal patterns in bacterial biomass and activity. Some studies exist that correlate bacterial biomass and activity with hydrogeological metrics, but they were either limited to the distance between the river and the groundwater wells or to water table changes (Brugger et al., 2001; Ellis et al., 1998). Important hydrogeological factors like aquifer characteristics and the hydraulic gradient were not taken into account. Furthermore, samples in these studies were taken along relatively small rivers and large increases in river water levels during flood events were not examined. Therefore, the main goal of our study was to examine surface water-groundwater interactions by assessing bacterial biomass and activity changes in a large and highly dynamic river over an extended period of time. The following questions were therefore addressed: (i) is microbial water quality in an RBF system vulnerable to surface water infiltration, especially during flood events? If so, (ii) are these changes primarily caused by the hydrological dynamics or do temperature and geochemical changes also play an important role? As the transition zone can extend up to several kilometres inland in large alluvial systems, another objective (iii) is to quantify the extent of the river's influence on the bacterial dynamics. For this purpose, river water and groundwater samples from six monitoring wells and one drinking water abstraction well in a porous aquifer (PGA) were taken on a monthly basis from October 2014 to May 2016. The monitoring wells were located along a gradient from the river towards the drinking water abstraction well. In order to account for changes in biomass and activity under extreme flow conditions, two flood events were sampled more extensively.

3.2 Materials and methods

3.2.1 Study site

The study site is a porous aquifer (PGA, Figure 2) along the river Danube, the second longest river in Europe and the most international river in the world with 19 countries within its catchment area. This alluvial backwater and floodplain area with forest, meadows and surface water bodies is located on the left bank of the Danube, downstream of the Austrian capital of Vienna. The floodplain is part of a national park and a Natura 2000 protected area as well as a drinking water protection zone with an area of approximately 50 km^2 (Derx et al., 2013) situated within one of the main groundwater bodies of Austria. Five groundwater abstraction wells are located in the floodplain, making the aquifer an important drinking water

resource. The local groundwater flow direction is from southwest to northeast. There is continuous infiltration of river water into the groundwater. The riverbank in this area consists of riprap. Due to clogging between these boulders, no or almost no infiltration directly through the riverbank occurs. River water can therefore only infiltrate into the groundwater through the riverbed (Blaschke et al., 2003). The backwater river is connected with the Danube above a water level of 150.5 meter above

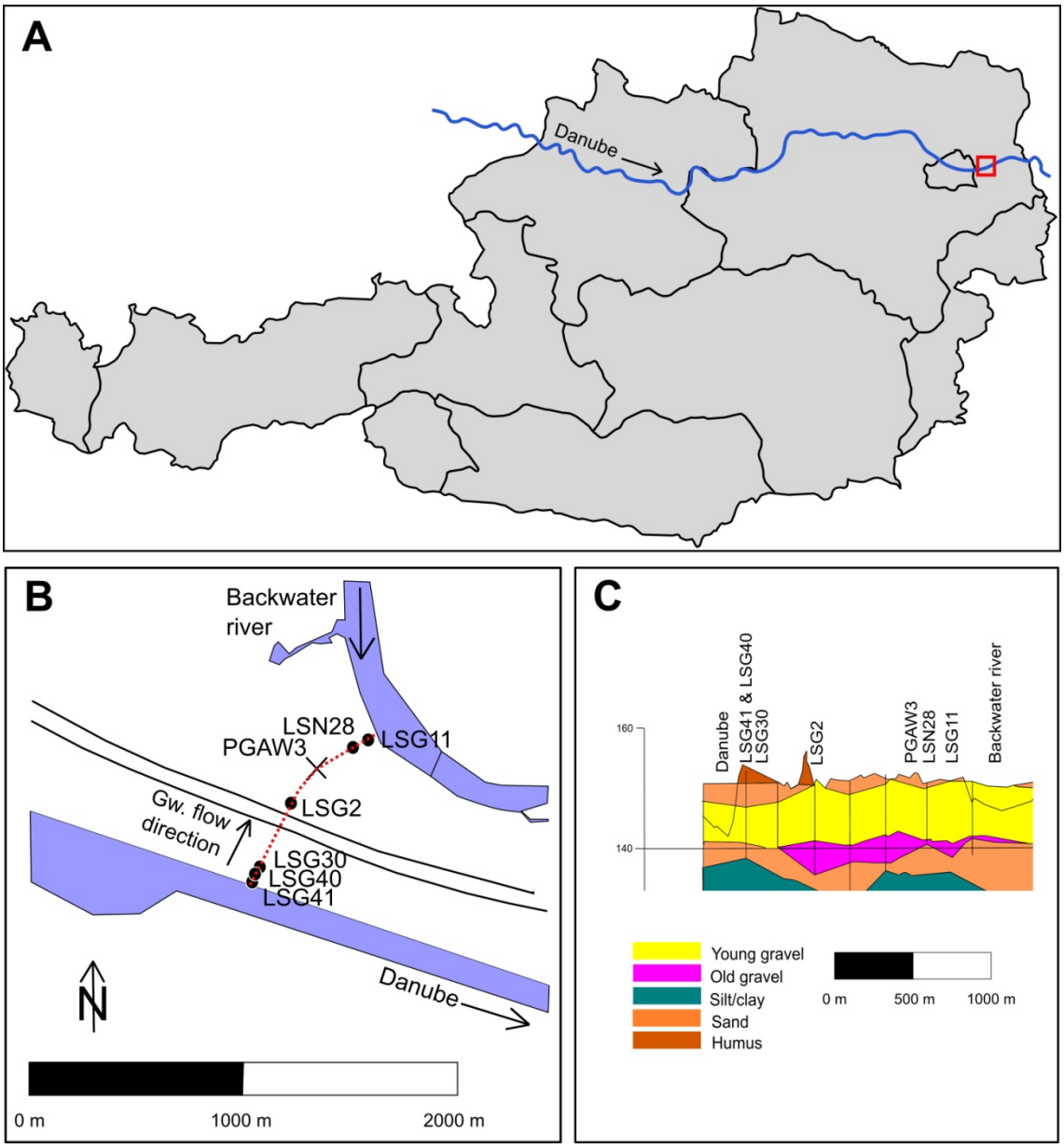

Figure 2 **a)** Situation of the Natura 2000 protected area (red square) in Austria, **b)** the sampled transect including monitoring wells LSG41, LSG40, LSG30, LSG2, LSN28 and LSG11. The groundwater abstraction well is depicted as PGAW3 and **c)** schematic cross section (dotted red line in b) of the transect with the hydrogeological layers and the groundwater monitoring wells (shown as black vertical lines)

the Adriatic Sea level (m a.A.) in the Danube at the station Fischamend (occurring just below a flood event with a recurrence of 1 year, Appendix B Figure 13).

By means of multiple borehole logs and topset bed exploration, 4 different soil layers are distinguished. Figure 2c shows a cross section of the studied transect. The upper layer of the PGA consists of sand (orange) and humus (dark orange) and has a thickness varying from 1 to 5 m. The main layers of the aquifer are young (yellow) and old Danube gravel (pink) and sand (orange). The aquifer has a thickness varying from 4 to 10 m along the transect. Underneath the aquifer there are alternating clay/silt (cyan) and sand layers (not shown). Hydraulic conductivities for the PGA were determined using a 3D groundwater flow and transport model that was calibrated to both steady flow conditions during high pumping rates of the wells and to transient flow conditions during a flood event (Farnleitner et al., 2014). The hydraulic conductivities in the entire PGA ranged from 5 x 10^{-4} m/s to 5 x 10^{-2} m/s and were also confirmed by pumping tests conducted in the area. In the studied transect, interpretation of the calibrated 3D model and geophysical measurements showed that the hydraulic conductivity (0.016 m/s) and the effective porosity (0.125) were constant. Mayr et al. (2014) showed conditions in the PGA were predominantly oxic. Groundwater gradients, flow velocities and travel times from the Danube towards the groundwater abstraction well were calculated using the measured water levels between the Danube and PGAW3 and equation 2. The water level gradients were calculated for each sampling date. The corresponding travel times ranged from a minimum of 11.5 days to a maximum of 47.4 days. These travel times correspond to the direct and thus shortest flow paths from the Danube to PGAW3.

In order to capture the dynamics of the system, groundwater gradients were estimated by calculating the differences in water levels between the wells where the measurements were taken on each sampling date. The gradient in monitoring well LSG41 was based on the water level difference between the Danube and the well. The gradient in LSG40 was based on the piezometric head difference between LSG41 and LSG40 etc. These values were then divided by the distance between the two points (Appendix B Table 15). The gradient is positive whenever the groundwater flow direction is from the Danube towards the groundwater abstraction well and further towards the backwater river. The flow velocities in the saturated zone were based on the gradients between the wells and calculated for each well pair according to the following equation:

$$v = \frac{K\Delta h}{n_e} \quad (2)$$

where v is the flow velocity (m/s), K is the hydraulic conductivity (with a value of 0.016 m/s), Δh is the gradient (-) and n_e is the effective porosity (with a value of 0.125).

3.2.2 Sampling

Monthly samples (n=18, Appendix B Figure 14) were taken from October 2014 to May 2016 in a transect extending from the Danube towards a drinking water abstraction well and the backwater river. In this period, river discharges ranged from 693 m^3/s to 6197 m^3/s (Appendix B Figure 14). During two flood events with a one-year return period in May 2015 (HQ2015) and February 2016 (HQ2016), samples were taken at an increased frequency in order to account for differences in infiltration during increased groundwater flow velocity (n=25, Appendix B Figure 14). Two surface water locations and 6 groundwater monitoring wells as well as the drinking water abstraction well were sampled during each monthly sampling event. During the flood events, samples were collected from the Danube and two wells close to the river (LSG41 and LSG30). Three of the groundwater monitoring wells (LSG41, LSG40 and LSG30) are situated close to the river to capture the high variability in river and groundwater levels in the system (Figure 2). LSG2 is located between these three wells and the drinking water abstraction well (PGAW3). LSN28 and LSG11 are situated between the drinking water abstraction well and the backwater river. All monitoring wells were screened from 1 m below the surface till the silt/clay layer, over a length of approximately 14 m. Groundwater levels were recorded manually during all sampling events. Additionally, hourly hydraulic pressure and temperature values were recorded continuously in all groundwater monitoring wells from October 2014 until May 2016. Furthermore, hourly recorded values for electrical conductivity were available for selected monitoring wells. Hourly river water level and discharge values from the station *Fischamend* (rkm 1908) between January 2014 and January 2017 were kindly provided by the Austrian federal waterway authority *viadonau*.

Groundwater samples for standard chemical parameters were taken after pumping of 3 well volumes, whereas samples for microbial parameters were taken after pumping of 15 well volumes (van Driezum et al., 2017). The samples were taken using a suction pump with an abstraction rate of 0.77 L/s. Temperature, pH, electrical conductivity and dissolved oxygen were measured in the field using a portable Sension+ MM150 sensor system (Hach-Lange, Austria) and a portable Profiline multi 3320 sensor system

(WTW, Germany). 250 mL of ground- and surface water was taken in clean plastic bottles to be analysed for standard chemical parameters, whereas autoclaved plastic gallons (4L) were used for the microbial parameters.

3.2.3 Organic and inorganic parameters

After pumping of 3 well volumes, 250 mL samples were filled in plastic bottles and transported to the lab in a cooling box of 4 °C for the analysis of inorganic parameters. The samples were stored in the lab at 4 °C before analysis. Samples were analyzed for a large set of organic and inorganic parameters (Appendix A Table 13). Anion and cation analysis was performed using ion chromatography. Absorption photometry was used to measure ammonium and nitrite (Appendix A Table 13).

3.2.4 Bacterial cell counts

Total bacterial cell counts (TCC) was measured using the slightly modified protocol of Riepl, et al. (2011). Depending on the type of water, between 1 mL (surface water) and 100 mL (groundwater) of sample was fixed with paraformaldehyde. 200 µL to 40 mL was filtered on a 0.2 µm membrane filter (Anodisc 25, Whatman, Germany) and stained with SYBR® Gold (Fisher Scientific, Austria). The slides were either stored at -20 °C or analysed immediately with a Nikon epifluorescence microscope (Nikon Eclipse 50i). Cells were classified in large cells (rod shaped cells and coccoid cells with diameter > 1.0 µm) and small cells (coccoid cells with a diameter < 1.0 µm).

3.2.5 Bacterial ^{3}H-leucine incorporation

Bacterial 3H-leucine incorporation (LI) was measured based on protocols of Kirschner and Velimirov (1999) and Simon and Azam (1989). Briefly, 3H-leucine was added to triplicate 10 mL samples at a final concentration of 10 nM. Duplicate control samples were stopped with trichloroacetic acid (TCA, 5% final conc., Sigma-Aldrich, Germany) directly after the addition of 3H-leucine. Both controls and samples were incubated for 30 min (surface water samples) to 24 hours (groundwater sample) in the dark at the measured temperature of the aquifer. At the end of the incubation, samples were also stopped by adding TCA. One-hundred µL of 35% NaCl was added to enhance precipitation of macromolecules inclusive proteins and all samples were incubated for 30 min at 18 °C. After incubation, the samples were filtered through a cellulose nitrate filter (0.45 µm) which was subsequently washed with 5 mL of 5% TCA, 80% ethanol and distilled water each for the purification of proteins. Filters were dried overnight in scintillation tubes. After adding 5 mL of scintillation cocktail, radioactivity was measured in a Perkin Elmer, TriCarb 2300 TR scintillation counter.

3.2.6 Bacterial Carbon Production, Biomass and Turnover time

Bacterial carbon production (BCP) was estimated according to (Simon and Azam, 1989) using the following equation:

$$BCP = LI * 131.2 /(Leu\ per\ protein) * (cell\ C\ per\ protein) * ID \quad (3)$$

where LI is the leucine incorporation rate (mol/L/h), 131.2 is the molecular weight of leucine, Leu per protein is 0.073 (the fraction of leucine in protein), cellular carbon (C) per protein is 0.86 (Simon and Azam, 1989) and ID is the isotope dilution. Sufficiently high concentrations of leucine were added to compensate the ID. BCP values were given in ng C/mL/h. A constant value of 20 fg C per large bacterial cell and 10 fg C per small cell was used to calculate biomass (Bott and Kaplan, 1985; Lee and Fuhrman, 1987). Biomass values were given in ng C/mL. The turnover times of the bacterial biomass were calculated by dividing biomass with bacterial carbon production. Turnover time values were given in days.

3.2.7 Total Viable Counts

Total viable active counts (TVAC) were estimated according to Riepl et al. (2011). To assess the amount of cells that actively contribute to biomass production, the number of TVAC was determined in all groundwater wells during three separate sampling campaigns conducted during spring 2017. Samples were taken from all groundwater monitoring wells during this sampling campaign. Briefly, 1 mL of groundwater sample was filtered through a black, 0.4 µm pore-size polyester filter (CB04) and counterstained with 1 mL of counterstain medium CSE/2 (Biomérieux, France). After incubation of 1 h ± 5 min at 37 °C on a ChemSol A4 saturated labeling pad in a petridish, the labeling pad was transferred on a labeling pad saturated with dye (Chemchrome V6). This was incubated for another 30 min at 30 °C before transferring the pad to a membrane holder. Then, it was immediately enumerated with a solid-phase cytometer (Chemscan RDI; Biomérieux, France) using the Bioburden discrimination settings according to the manufacturer's instructions (Catala et al., 1999). Positive signals detected and discriminated as viable active cells by the system were inspected and validated visually (all signals if n ≤ 100 or 100 representative signals if n > 100). All working steps were performed under laminar airflow.

3.2.8 Statistical analysis

Correlation analysis of microbial parameters with hydrological, physical and chemical variables was performed using the Pearson product correlation and the Spearman rank order correlation. Normality of the data was tested by visual examination of the quantile-quantile plots. A P-value of 0.05 was set as a significance threshold. A multiple linear regression was performed

between several chemical parameters and BCP. To determine whether there was a statistically significant difference between the percentage of large cells in the surface water samples and in the groundwater samples, an ANOVA test and its associated *post-hoc* test were used (functions aov and TukeyHSD). All statistical analyses were performed using R 3.1.1., partly using the *Hmisc* package (v. 4.1.1).

3.3 Results and Discussion

3.3.1 Both the Danube and the backwater river influence groundwater quantity and quality in the study area

In order to get an insight in which parameters may have an influence on bacterial biomass and activity dynamics in the groundwater, it is of profound importance to identify the dynamics of the main hydrological parameters in the studied aquifer that can be affected by the Danube and the backwater river. Substantial water table fluctuations and gradients in temperature, pH and chemical constituents like nitrate and DOC are common characteristics of the transition zone. During the studied period, the water table change of the Danube was almost as high as 6 m (maximum value of 153.38 m a.A., minimum value of 147.54 m a.A., Table 3 and Figure 3) with a peak in late October 2014.

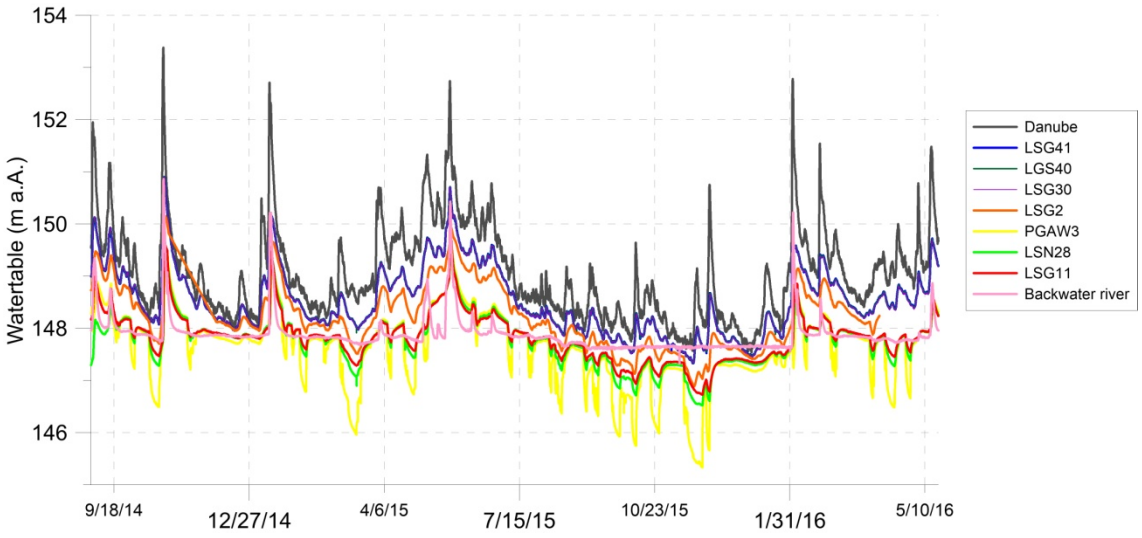

Figure 3 Watertables of both surface waters and all monitoring wells. Values of wells LSG41, LSG40 and LSG30 are very similar, therefore only the hydrograph of LSG41 can be distinguished

Water levels within the aquifer were consistently lower than surface water levels in the Danube. In the three nearest monitoring wells (LSG41, LSG40 and LSG30), groundwater tables exhibited striking hydrological dynamics (Figure 3), although the fluctuations were slightly lower than in the river

(maximum of 3.83 m). Due to pumping, groundwater tables were decreasing closer to the groundwater abstraction well PGAW3. The dynamics in wells PGAW3, LSN28 and LSG11 was similar with a maximum water table change of 3.7 m (Table 3). Groundwater gradients were calculated for all groundwater monitoring wells in the transect (Table 3). The gradients from the groundwater monitoring wells situated between the river and PGAW3 were predominantly positive, indicating that river water was infiltrating into the aquifer and groundwater flow was towards the groundwater abstraction well.

Table 3 Water tables, gradient, temperature and conductivity range of the surface and groundwater bodies during the studied period

	Water table difference (in m a.A.)	Gradient (%)[1]	Temperature range (in °C)	Electrical conductivity (µS/cm)
Danube	147.54-153.38	n.a.	2.8-23.2	329-882*
LSG41	147.33-151.16	1.97 - 18.6	7.5-16.1	367-757
LSG40	147.33-151.15	-0.26 - 0.28	8.2-15.3	317-914
LSG30	147.33-151.12	0.005 – 0.16	7.1-18.1	360-610
LSG2	146.89-150.15	0.04 – 0.37	9.9-14.4	418-575
PGAW3	145.33-149.96	0.13 – 0.64	10.9-14.1	480-544*
LSN28	146.52-150.22	-0.75 – 0.05	9.8-14.6	533-729*
LSG11	146.73-150.35	-0.26 – 0.06	9.8-14.5	438-548
Backwater	147.32-150.86	n.a.	0**-31.3	414-639*

[1] Gradient values are given as average values, with the minimum and maximum values given in parentheses. The gradient given at LSG41 is calculated from water table differences between the Danube and LSG41, at LSG40 water table differences between LSG41 and LSG40 were used etc. * Logger values for this parameter were not available. Instead, hand held measurements taken during the sampling campaigns were used. ** The backwater river was frequently frozen during the winter.

The gradient increased further towards PGAW3, due to constant pumping of the groundwater abstraction well. As expected, the gradient from PGAW3 towards LSN28 was predominantly negative, meaning groundwater flow towards PGAW3 from the direction of the backwater river (Figure 2). Contour maps created during different flow conditions of the Danube (Appendix B Figure 15) showed that the well capture zone of PGAW3 does not always include LSN28 and LSG11. Under certain conditions, the backwater was fed by the Danube (Appendix B Figure 13). This had an influence on the gradient between LSN28, LSG11 and the backwater river. During the rising limb of a flood event, water flows into the backwater river. Water levels in the backwater rise and cause an extension of the well capture zone towards LSN28 and LSG11. The gradient was negative and the groundwater flow direction was towards the groundwater abstraction well. The flow direction in the backwater reverses during the falling limb and the gradient simultaneously reversed. This was confirmed by a 3D groundwater flow and transport model (Farnleitner et al., 2014).

Table 4 Average values of (physico)chemical parameters and microbiological parameters during the monthly sampling campaigns. Values in brackets are min-max values. Values in meters are the distance to the Danube

	Danube	**LSG41**	**LSG40**	**LSG30**	**LSG2**	**PGAW3**	**LSN28**	**LSG11**	**Backwater**
Parameter	*0 m*	*10 m*	*13 m*	*24 m*	*283 m*	*551 m*	*704 m*	*782 m*	*882 m*
T (°C)	10.6 (3.5-22)	11.6 (7.9-19.4)	11.7 (8.3-18.2)	11.6 (8-17.1)	12.5 (8.1-14.8)	12.1 (11.2-14.4)	12.2 (10.6-14.8)	12.4 (11.5-14)	12.1 (4-29.1)
EC (µS/cm)	445 (329-882)	473 (411-575)	481 (413-582)	448 (398-518)	475 (401-549)	509 (480-544)	598 (533-729)	601 (529-689)	521 (414-639)
pH	8.0 (7.6-8.8)	7.4 (7.1-8.2)	7.4 (6.9-8.2)	7.5 (7.1-8.2)	7.4 (7.0-8.0)	7.4 (7.0-8.1)	7.3 (6.9-7.8)	7.3 (7.1-7.9)	8.1 (7.5-9.0)
DOC (mg/L)	2.4 (1.3-3.9)	0.8 (0.4-1.1)	0.8 (0.4-1.3)	0.9 (0.4-1.3)	0.6 (0.2-0.9)	0.5 (0.2-1.7)	0.7 (0.4-1.6)	0.7 (0.5-2.0)	2.2 (1.4-3.4)
NH4-N (mg/L)	0.024 (0.003-0.049)	0.010 (n.d.-0.031)	0.008 (n.d.-0.023)	0.006 (n.d.-0.019)	0.01 (n.d.-0.030)	0.012 (n.d.-0.041)	0.011 (n.d.-0.024)	0.009 (n.d.-0.019)	0.025 (0.004-0.069)
NO3- (mg/L)	8.4 (4.8-13.0)	6.6 (2.5-10.9)	6.8 (2.5-11.0)	7.0 (3.1-10.7)	7.2 (4.3-9.7)	5.7 (4.0-8.3)	1.5 (0.7-4.2)	1.2 (n.d.-5.1)	0.8 (n.d.-7.3)
Bacterial abundance (cells/mL)	3.78×10^{6} (1.77×10^{6}-6.14×10^{6})	1.99×10^{5} (1.35×10^{5}-3.08×10^{5})	1.89×10^{5} (9.77×10^{4}-4.04×10^{5})	1.55×10^{5} (5.83×10^{4}-2.94×10^{5})	9.14×10^{4} (4.64×10^{4}-1.45×10^{5})	7.31×10^{4} (4.80×10^{4}-9.55×10^{4})	1.85×10^{5} (1.37×10^{5}-2.41×10^{5})	1.69×10^{5} (8.21×10^{4}-2.81×10^{5})	8.75×10^{6} (2.35×10^{6}-2.45×10^{7})
Large cells (cells/mL)	1.83×10^{6} (9.5×10^{5}-2.68×10^{6})	6.09×10^{4} (2.65×10^{4}-1.64×10^{5})	5.92×10^{4} (2.54×10^{4}-1.62×10^{5})	4.09×10^{4} (1.73×10^{4}-1.02×10^{5})	2.30×10^{4} (1.51×10^{4}-4.10×10^{4})	1.34×10^{4} (9.94×10^{3}-2.03×10^{4})	3.86×10^{4} (2.54×10^{4}-5.18×10^{4})	4.47×10^{4} (2.16×10^{4}-1.64×10^{5})	2.81×10^{6} (1.53×10^{6}-5.76×10^{6})
Proportion large cells (%)	49.9 (33-71.1)	29.0 (18.8-63.7)	30.5 (19.5-56.4)	27.2 (12.8-56.6)	25.6 (17.6-34.8)	19.3 (12.4-26.8)	21.3 (11.8-31.0)	25.8 (10.6-58.5)	41.1 (15.5-65.1)
Biomass (ng C/mL)	56.1 (27.2-81.7)	2.60 (1.67-4.22)	2.48 (1.23-5.66)	1.96 (0.83-3.48)	1.14 (0.62-1.86)	0.87 (0.59-1.08)	2.23 (1.70-2.83)	2.13 (1.06-4.45)	116 (38.9-302)
Leucine incorporation (pmol/L/h)	87.3 (5.09-384)	0.276 (0.028-0.995)	0.249 (0.025-0.99)	0.069 (0.013-0.413)	0.015 (0.007-0.033)	0.008 (0.003-0.023)	0.037 (0.011-0.211)	0.104 (0.006-1.06)	90.5 (7.00-248)
BCP (ng C/mL/h)	1.35×10^{-1} (7.87×10^{-3}-5.93×10^{-1})	4.27×10^{-4} (4.35×10^{-5}-1.54×10^{-3})	3.85×10^{-4} (3.90×10^{-5}-1.53×10^{-3})	1.06×10^{-4} ($2.03\times^{-5}$-6.39×10^{-4})	2.26×10^{-5} (1.13×10^{-5}-5.16×10^{-5})	1.26×10^{-5} (4.18×10^{-6}-3.49×10^{-5})	5.72×10^{-5} (1.65×10^{-5}-3.26×10^{-4})	1.16×10^{-4} (9.73×10^{-6}-1.64×10^{-3})	1.40×10^{-1} (1.08×10^{-2}-3.83×10^{-1})

Temperature is another parameter frequently used to investigate the interaction between groundwater and surface water (Schmidt et al., 2006). Both the Danube and the backwater river showed pronounced seasonal changes in surface water temperature and had highest temporal variability (Table 3). A seasonal trend was also observed in wells LSG41, LSG40 and LSG30, albeit with a lag time of approximately 2 months (not shown). Less pronounced seasonality was shown in PGAW3, LSN28 and LSG11. A seasonal pattern was also observed for nitrate. Peak concentrations in the river were observed during the winter months, but were not correlated to the water table (not shown). Nitrate concentrations in the groundwater wells between the Danube and PGAW3 were only 20% to 30% less than in the Danube and seemed to be influenced by the river. Wells LSN28 and LSG11 (which were located between PGAW3 and the backwater river) in contrast, were influenced by the backwater river ($r=0.50$, $P=0.035$ for LSG11 and $r=0.55$, $P=0.019$ for LSN28). Other standard chemical parameters (NH_4^+, Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, HCO_3^-) and EC did not exhibit any seasonality.

A clear distinction between both surface waters and the groundwater could be seen for the DOC concentrations. Analysis of variance (one-way ANOVA) showed that the average concentrations and fluctuations in the Danube (2.35 ± 0.67 mg/L) and in the backwater river (2.17 ± 0.52 mg/L) were significantly higher than in the groundwater in all wells (average concentration of 0.71 ± 0.27 mg/L, Table 4). No clear seasonal DOC pattern could be observed in the surface waters nor in the groundwater, which was in contrast to other studies (Brugger et al., 2001; Ellis et al., 1998; Zhou et al., 2012). No statistically significant correlations were found between groundwater flow velocity and DOC in any of the wells.

3.3.2 Enhanced surface water infiltration during flood events governs the seasonal dynamics of bacterial biomass and carbon production during RBF

After tracing which river characteristics are of major influence on groundwater quality in the study area, the next step was to identify the spatiotemporal dynamics of bacterial biomass and BCP in both surface water and groundwater.

3.3.2.1 Total cell counts

Total bacterial cell counts (TCC) in the Danube ranged from 1.77×10^6 to 6.14×10^6 cells/mL and from 2.35×10^6 up to 2.45×10^7 cells/mL in the backwater river (Table 4), with corresponding biomass values ranging from

27.2 up to 81.7 ng C/mL and from 38.9 up to 302 ng C/mL, respectively. These values were in the same range as found during the Joint Danube Survey 2007 (Velimirov et al., 2011) and in rivers of similar discharge such as the Pearl river, the river Rhine and the river Meuse (Duan et al., 2007; Scherwass et al., 2010; Servais, 1989). TCC and bacterial biomass in the Danube were positively correlated to temperature (r=0.61, P=0.007, Appendix B Table 16). A similar trend, but no significant correlation, was observed in the backwater river. The variation in TCC was higher in the backwater due to the discontinuous inflow of river water (Kirschner and Velimirov, 1997). DOC concentrations in both the Danube and the backwater river were in a similar range during summer, but were not correlated to TCC. The correlations with other nutrients, which were mainly negative, were more pronounced in the Danube than in the backwater river (not shown).

In the groundwater wells, mean TCC were significantly lower than in the surface waters, ranging from 4.64 x 10^4 cells/mL up to 4.04 x 10^5 cells/mL (Table 4 and Figure 4). They were in a similar range as reported by Alfreider et al. (1997), Brugger et al. (2001) and Zhou et al. (2012), even though the infiltrating rivers or lakes in those studies were significantly smaller than the Danube river. No clear seasonal patterns in TCC were observed in our study, although this has been found elsewhere (Velasco Ayuso et al., 2009a). The corresponding bacterial biomass values in the groundwater (Table 4) ranged from 0.59 ng C/mL in PGAW3 up to 5.66 ng C/mL in LSG40. Highest bacterial cell counts and biomass was found in the wells closest to the river (up to a maximum distance of 24 m). In these first meters, only 5% of TCC measured in the river was found in the groundwater and further decreased to only 2% in the groundwater abstraction well. Brugger et al. (2001) found a similar decrease in bacterial abundance along the flow path; a less pronounced decrease was shown for the Flathead river in Ellis et al. (1998), caused by 10-fold lower TCC values in this river. The absolute numbers however were in the same order of magnitude. Not only did the absolute values of TCC and biomass show a clear decrease towards the groundwater abstraction well PGAW3, the temporal variability also decreased significantly. The highest temporal variability of TCC and biomass in the groundwater was observed in wells LSG41, LSG40 and LSG30 next to the Danube, and in well LSG11 next to the backwater (Figure 5a). Both the river and the backwater river showed a similar temporal pattern as the wells close to the surface water bodies. This variability could therefore be attributed to the input of water from either the river or the backwater river.

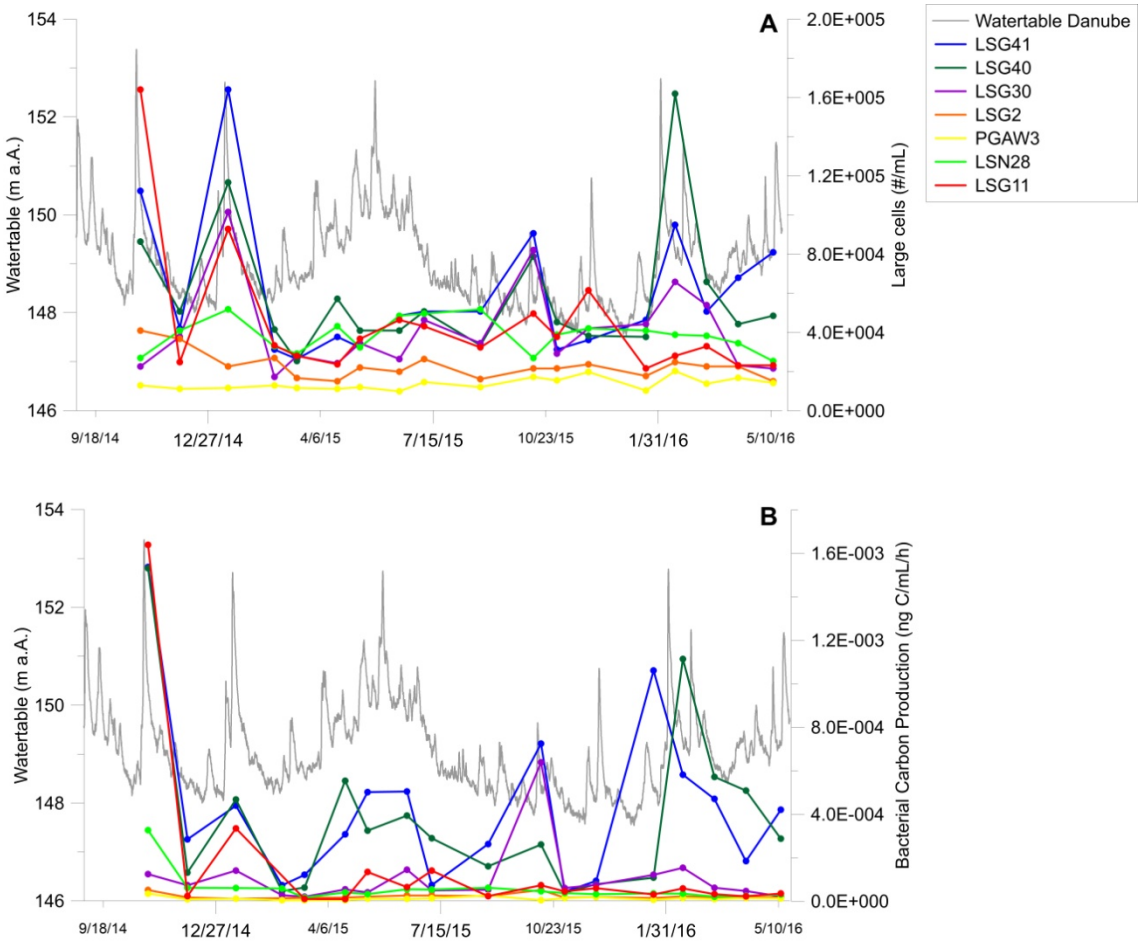

Figure 4 **a)** Abundance of large bacterial cells and **b)** the bacterial carbon production in all groundwater monitoring wells versus the water table of the Danube

Lowest values and temporal variability in TCC and biomass were observed in the wells with the highest distance to the river (LSG2, PGAW3) corresponding to observations made earlier (Brugger et al., 2001; Ellis et al., 1998). This part of the aquifer is relatively pristine and the concentrations of most nutrients were lowest. In contrast to the surface waters, no correlation between bacterial abundance and standard chemical parameters and also no correlation with temperature was observed for the groundwater samples.

3.3.2.2 Bacterial carbon production

BCP varied from 7.87 x 10^{-3} up to 0.59 ng C/mL/h in the surface water samples. This was well within the range of other rivers (Bernard et al., 2000; Brugger et al., 2001; Fischer and Pusch, 2001), but slightly lower than during the Joint Danube Survey 2007 (Velimirov et al., 2011), which was a snapshot of the river Danube in autumn. BCP in the Danube coincided with peaks in water level ($r=0.82$, $P=3.3 \times 10^{-5}$) but did not show significant seasonality. The backwater river on the contrary showed a temperature dependence ($r=0.64$, $P=0.004$), but no significant correlation between BCP

and water level. In both the Danube and the backwater river, BCP was positively correlated to DOC (r=0.59, P=0.013; r=0.77, P=3×10^{-4} respectively). Only few other chemical parameters (HCO_3^- and Cl^-) correlated with BCP in both surface waters. Due to the lower quantity and quality of DOC, BCP values in groundwater are typically much lower than in surface water. Indeed, BCP was much lower (up to 4 orders of magnitude) in the groundwater of the investigated PGA, ranging from 4.18 x 10^{-6} in PGAW3 up to 1.64 x 10^{-3} ng C/mL/h in LSG11 (Table 4). A broad range of BCP values for groundwater samples was found in similar studies, ranging from below the detection limit up to 1.82 ng C/mL/h (Alfreider et al., 1997; Brugger et al., 2001; Velasco Ayuso et al., 2009b). The very high values measured by Velasco Ayuso et al. (2009b) were most likely due to the high carbon production and the high amount of readily degradable DOC in the coastal environment that infiltrated into the aquifer. Similar to biomass, BCP decreased further along the flow path towards PGAW3 (Figure 5b). The temporal variability in each well concurrently decreased and was lowest in LSG2 and PGAW3. As for bacterial numbers, no significant correlations could be found between BCP and DOC or other nutrients once the river water infiltrated into the groundwater. In addition, no correlation with water temperature was observed.

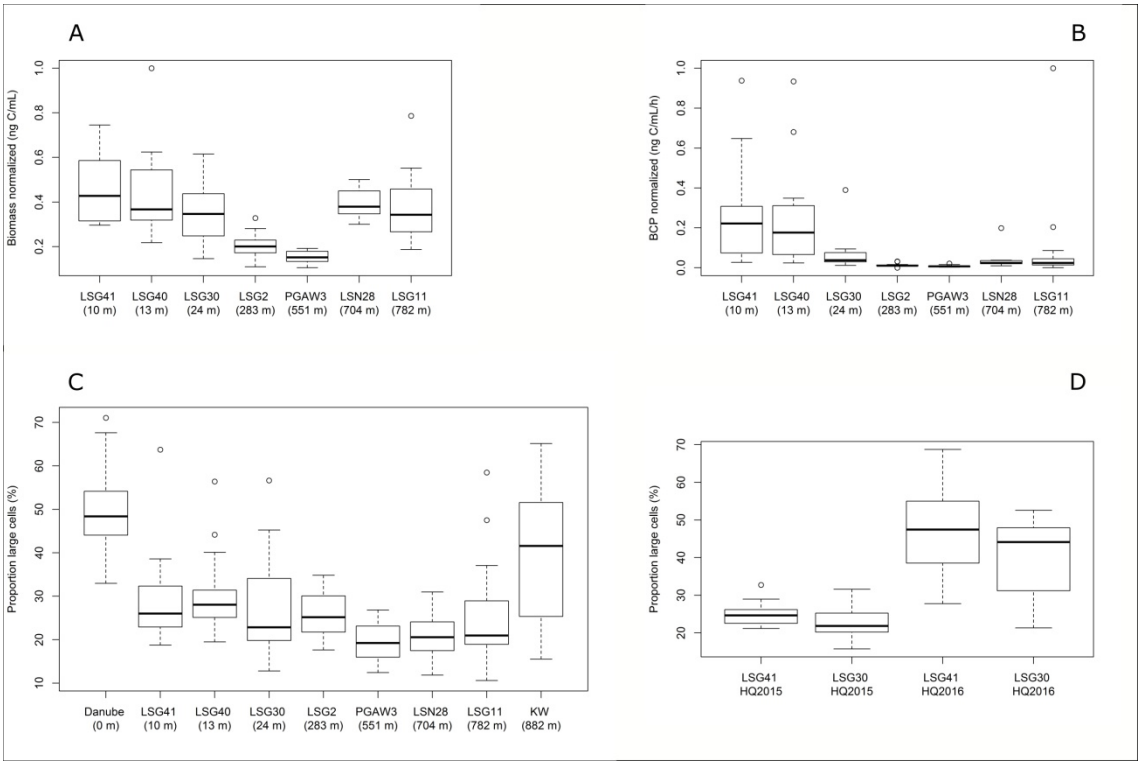

Figure 5 Boxplots of **a)** normalized biomass in the groundwater monitoring wells during the monthly sampling campaign, **b)** normalized BCP in the groundwater monitoring wells during the monthly sampling campaign **c)** the proportion of large cells during the monthly sampling campaign and **d)** the proportion of large cells during HQ2015 and HQ2016

Several explanations can be proposed for the lack in correlations between these parameters. Most likely, stochastic ecological processes govern the microbial communities in groundwater aquifers (Stegen et al., 2016). Only when readily available labile organic carbon enters the aquifer, the microbial community responds. It can be hypothesized that due to a relatively low amount of readily degradable DOC in our investigated groundwater, no correlation was found between DOC and any of the microbial parameters during the monthly sampling period. A multiple regression analysis between several nutrients and the microbial parameters further confirmed the lack of correlations between these parameters in groundwater samples, as also observed by Zhou et al. (2012). Under regular discharge conditions of the Danube, when flow velocities in the groundwater body are low, the high quality DOC does not reach far into the aquifer and bacterial production values stay in a low range. Only when groundwater flow velocities increase significantly, caused by a flood event in the river and concomitant infiltration of surface water into the groundwater body, nutrients - but also bacteria - are effectively pressed into the groundwater and a correlation between bacterial production and bacterial numbers with flow velocity would occur. In well LSG41 located nearest to the river, such correlation was indeed observed ($r=0.69$, $P=1.4 \times 10^{-3}$ and $r=0.47$, $P=0.048$, for BCP and large cells, respectively; Appendix B Table 16). For the wells further towards the groundwater abstraction well however, no correlation between flow velocity and BCP or large cells was found.

3.3.2.3 Cell size distribution as indicator of surface water infiltration

Besides the significant differences between TCC and BCP in surface- and groundwater, the proportion of large cells may be used as an indicator of surface water infiltration. It has been shown that size distribution of hyporheic bacteria can be very similar to river samples, but changes while moving further away from the river into the aquifer (Ellis et al., 1998). To test this hypothesis, cell counts were classified into large cells (rod shaped cells and coccoid cells with a diameter > 1.0 μm) and small cells (cocci with a diameter < 1.0 μm). In both surface water and groundwater, TCC were dominated by small cells. There was however a distinct difference in the proportion of large cells in the surface waters relative to the proportion of large cells in the groundwater (Figure 5c). The river water consisted of a much larger proportion of large cells, which may be attributable to higher availability of nutrients. During subsurface passage, the proportion of large cells in the water matrix decreased due to the lack of nutrients and readily degradable organic carbon (Zhou et al., 2012). A one-way ANOVA showed that the difference in proportion of large cells was statistically significant between the river samples and the groundwater samples taken during the

monthly sampling campaigns. Our hypothesis was that during flood events, due to the higher flow velocities and the increased amount of water entering the aquifer, the proportion of large cells in the groundwater close to the river would be much more similar to river water than under normal flow conditions.

3.3.3 Flood events lead to a short-time response of the bacterial groundwater community and a concomitant increase in TCC and large cells

We hypothesized that during flood events, the influence of the river on the groundwater is higher than under regular discharge conditions and this influence reaches more distant wells. In order to account for changes in TCC and size distribution of bacterial cells entering the aquifer under high flow velocities, additional samples were taken during two flood events. Although during both sampled flood events peak water levels in the Danube were similar (Table 5), minimum surface water levels were distinctly different, which was also seen in the dynamics of the groundwater levels (Table 5).

Another difference between the two flood events was seen in the gradient and flow velocities. The maximum gradient during HQ2015 between the Danube and LSG41 was 20.4%, whereas the maximum gradient between the Danube and LSG41 during HQ2016 was 31%. Flow velocities were approximately 1.5 fold higher during HQ2016 (0.040 m/s) than during HQ2015 (0.026 m/s). In contrast to the Danube, TOC concentrations in the groundwater were stable throughout both flood events and did not significantly differ between the two events. Brugger et al. (2001) showed that a peak in DOC concentration in the Enns river resulted in higher DOC concentrations only at the stations near the river (up to 6 m), but had no effect on the more distant stations, which could explain the lack of correlation in our groundwater wells. Nitrate concentrations in the groundwater during HQ2016 were very similar to those in the river and were significantly higher than under regular discharge conditions. A significant correlation between flow velocity and nitrate was however not present. None of the other nutrients showed significant changes during the flood events in the groundwater samples. During HQ2015, TCC in the groundwater (Table 5) were in the same range as the average TCC values measured during the monthly sampling campaigns (Table 4). During HQ2016, TCC in the groundwater was twice as high as during HQ2015 (Table 5). In the Danube however, average TCC values were in a similar range as during the monthly sampling campaigns (Table 4 and Table 5). TCC in the river started to increase as water levels rose and stayed fairly constant during the six following days. TCC in the nearest monitoring well,

LSG41, showed a similar clear increase which was associated with the low travel times from the Danube towards LSG41. Although no clear decrease was seen in the river, the bacterial abundance in LSG41 decreased rapidly after the peak in water level (Figure 5b), obviously linked to a decrease in the gradient. In LSG30, a similar but attenuated pattern could be found. Probably due to the lower gradient, travel times from LSG41 towards LSG30 were much longer. Therefore, the rise in TCC was less evident than in LSG41. The variability in the proportion of large cells in the groundwater during HQ2016 was much higher than during HQ2015 and was caused by the response of the bacterial community to the increased gradient and flow velocity (Figure 6). Because the response was temporally shifted, no correlation was found between large cells and the flow velocity during HQ2016 (Figure 6c). For biomass however, a statistical significant correlation with flow velocity was present in both wells (LSG41: $r=0.853$, $p=1.7 \times 10^{-3}$; LSG30: $r=0.664$, $p=0.036$; Appendix B Table 17). These correlations were much higher than under regular discharge conditions. During HQ2015, these correlations were not present due to the lower gradients and flow velocities. This suggests that during flood events with high gradients, an increased and extended influence (up to a distance of 24 m) of the river can be observed. The proportion of large cells in the monthly samples (Figure 5c) was significantly different between the surface water and groundwater samples. During HQ2016, the proportion of large cells in the Danube increased significantly (Figure 6c) and was much higher than during the monthly sampling campaigns (Table 4) or during HQ2015. Peak values in both wells were reached one day after the peak in gradient (which corresponded to the travel time from LSG41 to LSG30; Figure 6c).

3.3.4 Turnover times of the bacterial biomass are too long to explain the observed increase in TCC in the groundwater wells during flood events

Lin et al. (2012b) showed the influence of the temporal dynamics in water level on the community composition of the Hanford aquifer. During higher water levels two groups of Actinobacteria were found which were not present under lower water levels. A distinction between inflow of riverine bacteria, elution from the lower vadose zone, or environmental selection of aquifer bacteria by the riverine nutrients could however not be made since the study did not analyze the riverine microbial population. We hypothesize that only when large amounts of surface water flow into the aquifer and when flow velocities are high, riverine bacteria enter the aquifer. It is less likely that bacteria detach from the subsurface sediments of the lower vadose zone, since this would have also meant an increase in abundance during the HQ2015 flood.

	Danube 0 m		LSG41 10 m		LSG30 24 m	
	HQ2015	HQ2016	HQ2015	HQ2016	HQ2015	HQ2016
Water table difference (m a.A.)	150.10-152.74	147.95-152.78	149.63-150.70	147.80-149.74	149.62-150.71	147.80-149.71
Gradient (%)	n.a.	n.a.	5.77-20.4	1.50-31.0	-0.08-0.10	0.01-0.19
T (°C)	13.0	6.18	11.6	8.41	12.7	7.53
	(11.7-15.2)	(5.40-7.00)	(11.0-13.9)	(7.80-9.20)	(12.0-13.9)	(7.00-8.30)
EC (µS/cm)	345	384	505	440	397	426
	(329-365)	(359-440)	(425-560)	(432-453)	(391-401)	(420-435)
pH	7.94	7.78	7.28	7.64	7.40	7.65
	(7.52-8.05)	(7.60-8.00)	(7.05-7.45)	(7.60-7.70)	(7.27-7.52)	(7.40-7.80)
TOC (mg/L)	2.73	4.40	0.90	1.10	1.00	1.30
	(1.60-3.60)	(2.70-6.40)	(0.80-0.90)	(1.00-1.20)	(1.00-1.00)	(1.20-1.40)
NO3- (mg/L)	7.44	11.0	5.40	9.97	6.60	10.9
	(6.00-8.10)	(9.80-12.0)	(4.30-6.60)	(8.80-11.0)	(6.30-6.80)	(10.0-12.0)
Bacterial abundance (cells/mL)	n.a.	3.80×10^6	2.12×10^6	4.48×10^6	1.06×10^6	2.63×10^6
		$(1.87\times10^6$-	$(1.46\times10^6$-	$(2.46\times10^6$-	$(9.56\times10^4$-	$(1.88\times10^6$-
		$4.97\times10^6)$	$2.58\times10^6)$	$7.32\times10^6)$	$1.20\times10^5)$	$3.46\times10^6)$
Large cells (cells/mL)	n.a.	3.30×10^6	5.29×10^4	2.28×10^6	2.44×10^4	1.08×10^6
		$(1.26\times10^6$-	$(3.40\times10^4$-	$(9.50\times10^4$-	$(1.51\times10^4$-	$(6.26\times10^4$-
		$4.48\times10^6)$	$6.37\times10^4)$	$4.32\times10^6)$	$3.38\times10^4)$	$1.66\times10^6)$
Proportion large cells (%)	n.a.	85.0	25.1	49.1	23.1	41.0
		(67.6-94.0)	(21.2-32.7)	(31.7-68.7)	(15.7-31.6)	(21.3-52.6)
Biomass (ng C/mL)	n.a.	71.0	2.65	7.22	1.30	3.86
		(31.3-94.4)	(1.80-3.22)	(3.41-11.4)	(1.11-1.46)	(2.54-5.12)

Table 5 Minimum and maximum values of the water table difference and gradient and average values of (physico)chemical and microbiological parameters during HQ2015 and HQ2016. Values in brackets are min-max values Values in meters are the distance to the Danube

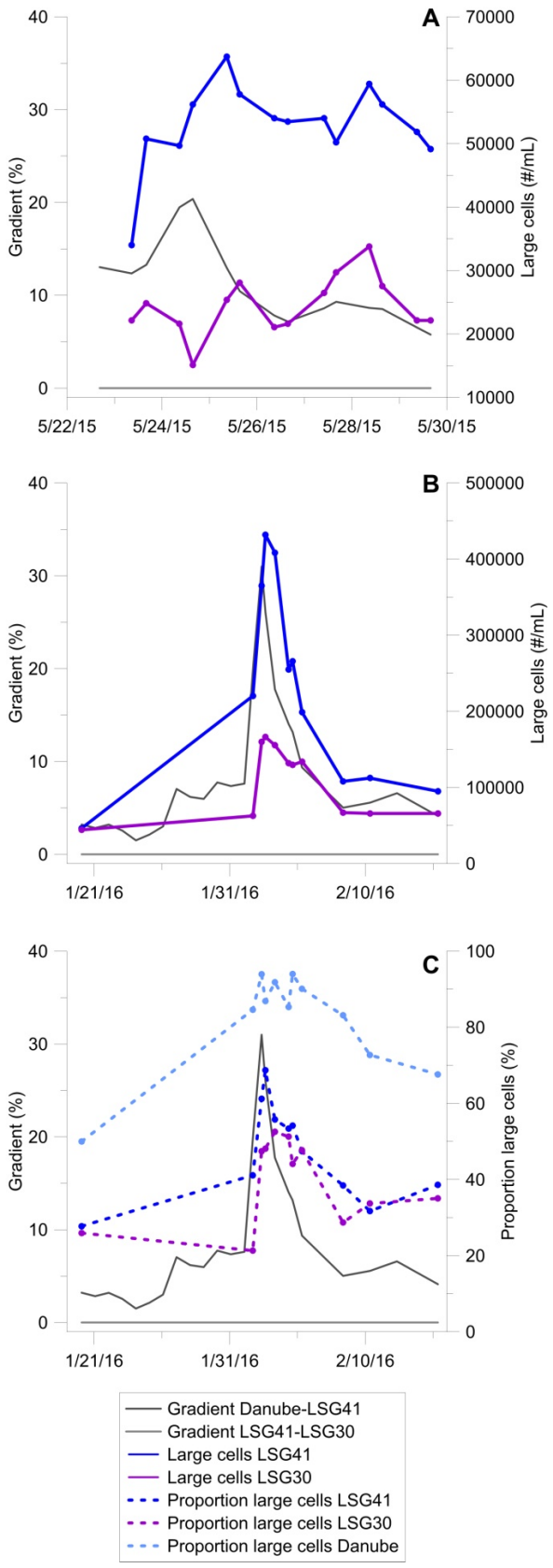

Figure 6 Large cells versus gradient during **a)** HQ2015 and **b)** HQ2016 and **c)** the proportion of large cells versus gradient during HQ2016

This was, however, not observed. Due to the lack of correlations between the chemical parameters and the microbiological parameters, it was unlikely that the riverine nutrients were the source of the increasing abundances. Moreover, turnover times of the bacterial biomass are too long to explain the observed increase in bacterial numbers in the groundwater wells during flood events. Turnover times varied from 3.72 up to 201 days in the Danube to 12.7 up to 200 days in the backwater river. Lowest values measured in the Danube were measured following peaks in discharge ($r=-0.64$, $P=0.004$) and were in a similar range as during peak discharges in other rivers (Bernard et al., 2000; Billen et al., 1990; Brugger et al., 2001). Turnover times in the groundwater (84 – 10514 days) were much longer than in both surface waters. They were shortest in the wells close to the river (LSG41 and LSG40) and increased significantly towards PGAW3 (Table 4). On average they were generally one order of magnitude higher than in similar studies (Brugger et al., 2001; Ellis et al., 1998). The calculated turnover times were based on total cell counts. These however, do not only include viable cells, but also a mixture of dormant and dead cells. TVAC counts (Appendix B Table 18) constituted only a low percentage (below 1%) of total cell counts. In the wells

next to the river (LSG41 and LSG40), the percentage of TVAC was highest, whereas it was lowest in the most distant wells (LSG11, LSN28). When turnover times were calculated on the basis of the viable active cells, they were in the range of only a couple of days (Appendix B Table 19). The highest turnover times were measured in well LSG2 and in PGAW3. The lowest turnover times were measured in the wells closest to the Danube.

With the calculated turnover times, based on the total bacterial biomass, the observed increase in bacterial numbers/biomass in the wells in close proximity to the river during a flood event cannot be explained. During HQ2016 a 4.4 fold increase in TCC from 1.67×10^5 to a maximum of 7.32×10^5 cells was observed within a period of 4 days.

Minimum turnover times observed within the monthly sampling campaign (including flood events) were around 100 days for the groundwater samples and it would thus need > 400 days to achieve a 4.4 fold increase in bacterial numbers by the growth of the bacterial community from an additional nutrient input. Thus the observed increase has to be caused by the input of bacterial cells from the river or from detachment of bacterial cells from subsurface biofilms from the lower vadose zone due to water table changes. As the percentage of large cells during HQ2016 was similar in the groundwater and the surface water we assume that surface water infiltration is the responsible factor. Community composition profiling could prove this hypothesis.

3.4 Summary and Conclusions

During a 20 month sampling campaign considerable spatiotemporal fluctuations were observed in bacterial cell numbers, biomass and carbon production in a porous aquifer. Under regular discharge conditions, bacterial abundance, the percentage of large cells, bacterial biomass and bacterial carbon production decreased significantly from the river and the backwater river towards the groundwater abstraction well due to processes like filtration or die-off. Despite the tendency of many environmental biota to exhibit seasonal responses and responses to nutrient stimuli, temporal changes in microbial metrics monitored in this study were more closely aligned with fluctuations in groundwater flow velocities. The observed increase in bacterial cell numbers during flood events was most likely attributable to the infiltration of surface water bacteria. Calculated turnover times of the bacterial biomass were too long to explain the observed increase in bacterial numbers in the groundwater wells. Moreover,

during flood events, the percentage of large cells in the groundwater wells was similar to the surface water. This infiltration was markedly visible in the well 10 m away from the riverbank at several occasions during the investigation period, and was extended in an attenuated way towards the well situated 24 m away from the riverbank during flood events. The drinking water abstraction well situated at a distance of approx. 550 m was never significantly affected. In contrast, the two wells close to the backwater river also showed considerable variability in microbiological parameters over the year. This was related to the influence from the backwater river that showed pronounced hydrological variability in relation to its connectivity to the main river.

The use of the bacterial abundance, biomass and activity as indicators for surface water – groundwater interaction is of high relevance for drinking water management. Bacterial cell numbers and biomass can be measured near-real time using (for example) flow cytometry. Together with information on hydrogeological characteristics of the aquifer, such as hydraulic conductivity and porosity, water utilities can use the microbiological data to improve their water abstraction strategies and react quicker to changing hydrological conditions in the RBF system.

3.5 Acknowledgements

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4. Spatiotemporal resolved sampling for the interpretation of micropollutant removal during riverbank filtration

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Key messages

- OMP fate was studied along an RBF system under normal and elevated conditions
- Benzotriazole was almost fully removed during RBF under oxic conditions
- Carbamazepine and Sulfamethoxazole showed a relatively persistent behavior
- Increase in load of several OMPs in the river observed during flood events
- OMP concentrations in the groundwater were far below drinking water guideline values

Abstract

Riverbank filtration (RBF) systems along rivers are widely used as public water supplies. In these systems, many organic micropollutants (OMPs) are attenuated, but some compounds have shown to be rather persistent. Their fate and transport has been studied in RBF sites along lakes and small rivers, but not extensively along large and dynamic rivers. Therefore, the influence of flood events on OMP behavior in these large and dynamic RBF sites was investigated. Monthly samples were taken from surface- and groundwater up to a distance of 900 m from the riverbank of the Danube from March 2014 till May 2016. Two flood events were sampled more extensively nearby the river. Results showed that changes in flow conditions in the river not only caused changes in OMP concentrations, but also in their load. It was seen that the load of benzotriazole, carbamazepine and sulfamethoxazole in the river increased with increasing river discharges. After a relatively long, oxic groundwater passage, several OMPs were reduced. In contrast to previous work, we found that benzotriazole was almost fully removed under oxic conditions. When entering the aquifer, benzotriazole concentrations were significantly reduced and at a distance of 550 m from the river, >97% was degraded. Carbamazepine and sulfamethoxazole showed relatively persistent behavior in the aquifer. The concentrations measured during flood events were in the same range as seasonal sampling. Furthermore concentrations in the groundwater were higher during these events than in the Danube and can reach further into the aquifer. During flood events some highly degradable compounds (i.e. diclofenac) were found up to a distance of 24 m from the river. These results implied that drinking water utilities with RBF wells in oxic, alluvial aquifers located close to highly dynamic rivers need to consider a potential reduction in groundwater quality during and directly after flood events.

4.1 Introduction

Along large rivers such as the Danube, millions of people use drinking water from riverbank filtration (RBF). RBF systems are used in many countries (Heberer et al., 2001; Hiscock and Grischek, 2002; Ray et al., 2002; Tufenkji et al., 2002) due to the availability of large quantities of potential drinking water. They are however under much more anthropogenic stress than other, pristine groundwater sources. Due to the infiltration of low quality river water, the pristine groundwater can get contaminated with chemical and

microbial substances. Substances that have been receiving increased attention are the organic micropollutants (OMPs) (Schwarzenbach et al., 2006). These pollutants comprise many different substances, such as industrial chemicals, pharmaceuticals and personal care products, but also pesticides or herbicides. Most of the pharmaceuticals and personal care products enter the environment through wastewater treatment plants (WWTPs) where they – depending on their persistence – are removed or remain in the effluent to a certain extent (Joss et al., 2005; Radjenović et al., 2009). Due to their persistence during treatment and their widespread presence in wastewater as main pathway to the aquatic environment, certain compounds have been suggested as indicators for impacts from waste water (Jekel et al., 2015), such as the corrosion inhibitor benzotriazole (BTri), the antiepileptic drug carbamazepine (CBZ) and the antibiotic sulfamethoxazole (SMZ).

Several studies have examined the behavior of these and other OMPs during RBF or similar systems (Heberer et al., 2008; Kahle et al., 2009; Rauch-Williams et al., 2010; Reemtsma et al., 2010; Regnery et al., 2015; Scheurer et al., 2011). Many of these studies however were conducted in RBF systems connected to small rivers or lakes. The range of water level fluctuations in these systems was much lower than along dynamic rivers such as the Danube (with water level fluctuations of up to 8 meter).

Several factors such as groundwater residence times, redox conditions and mixing with pristine groundwater have shown their importance for the attenuation of OMPs (Burke et al., 2014b; Epting et al., 2018; Massmann et al., 2008, 2006; Storck et al., 2012; Wiese et al., 2011). Changing redox conditions and groundwater residence times for example can have an effect on removal rates of OMPs in the groundwater (Bertelkamp et al., 2016b) due to their effect on the biodegradation processes taking place in the aquifer. Not only the seasonal dynamics can influence the transport of the OMPs in the groundwater, flood events can also have an effect on their behavior. During a flood event, groundwater residence times can be shortened due to increased flow velocities (Derx et al., 2013; Sprenger et al., 2011). Furthermore, the composition of the infiltrating surface water can change the redox conditions in the aquifer and simultaneously have an influence on the micropollutant removal. Electron acceptors or donors can react abiotically with OMPs in the environment. The feasibility of these reactions is dependent on the prevailing environmental (redox) conditions (Schwarzenbach et al., 2017). Under oxic conditions for example, aerobic respiration can take place and OMPs can be oxidized. Especially oxic RBF systems are highly vulnerable to flood events due to a possible shift in redox

conditions (Sprenger et al., 2011). Unfortunately, little is known so far on this removal in large and dynamic RBF systems. Therefore it is of paramount importance to gain more insight in the behavior of OMPs in these RBF systems. The aim of this paper was therefore to investigate the influence of flood events on the behavior of OMPs along a large and dynamic river. This was done by addressing the following questions: (i) What is the behavior of OMPs in an alluvial porous aquifer during RBF along a large and highly dynamic river? and (ii) Do flood events change the presence and behavior of OMPs in surface- and groundwater along this large and dynamic river? For this purpose, river and groundwater samples were taken from two surface water locations, six groundwater monitoring wells and a drinking water abstraction well in an alluvial porous aquifer (PGA). Seasonal samples were taken monthly between March 2014 and May 2016 and were analyzed for a mixture of 7 OMPs and standard chemical parameters. To account for changes during extreme river level fluctuations, two flood events with water level fluctuations of up to 5 m (with a recurrence of 1 year) were sampled at a higher temporal resolution.

4.2 Materials and methods

4.2.1 Study area and instrumentation

The study was conducted at an RBF system on the left bank of the Danube, downstream of the Austrian capital of Vienna, as previously described by van Driezum et al., (2018)(Figure 7). The water quality in the studied section of the Danube is impacted by upstream wastewater treatment plant discharges (Frick et al., 2017). The total amount of wastewater discharges is based on 13 million inhabitants and a corresponding PE of 20 million inhabitants (Zessner and Lindtner, 2005). It thus contributes to 2.5% of the discharge of the Danube under mean flow conditions. Discharges of the Danube in Vienna can range from 700 m^3/s during low flow conditions up to 11,000 m^3/s, such as during the 2013 flood (Blöschl et al., 2013). The discharge regime of the river at this point was classified as alpine influenced (Wimmer et al., 2012). The RBF system is part of an alluvial backwater and floodplain area containing five groundwater abstraction wells used for drinking water. The daily extraction capacity of all five wells is 109,000 m^3. A transect containing several monitoring wells and a groundwater abstraction well was chosen which was continuously fed by the infiltrating Danube, resulting in predominantly oxic conditions (Mayr et al., 2014). The main layers of the unconfined aquifer consist of gravel and sand and have a thickness varying from 3 to 15 meters. Hydraulic conductivities in the

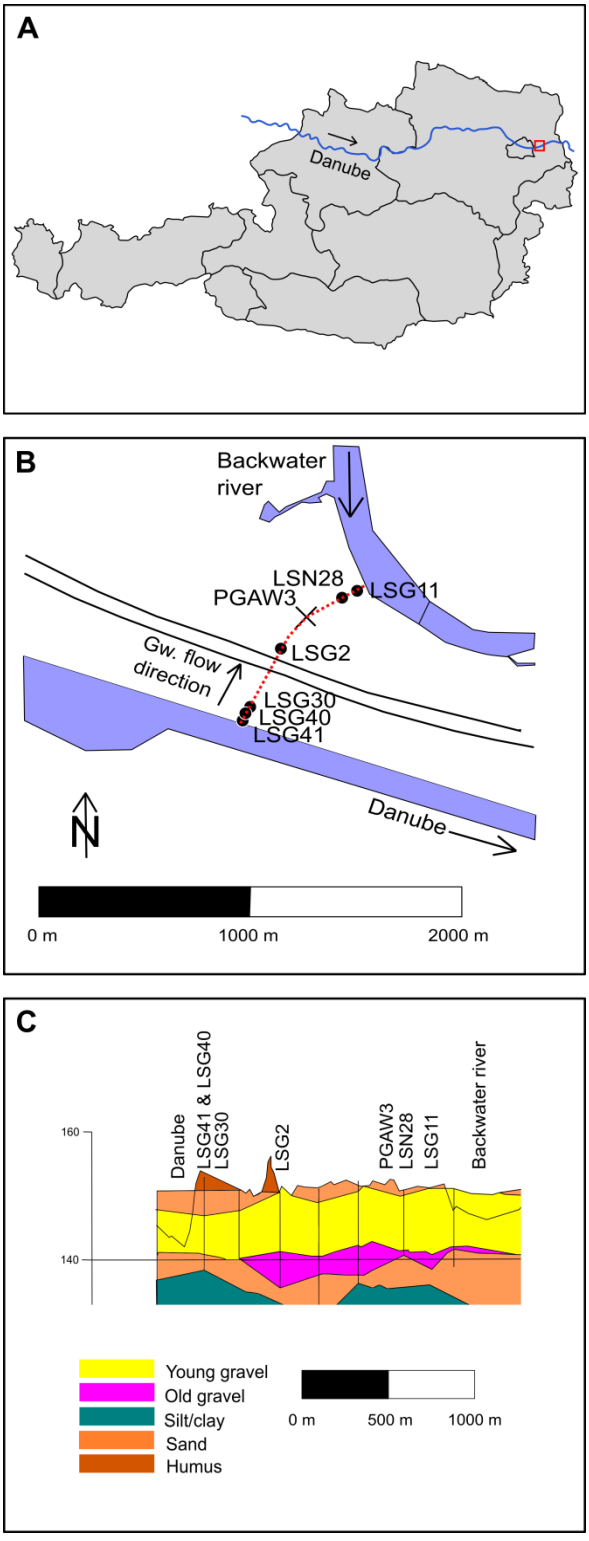

Figure 7 **a)** situation of the Natura 2000 protected area (red sqaure) in Austria, **b)** the sampled transect including monitoring wells LSG41, LSG40, LSG30, LSG2, LSN28 and LSG11. The groundwater abstraction well is depicted as PGAW3 and **c)** schematic cross section (dotted red line in b) of the transect with the hydrogeological layers and the groundwater monitoring wells (shown as black vertical lines)

transect ranged from 5 x 10^{-4} m/s to 5 x 10^{-2} m/s, determined by pumping tests conducted in the area and a 3D groundwater flow and transport model (for calibration details refer to Farnleitner et al. (2014)). Local groundwater flow is directed from the southwest to the northeast. Underneath the aquifer are alternating sand and clay/silt layers with hydraulic conductivities of at least 2 orders of magnitude lower. The transect with the sampled wells extends from the Danube towards the backwater river. It consists of 2 surface water locations, 6 groundwater monitoring wells and one groundwater abstraction well with a maximum extraction capacity of 0.28 m3/s (PGAW3, Figure 7). Three wells (LSG41, LSG40 and LSG30) are located close to the river and are subjected to high variability in river and groundwater levels.

One well (LSG2) is located between these three wells and PGAW3. Wells LSN28 and LSG11 are located between PGAW3 and the backwater river. All wells are screened over the full length of the saturated aquifer. Travel times from the Danube toward PGAW3 based on the hydraulic gradient can be found in van Driezum et al., (2018) and range between 11.5 days to 47.4 days. Travel times toward the three nearest monitoring wells (LSG41, LSG40

and LSG30) ranged from 1 hour to LSG41 (10 m away from the Danube) to 5.4 days to LSG30 (24 m away from the Danube) during the monitoring period from March 2014 to May 2016.

The backwater river is a sequence of connected ponds which is connected to the Danube when water levels in the river exceed 150.5 m a.A. (meter above the Adriatic Sea) at the river gauge station *Fischamend* (river kilometer 1908, occurring just below a flood event with a recurrence of 1 year).

Hourly hydraulic pressures and water temperatures were recorded continuously during the monitoring period in all groundwater monitoring wells. Hourly Danube water level and discharge values were measured at the station *Fischamend*.

4.2.2 Sampling strategy

Monthly samples were taken at all sampling locations from March 2014 to May 2016 (n=22, Appendix C Figure 16). During this period, discharges in the Danube ranged from 693 m^3/s to 6197 m^3/s. In addition to the monthly samples, two flood events with a one-year return period (HQ2015 and HQ2016) were sampled with an increased sampling frequency (n=25) in the Danube and in wells LGS41 and LSG30.

Groundwater samples for micropollutants and standard chemical parameters were taken after pumping 3 well volumes at an abstraction rate of 0.77×10^{-3} m^3/s (van Driezum et al., 2017). A portable Sension+ MM150 sensor system (Hach-Lange, Austria) and a portable Profiline multi 3320 sensor system (WTW, Germany) were used in the field to measure temperature, pH, electrical conductivity and dissolved oxygen.

4.2.3 Chemical analysis

4.2.3.1 Inorganic and organic parameter analysis

A volume of 250 mL of ground- and surface water was taken in clean plastic bottles which were cooled at 4 °C and immediately transported to the lab. Anion and cation analyses were performed using ion chromatography. Absorption photometry was used to measure ammonium and nitrite (Appendix A Table 13).

4.2.3.2 OMP analysis and quantification

For this study, seven OMPs were selected based on their potential to serve as indicator substances for wastewater sources (Jekel et al., 2015). One-liter samples were filled in cleaned, clear glass bottles and transported to the lab in cooling boxes at 4 °C immediately. All samples were stored at 4 °C until analysis. Analysis of OMPs by solid phase extraction (SPE) followed by high

performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was performed using the method described in van Driezum et al. (2017). The LC-MS/MS system consisted of a Primaide HPLC with 1210 Autosampler (Hitachi High Technologies, USA) coupled to a hybrid triple quadrupole linear trap ion trap tandem mass spectrometer Q Trap 3200 (Applied Biosystems, Foster City, CA, USA) equipped with electrospray ionization (ESI) source operated in negative- and positive-ion mode. Details on analyte data and precursor-product ion can be found in Appendix C Table 20. The compounds were identified by retention time match and their specific HPLC-MS/MS transitions. The recoveries and LOQs of the different compounds can be found in the supporting information (Appendix C Table 21).

4.2.4 Mixing ratios of Danube and backwater river in PGAW3

In order to give an indication of the behavior of the OMPs, mixing ratios of the Danube and backwater river in PGAW3 were calculated based on daily 2-D groundwater flow simulations during the study period. As stated previously, river water enters the backwater when water levels exceed a certain threshold value. When water levels in the backwater rise, groundwater flow paths towards PGAW3 might change and water of a different composition can be extracted in PGAW3. The 2-D variable saturated groundwater flow model was previously developed for the studied transect (Naus, 2015). The mean deviation between measured and simulated groundwater levels was 0.2 m at maximum after calibration. For calculating the daily mixing ratios the simulated inflow rates were summed along river beds of the Danube and the backwater, respectively, over the full simulation time. With the results from the mixing ratio procedure, OMP concentrations were calculated in PGAW3. Calculations were made for 6 mixing scenarios. Two scenarios with solely Danube water and solely backwater river water were taken as extremes, whereas the scenarios with a mixture of both sources were more likely to occur (Appendix C Table 22, van Driezum et al., 2018). For CBZ, no degradation is assumed (Clara et al., 2004). SMZ removal under oxic conditions is slow and only partial (Table 9). For simplification, no degradation was assumed. Danube and backwater river concentrations were taken for the calculation of CBZ and SMZ. Calculated BTri concentrations were based on concentrations measured in LSG2 and in the backwater river.

4.2.5 Flow intervals and load calculation

The dataset was divided in classes depending on flow intervals for comparison of OMP loads in the Danube during the studied period. The flows were categorized according to the percentage of exceedance, as follows

(US Environmental Protection Agency, 2007): *flood events* (0-2.5%), *high flows* (2.5-5%), *moist conditions* (5-10%), *mid-range flows* (10-40%), *dry conditions* (40-70%) and *low flows* (70-100%). The calculation of the cumulative frequency was based on hourly mean discharges measured at the gauging station *Fischamend* from March 2014 till May 2016. The discharges corresponding to these intervals are shown in Table 6. The hourly loads *L* were calculated based on the method using flow intervals, as described by Zoboli et al. (2015).

Table 6 Range in discharge at *Fischamend* for the flow intervals, *n* is the amount of water quality samples per flow interval

	Low flows (Q_L)	Dry conditions (Q_d)	Mid-range flows (Q_m)	Moist conditions (Q_{mo})	High flows (Q_h)	Flood events (Q_{FL})
Discharge (m^3/s)	<1250	1250-1700	1700-2500	2500-3000	3000-3500	>3500
N	*10*	*6*	*6*	*7*	*10*	*8*

4.2.6 Data analysis and statistics

Correlation analyses of micropollutants with hydrological, physical and chemical variables were performed using the Pearson product correlation and the Spearman rank order correlation. A P-value of 0.05 was set as a significance threshold for all parameters. One-way analysis of variance (ANOVA) tests and the associated *post-hoc* Tukey's range test were used (function aov and TukeyHSD) to determine if any significant difference existed between the OMP concentrations in the surface water samples and in the groundwater samples. All statistical analyses were performed using R 3.1.1., partly using the *Hmisc* package (v. 4.1.1). All graphs were prepared using Grapher 10.5 (Golden Software, Colorado, USA).

4.3 Results

4.3.1 Hydrological and chemical characterization of surface water and groundwater

The Danube showed strong fluctuations during the studied period with changes in water levels as high as 6 m (Appendix C Figure 17). Continuously low flow periods were observed early 2014 and from July 2015 till January 2016. Discharges in these periods were mostly below 1500 m^3/s. Higher discharges were observed during spring and summer 2014 and in spring 2015 and 2016. The discharges on the days when seasonal sampling took place ranged from 862 m^3/s to 2960 m^3/s. Water level fluctuations during HQ2015 were 2.6 m and discharges ranged from 2500 m^3/s to 5200

m^3/s. Water level fluctuations during HQ2016 (4.8 m) were almost twice as high and discharges ranged from 1000 m^3/s to 5200 m^3/s.
The backwater river was only connected to the Danube during flood events. During these events, water levels increased up to 3 m, much less than in both the Danube and the groundwater (Appendix C Figure 17).

Water level fluctuations in all groundwater monitoring wells were nearly 4 meters over the entire study period. During both flood events, groundwater levels close to the river fluctuated >1 m during HQ2015 and >2 m during HQ2016. Long-term oxygen concentrations of PGAW3 (minimum of 1.9 mg/L, maximum of 4.6 mg/L, data not shown) and the measured oxygen concentrations during sampling showed conditions in the aquifer were oxic. Average nitrate concentrations (Appendix C Table 23) in the wells between the Danube and PGAW3 were well above 5 mg/L, further confirming oxic conditions. Manganese and iron concentrations taken in the PGA were predominantly below 0.1 mg/L (Mayr et al., 2014).

The portion of groundwater at well PGAW3 coming from the backwater river showed large variations from January 2014 till May 2016 (Appendix C Figure 18). By the end of June and beginning of July 2014, the water flowing into the aquifer was almost solely coming from the backwater river. From March to July 2015 on the contrary, most of the groundwater originated from the Danube except during HQ2015 when the proportion of backwater river increased shortly to 20% (Appendix C Figure 18).

4.3.2 OMP occurrence in surface waters

4.3.2.1 Seasonal sampling

All seven OMPs were found in both the Danube and the backwater river (Table 7) with substantial higher detection frequencies for all compounds in the Danube. Highest concentrations in the Danube were found for BTri, ranging from 58 ng/L up to 402 ng/L. The concentrations of CBZ and SMZ were 1 order of magnitude lower, ranging from 7.48 ng/L to 42.0 ng/L and from 1.86 ng/L to 15.1 ng/L respectively. Although the detection frequencies of BTri and CBZ in the backwater river were high, the concentrations were substantially lower than in the Danube (Figure 8). Although a negative correlation with water levels was present for these compounds in the Danube ($r=-0.58$, $P=0.005$ for BTri and $r=-0.62$, $P<0.005$ for CBZ), the backwater showed a positive correlation between water levels and the compounds ($r=0.87$, $P<0.005$ for BTri and $r=0.87$, $P<0.005$ for CBZ, Pearson correlation). SMZ had a much lower detection frequency in the backwater river than BTri and CBZ. No clear seasonal patterns could be seen for BTri, CBZ and SMZ (Figure 8).

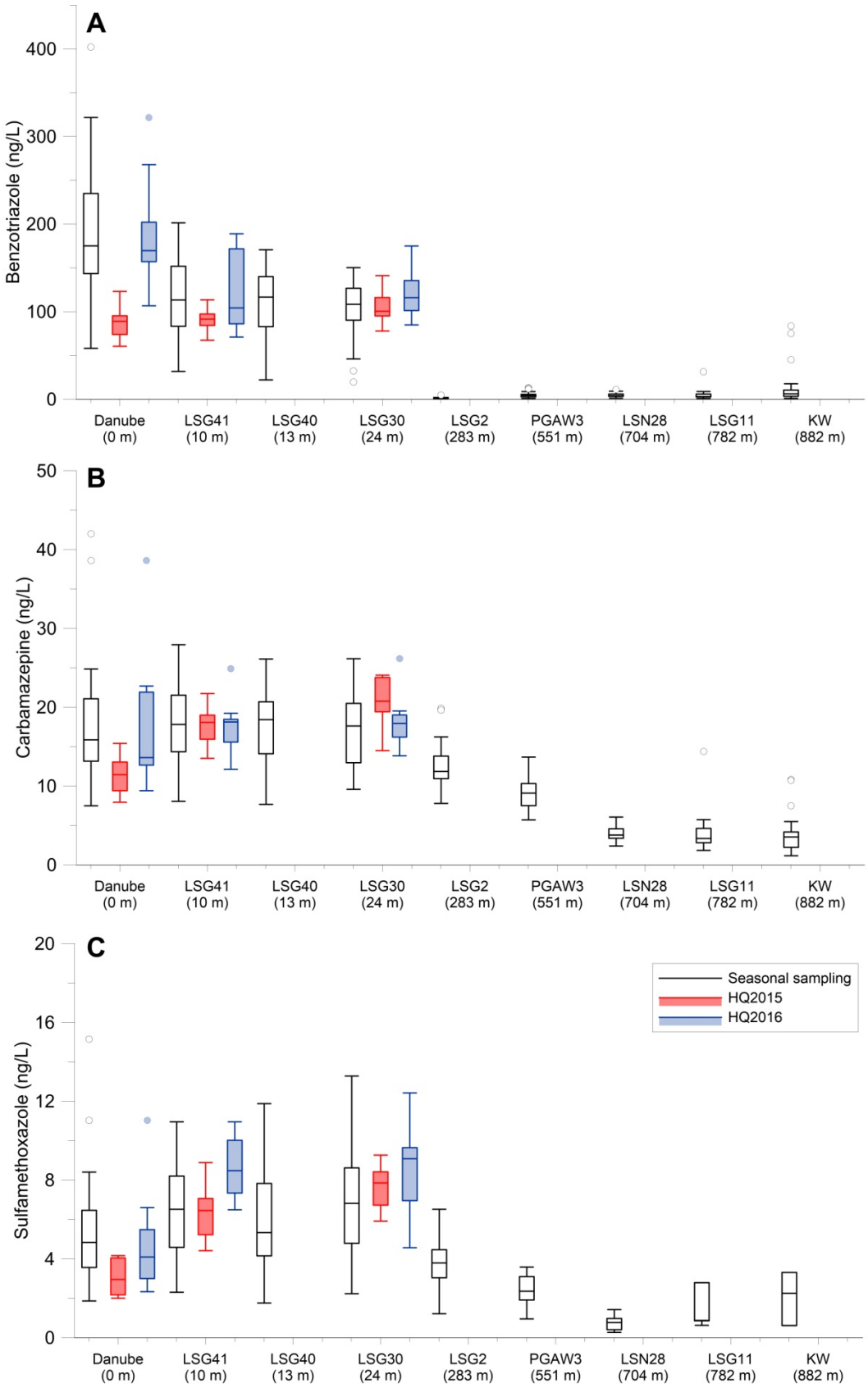

Figure 8 Boxplots of **a)** benzotriazole, **b)** carbamazepine and **c)** sulfamethoxazole. White boxes represent the seasonal samples, red boxes the HQ2015 samples and blue boxes the HQ2016 samples. The boxes cover the 25th and 75th percentile, the line within the boxes the median and whiskers the 10th to 90th percentile

Bezafibrate and diclofenac were frequently measured in the Danube, but had much lower detection frequencies in the backwater river. Similarly to BTri, CBZ and SMZ, no seasonal patterns were observed. Peak concentrations were encountered at the same time as the previously mentioned compounds. Bisphenol A and ibuprofen were only sporadically measured in both surface waters.

4.3.2.2 Flood event sampling

The same detection frequencies of BTri, CMZ and SMZ in the Danube were observed during both flood events as during seasonal sampling. Concentrations in the Danube were slightly lower during HQ2015 than during seasonal sampling (Figure 8). The concentrations of BTri, CBZ and SMZ in the Danube measured during HQ2016 were in a similar range as the seasonal samples (Table 8).

Bezafibrate, bisphenol A, diclofenac and ibuprofen were sporadically detected in the Danube during HQ2015. During HQ2016, detection frequencies of these compounds were similar as during the seasonal sampling campaign, although their concentrations were clearly lower during the flood events than during seasonal sampling.

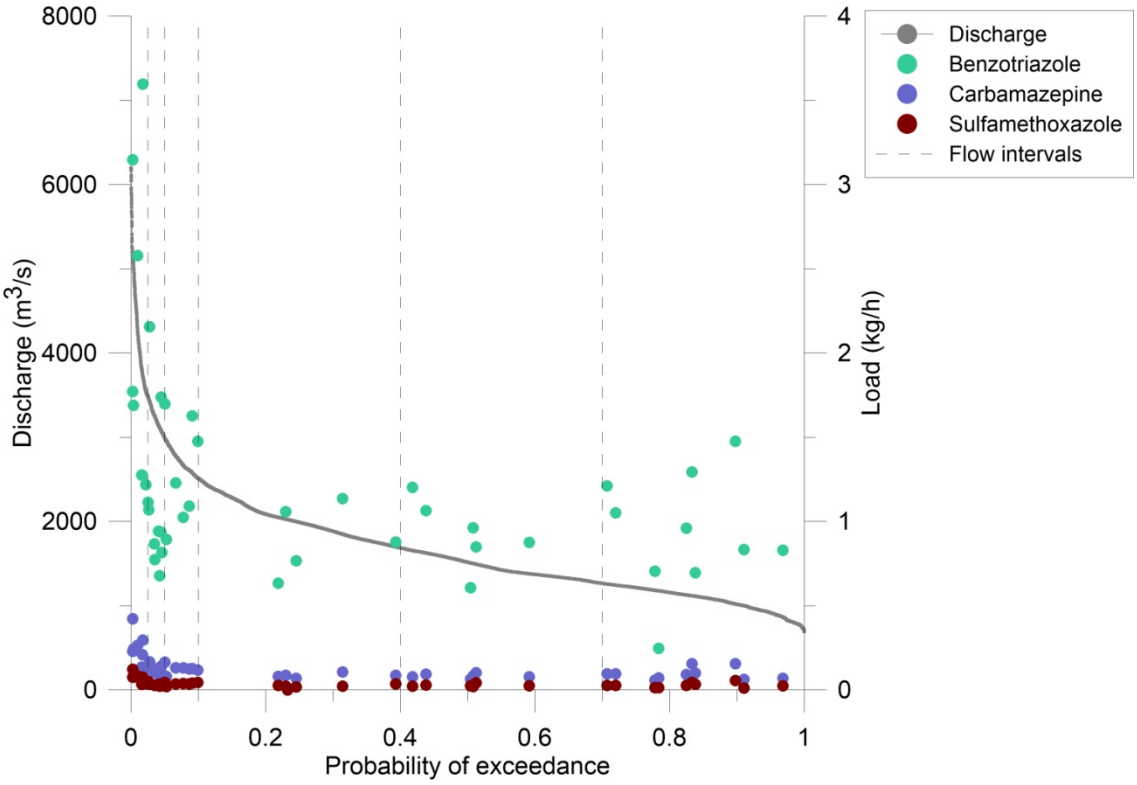

Figure 9 Flow duration curve showing the flow intervals and the corresponding loads during these days. BTri is shown in green, CBZ in blue and SMZ in dark red

4.3.2.3 Load calculations

The load of these compounds was calculated in order to gain more understanding of OMP dynamics under different discharges in the Danube. A pearson correlation performed using log-transformed loads of BTri, CBZ and SMZ indeed showed all loads significantly correlated with discharges (r=0.58, 0.77 and 0.66 for BTri, CBZ and SMZ, respectively, with P<0.005) and during HQ2015, they even doubled (Figure 9).

4.3.3 OMP attenuation during RBF

4.3.3.1 Seasonal sampling

BTri, CBZ and SMZ had similar detection frequencies in the groundwater as in the surface water (Table 7). Bezafibrate was only sporadically detected in the groundwater close to the river, with values around the LOQ. Bisphenol A, diclofenac and ibuprofen were not detected in any of the groundwater samples during the seasonal sampling campaigns.

Figure 10 shows the results of the seasonal sampling for BTri, CBZ and SMZ. As can be seen, the highest OMP concentrations were found for BTri. During the first 24 m of aquifer passage (wells LSG41 (10 m), LSG40 (13 m) and LSG30 (24 m)), BTri concentrations decreased from an average value of 183 ng/L in the Danube to 103 ng/L in LSG30, which was an average removal of 44%. After another 260 m of aquifer passage (LSG2), BTri dropped to an average concentration of 1.42 ng/L and was only detected 10 out of 22 times. In PGAW3, after another 268 m of aquifer passage, BTri remained at a similarly low level and detection frequencies increased simultaneously. The removal in PGAW3 was up to 97%. Concentrations in LSN28 and LSG11 were slightly higher than in PGAW3 and seemed to be influenced by the backwater river. The temporal variations in BTri concentrations seen after 260 m of aquifer passage (LSG2) were substantially lower than in the first 24 m. Figure 10 shows that the removal of BTri is not constant throughout the year. During an extended period of higher discharges (for example from April 2015 till the end of June 2015), the groundwater in the first meters of aquifer passage had a higher BTri concentration than the Danube.

CBZ was found in all groundwater samples and reached a maximum concentration of 27.9 ng/L in well LSG41, which was closest to the river. Concentrations were stable during the first 24 m of aquifer passage, but a decrease of up to 48% was observed towards PGAW3 (Figure 8). The results of an ANOVA test further indicated that CBZ was not fully persistent in the PGA. According to these results, LSG41, LSG40 and LSG30 group together (P=0.98), just as LSG2 and PGAW3 (P=0.28). The concentrations and

temporal variation in LSG2 and PGAW3 were higher than in wells LSN28 and LSG11.

A similar pattern can be seen for SMZ. The concentrations in the first 24 meters of the aquifer passage stayed stable (1.76 ng/L – 15.1 ng/L) and decreased towards PGAW3 (up to 56% attenuation). The temporal variability in SMZ concentrations simultaneously decreased with longer groundwater residence times. In LSN28 and LSG11, SMZ was only sporadically above the LOQ.

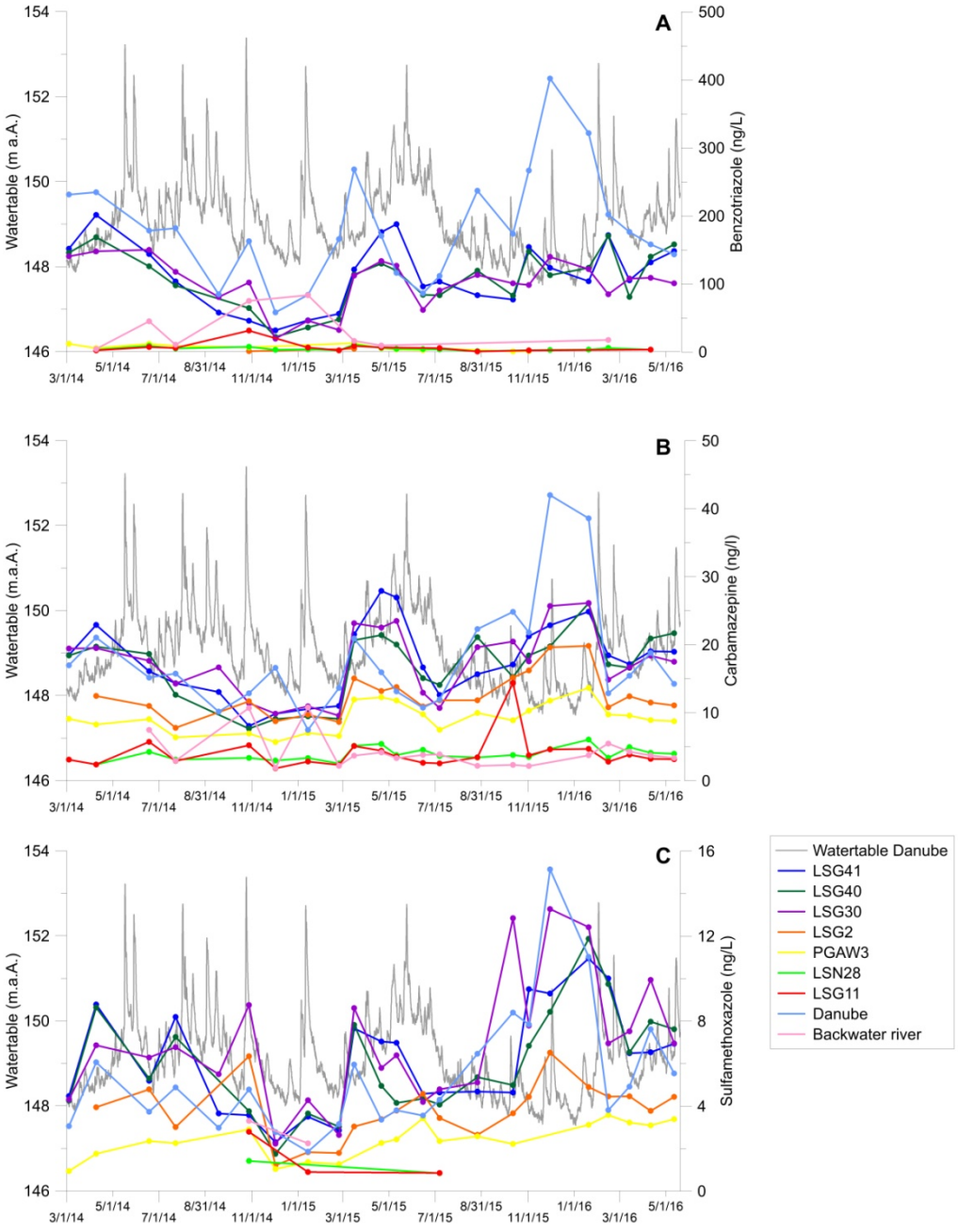

Figure 10 Seasonal sampling results for **a)** BTri, **b)** CBZ and **c)** SMZ. Concentrations were given for both surface waters and all monitoring wells

seasonal sampling	Danube	LSG41	LSG40	LSG30	LSG2	PGAW3	LSN28	LSG11	Backwater
Concentration in ng/L	*0 m*	*10 m*	*13 m*	*24 m*	*283 m*	*551 m*	*704 m*	*782 m*	*882 m*
Benzotriazole	58.0-402 (22/22)	31.6-201 (22/22)	22.0-171 (22/22)	19.4-150 (22/22)	n.d.-4.65 (10/22)	<LOQ-12.9 (22/22)	LOD-10.9 (19/22)	<LOQ-31.3 (22/22)	LOD-83.4 (21/22)
Carbamazepine	7.48-42.0 (22/22)	8.05-27.9 (22/22)	7.66-26.1 (22/22)	9.58-26.1 (22/22)	7.79-19.8 (22/22)	5.70-13.7 (22/22)	2.38-6.05 (22/22)	1.82-14.4 (22/22)	<LOQ-10.8 (22/22)
Sulfamethoxazole	1.86-15.1 (22/22)	2.30-11.0 (22/22)	1.76-11.9 (22/22)	2.23-13.3 (22/22)	1.21-6.51 (22/22)	<LOQ-3.58 (22/22)	n.d.-1.42 (17/22)	n.d.-2.79 (7/22)	n.d.-3.31 (4/22)
Bezafibrate	LOD-10.1 (21/22)	n.d.-<LOQ (3/22)	n.d.-<LOQ (1/22)	n.d.-<LOQ (2/22)	n.d.(0/22)	n.d.(0/22)	n.d.(0/22)	n.d.(0/22)	n.d.-3.94 (3/22)
Bisphenol A	n.d.-155 (4/22)	n.d. (0/22)	n.d.-32.2 (1/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-<LOQ (1/22)
Diclofenac	<LOQ-88.2 (22/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-LOD (1/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-22.6 (3/22)
Ibuprofen	n.d.-7.29 (7/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-LOD (1/22)
OMP ratios (-)									
Benzotriazole /carbamazepine	3.49-15.7 (*10.5*)***(r=0.85)**	3.21-9.31 (*6.42*)	2.43-9.98 (*6.29*) ***(r=0.78)**	1.97-8.93 (*5.89*) ***(r=0.75)**	N/A	0.07-1.35 (*0.79*)	0.33-2.21 (*1.21*)	0.18-6.00 (*1.71*) ***(r=0.57)**	2.28-7.74 (*4.86*) ***(r=0.96)**
Benzotriazole/ sulfamethoxazole	20.7-75.6 (*36.7*) ***(r=0.80)**	9.49-33.9 (*18.9*) ***(r=0.65)**	9.84-34.2 (*18.5*) ***(r=0.68)**	7.84-32.6 (*15.6*) ***(r=0.50)**	N/A	0.75-12.4 (*4.59*)	N/A	N/A	N/A
Carbamazepine/ Sulfamethoxazole	2.47-6.00 (*3.54*) ***(r=0.93)**	1.75-4.28 (*3.00*)	1.74-5.17 (*3.06*) ***(r=0.69)**	1.30-4.51 (*2.74*) ***(r=0.66)**	1.84-7.21 (3.60) ***(r=0.56)**	2.39-9.63 (*4.28*)	N/A	N/A	N/A

Table 7 Min-max concentration values and min-max values of the ratios between the OMPs in the surface- and groundwater samples during the seasonal sampling The values in brackets for the concentrations represent the detection frequencies; the *italic* values in brackets for the ratios represent the average values. Ratios were only given between values above LOQ and when more than 30% of the concentrations could be determined. A statistically significant correlation (P<0.05, based on the Pearson correlation) between the compounds was indicated by an asterisk. Values in meters are the distances to the Danube

	Danube 0 m		LSG41 10 m		LSG30 24 m	
	HQ2015	HQ2016	HQ2015	HQ2016	HQ2015	HQ2016
Water table difference (m a.A.)	150.10-152.74	147.95-152.78	149.63-150.70	147.80-149.74	149.62-150.71	147.80-149.71
Gradient (%)	n.a.	n.a.	5.77-20.4	1.50-31.0	-0.08-0.10	0.01-0.19
Benzotriazole (ng/L)	60.4-123 (15/15)	107-268 (9/9)	67.3-118 (15/15)	70.9-189 (9/9)	77.8-141 (15/15)	85.1-175 (9/9)
Carbamazepine (ng/L)	7.93-15.4 (15/15)	9.40-22.7 (9/9)	13.5-21.7 (15/15)	12.1-19.2 (9/9)	14.5-30.6 (15/15)	13.8-19.5 (9/9)
Sulfamethoxazole (ng/L)	2.00-4.16 (15/15)	2.33-6.60 (9/9)	4.41-8.88 (15/15)	6.49-10.3 (9/9)	5.91-9.26 (15/15)	4.56-9.95 (9/9)
Bezafibrate (ng/L)	n.d.-0.58 (5/15)	3.58-5.92 (9/9)	n.d.	n.d.-<LOQ (2/9)	n.d.	n.d.-<LOQ (3/9)
Bisphenol A (ng/L)	n.d.-93.2 (1/15)	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac (ng/L)	6.90-21.6 (15/15)	31.0-50.2 (9/9)	n.d.	n.d.-LOQ (4/9)	n.d.	n.d.-8.60 (5/9)
Ibuprofen (ng/L)	n.d.	LOD-<LOQ (9/9)	n.d.	n.d.	n.d.	n.d.

Table 8 Min-max values of the water table difference and gradient and of the OMPs in the surface- and groundwater samples during HQ2015 and HQ2016. The values in brackets represent the detection frequency. Values in meters are the distances to the Danube

4.3.4 Flood event sampling

Samples were taken with a higher frequency during two flood events (HQ2015 and HQ2016) and analyzed for the studied OMPs (Table 8). It can be seen that the detection frequencies of BTri, CBZ and SMZ in the groundwater were similar during both events and comparable with the seasonal sampling campaign. No statistically significant difference between the seasonal and flood sampling events (ANOVA, $P>0.05$) was observed for all three compounds (Figure 8). Concentrations of the three compounds at LSG41 (10 m from the riverbank) and LSG30 (24 m from the riverbank) were even higher than in the Danube during both events. This was especially seen for the more conservative compounds CBZ and SMZ and was most evident during HQ2015. A much lower attenuation of BTri was observed during HQ2015 (30%) and HQ2016 (0%) than during seasonal sampling (44%) after 24 m of groundwater passage. In contrast to the seasonal sampling and HQ2016, where BTri significantly correlated with CBZ and SMZ in both the Danube and the groundwater, this was only the case in the Danube during HQ2015. CBZ and SMZ significantly correlated with each other during all events in both surface- and groundwater.

Of the other measured OMPs, only bezafibrate and diclofenac were detected in the groundwater up to a distance of 24 m (LSG30) during HQ2016.

4.3.5 OMP ratios

In order to assess the fate of biodegradable compounds, their ratios can be calculated (Scheurer et al., 2011). The ratios between BTri, CBZ and SMZ concentrations were calculated for all samples taken in the river and the groundwater wells for both the seasonal sampling (Table 7) and the two flood events (not shown). For BTri, it can be seen that the ratios of this compound with either CBZ or SMZ decrease from the river towards the groundwater. During the first 24 m of aquifer passage, the ratios between BTri and CBZ or SMZ stayed stable and the compounds correlated significantly with each other. When moving towards PGAW3, the ratios decrease and a relatively higher amount of CBZ and SMZ is found. In contrast, the ratios between CBZ and SMZ stayed stable from the river towards PGAW3, which was confirmed by ANOVA. The compounds also correlate significantly with each other in both the groundwater and the surface water samples.

The ratios calculated for the flood events were not consistently different than during seasonal sampling. OMP concentrations and the corresponding ratios in the Danube during HQ2015 were substantially different from HQ2016 and the seasonal sampling while the difference in concentrations of the compounds was not similar. During groundwater infiltration, the OMP

ratios only differed significantly ($P<0.05$, ANOVA) between HQ2015 and HQ2016, with higher ratios during HQ2016.

4.4 Discussion

4.4.1 The behavior of OMPs during RBF along a large dynamic river

Concentrations of BTri, SMZ, bezafibrate, diclofenac, bisphenol A and ibuprofen found in the Danube samples were consistent with previous measurements in the Danube (Loos et al., 2017, 2010b) and other large European rivers (Ruff et al., 2015; Sjerps et al., 2017; Wolschke et al., 2011). The median concentration of CBZ was also consistent with previous measurements in the Danube (Loos et al., 2010) but slightly lower than in the Rhine (Ruff et al., 2015) and much lower than in the river Thames (Nakada et al., 2017). OMP concentrations and detection frequencies were generally much lower in the backwater river than in the Danube. Since the backwater river can be seen as a series of connected ponds fed by groundwater and precipitation rather than as a river, an increase in OMP concentrations was only detected during irregular inflows of Danube water.

Bezafibrate, bisphenol A, diclofenac and ibuprofen were not found in the oxic groundwater. These compounds have been known to be fully removed during RBF under oxic conditions (Burke et al., 2014b; Heberer et al., 2004; Rauch-Williams et al., 2010; Wiese et al., 2011). Concentrations of BTri, CBZ and SMZ in the groundwater were in a similar range as in other studies (Huntscha et al., 2013; Loos et al., 2010a; Scheurer et al., 2011), but they were attenuated differently. Previously, they were known to be either fully or mostly persistent under different hydrogeological conditions (Table 9). Concentrations of these compounds in the drinking water abstraction well were far below (provisional) drinking water guideline values derived in several EU countries (Baken et al., 2018).

As for BTri, most of the degradation was found to take place in the first few meters of the aquifer. In contrast to our results, BTri was previously found to be never fully removed except for the study of Reemtsma et al. (2010), which had unstable redox conditions in the aquifer. We found an average removal of 44% after 24 m of aquifer passage. A similar removal was found under similar hydrogeological characteristics as at our site at the Thur river (Huntscha et al. 2013). In contrast to Huntscha et al. (2013), BTri was almost fully removed at the drinking water abstraction well at our site. This difference can be explained because we sampled at wider distances (and higher residence times) from the river and therefore observed significantly

more BTri removal. Other studies showed no significant removal under oxic conditions (Table 9), e.g., as shown by Bertelkamp et al. (2016a) in a low conductive aquifer, even after up to 4 months of groundwater travel times. This suggests that travel time combined with oxic conditions alone does not explain BTri removal. Reported literature found no or very little removal of BTri in the environment. Under conditions with a highly active microbial community, like a WWTP, partial removal was shown (e.g. Mazioti et al., 2015). Sorption was found to be negligible (Yu et al., 2009). In our study, a high degree of river-groundwater interaction was apparent due to the high conductivity of the aquifer.

Furthermore, the microbial activity was found to be relatively high (van Driezum et al., 2018). Based on previous studies, we therefore conclude that biodegradation was the main mechanism responsible for the high BTri removal. Highly conductive RBF systems, such as the PGA and along the river Thur (Huntscha et al., 2013), are more favorable to biodegradation of compounds like BTri.

CBZ has generally been classified as persistent (Table 9). Some attenuation was sporadically found, e.g. studies from lake Tegel and lake Wannsee in Berlin showed that some degradation of CBZ can occur during aquifer passage (Burke et al., 2014a; Henzler et al., 2014; Wiese et al., 2011). Bertelkamp et al. (2016a) did not find attenuation of CBZ directly in the field, but column tests indicated some removal of the compound. Our study showed that CBZ concentrations are stable during the first 24 m of aquifer passage but then slightly decreased during an extra 527 m of aquifer passage towards PGAW3. A possible explanation for this decrease in CBZ could have been mixing of the Danube and backwater river at PGAW3. This was only partially confirmed by the mixing ratio calculations (Appendix C Table 22). The proportion of backwater river must be between 30-60% assuming a conservative behavior of CBZ, but this is not very likely for our system (Appendix C Figure 18).

Also SMZ was only partially removed under oxic conditions. A similar behavior was shown along the river Rhine (Storck et al., 2012), although concentrations were slightly lower in the PGA. A full removal of SMZ during RBF was previously found only under anoxic conditions (Table 9). Mixing with backwater river water could again only account for part of the decrease in concentration of SMZ as shown for CBZ.

Based on the marker ratios during seasonal sampling, the difference in attenuation between BTri on one hand and CBZ and SMZ on the other hand was clearly visible, with the latter two being similar. Several studies have

shown differences in biodegradation and retardation of CBZ and SMZ (Bertelkamp et al., 2016b; Hamann et al., 2016; Henzler et al., 2014; Nham et al., 2015). Since no distinction was made between retardation and biodegradation in this study only an indication of a similar rate of attenuation between CBZ and SMZ can be given.

4.4.2 Do flood events change the presence and behaviour of OMPs in surface and groundwater?

As was seen in Van Driezum et al. (2018), the flood events had an influence on the microbial activity and increased cell counts in the Danube. It was expected, that OMP concentrations in the Danube, on the contrary, were lower during the flood events than during seasonal sampling due to dilution. CBZ for example, is not removed during wastewater treatment (Joss et al., 2005; Radjenović et al., 2009; Zhang et al., 2008), so its load into the river is expected to be stable even when processes like combined sewer overflow (CSO) occur. BTri and SMZ are partly removed during wastewater treatment (Huntscha et al., 2014; Radjenović et al., 2009) and their loads might therefore slightly increase during flood events due to CSOs. A stable load, especially of CBZ, was however not seen in our study. Since CSOs could not be primarily responsible for the increase in OMP loads, another explanation was proposed.

During flood events, total suspended solids (TSS) can be mobilized. The TSS concentration, and also in stream phosphorus (P) concentrations can therefore increase significantly, as was seen previously in the Danube (Zessner et al., 2005, Zoboli et al. 2015). A significant trend was shown between discharge of the Danube and the TSS concentration (Nachtnebel et al., 1998). Some OMPs are partly sorbed to TSS and can desorb under conditions like flooding (Silva et al., 2011). Consequently, the amount that can desorb is higher during flood events and can lead to an increase in OMP loads (Rivetti et al., 2015). The positive relationship of CBZ concentration (but also other pharmaceuticals) to phosphorus dynamics and TSS was also shown by Acuna et al. (2015). An increased and extended influence of the Danube on the microbial compartment of the groundwater was observed during HQ2016 as compared to HQ2015 (van Driezum et al., 2018), due to the higher increase in river water levels during the event. Because of the influence of flood events on the microbial compartment, we expect that OMP concentrations could be similarly influenced by the infiltrating surface water. It was shown that groundwater concentrations of BTri, CBZ and SMZ during the flood events slightly increased and were even higher than in the surface water. A similar increase in OMP concentrations in groundwater was also observed by Huntscha et al. (2013) along the river

Compound	Removal	Aquifer properties		Travel time	Hydrological characteristics	Comments
		Hydrogeology	redox conditions			
Benzotriazole	No removal [b), d), h)]	unconsolidated sandy gravels [b)], predominantly sand [h)], undisturbed sandy core [d)]	Oxic [b), h)], fluctuation between oxic and anoxic [d)]	67-113h [b)], 1-3 months [h)]	Q_{max} 160 m³/s [b)], undisturbed core [d)], river Ijssel [h)]	Redox dependent removal, probably only partial removal under anoxic conditions
	partial removal [a), e), f), g), i)]	unconsolidated sandy gravels [a)], microcosms with aquifer sediment [f)], alluvial aquifer [i)]	Oxic [a)], fluctuation between oxic and anoxic [e)]	7h-3d 4h transect A; 0-35h transect B [a)],	Q_{max} 160 m³/s [a)], microcosm [f)], river Rhine [g)], Q_{max} 100 m³/s [i)]	
	90% [c)]	predominantly sand [c)]	fluctuation between oxic and anoxic [c)]	4-5 months [c)]	lake [c)]	
Carbamazepine	Persistent [a), b), e), g), h), k), i), m), n), o), p)]	unconsolidated sandy gravels [a), b)], predominantly sand [h), k)], sand and gravel [n), o)]	Oxic [a), b), h), n), o)], fluctuation between oxic and anoxic [e), m), o), p)], anoxic [k), o)]	7h-3d 4h transect A; 0-35h transect B [a)], 67-113h [b)], 1-3 months [h)], 1.65 – 3.65 years [k)], 7-30 days [n)], 25 days [o)]	Q_{max} 160 m3/s [a), b)], lake [m)], river Rhine [g)], river Ijssel [h)], Q_{max} 100 m³/s [i)], river Lek [k)], Q_{max} 120 m³/s [o)], $Q_{average}$ 3300 m³/s [o)], $Q_{average}$ 106 m³/s [o)], infiltration ponds [p)]	Overall low removal, probably due to retardation; no removal at high discharges
	slight attenuation [d), l), m)]	predominantly sand [l), m)], undisturbed sandy core [d)], alluvial aquifer [l)]	fluctuation between oxic and anoxic [d), l)]	4-5 months [l), m)]	lake [l), m)], undisturbed core [d)], Q_{max} 100 m³/s [i)],	
	degradation [j)]	predominantly sand [j)]	fluctuation between oxic and anoxic [j)]	4-5 months [j)]	lake [j)]	
Sulfamethoxazole	Rather persistent [a), b), n)]	unconsolidated sandy gravels [a), b)], sand and gravel [n)]	Oxic [a), b), n)]	7h-3d 4h transect A; 0-35h transect B [a)], 67-113h [b)], 7-30 days [n)]	Q_{max} 160 m3/s [a), b)],	Redox dependent removal; slow removal; and only partial under oxic conditions, up to full removal under anoxic conditions
	partial removal [h), i), l), q), r)]	predominantly sand [h), i), l), q), r)]	Oxic [h)], fluctuation between oxic and anoxic [i), l), q), r)]	4-5 months [i), l), q), r)], 1-3 months [h)]	lake [i), l), q), r)], river Ijssel [h)],	
	full removal [i), k), q), r)]	predominantly sand [i), k), q), r)]	fluctuation between oxic and anoxic [i), q), r)], anoxic [k)]	1.65 – 3.65 years [k)], 4-5 months [i), q), r)],	lake [i), q), r)], river Lek [k)]	

Table 9 Literature values for attenuation of BTri, CBZ and SMZ

a) (Huntscha et al., 2013), b) (Huntscha et al., 2012), c) (Reemtsma et al., 2010), d) (Burke et al., 2014a), e) (Kahle et al., 2009), f) (Liu et al., 2013), g) (Scheurer et al., 2011), h) (Bertelkamp et al., 2016a), i) (Epting et al., 2018), j) (Henzler et al., 2014), k) (Hamann et al., 2016), l) (Wiese et al., 2011), m) (Heberer et al., 2004), n) (Storck et al., 2012), o) (Hoppe-Jones et al., 2010), p) (Massmann et al., 2006), q) (Heberer et al., 2008), r) (Grünheid et al., 2005)

Thur after flood events. We observed higher OMP concentrations in the Danube and the groundwater prior to HQ2016 and to a lesser extent prior to HQ2015 relative to periods without flood events. During these events, more surface water can enter the aquifer, i.e. during HQ2015 and HQ2016 over 3 and 24 times more "fresh" water respectively can enter the aquifer during the flood peak than during the days prior to the peak as can be calculated following the procedure of Ubell (1987). This "fresh" surface water with lower concentrations mixes with older groundwater with higher OMP concentrations. Mixing occurs at a slower pace than the flow velocities during these events. This can explain why OMP concentrations reached further into the aquifer and were higher in groundwater than in the Danube during the flood events, even more so during HQ2016 than during HQ2015.

Similar to bacterial abundance (van Driezum et al., 2018), an increase of several OMPs was found in the groundwater up to 24 m away from the river during HQ2016. Bezafibrate and diclofenac were observed in the groundwater, although no correlation was found with groundwater flow velocity. Although no measurements were taken in the drinking water abstraction well during the flood events, we expect a negligible impact of the river on the groundwater quality in the abstraction well at 550 m from the river. This was supported by the lack of substantial variations in OMP concentrations in the drinking water abstraction well throughout the entire study period. The observation wells closer to the river however did show an extended impact of the river on groundwater quality. Drinking water abstraction wells that would be located closer to the river in highly conductive RBF systems can therefore be under direct stress during flood events. In these cases, more intensive monitoring of OMPs is proposed during flood events.

4.5 Conclusion

The results show that drinking water abstraction wells in particular close to the river and under oxic conditions can be vulnerable to an extended contamination during flood events, even from highly degradable compounds.

In contrast to previous studies, this study showed that BTri is almost fully removed by the time it reaches the drinking water abstraction well. CBZ and SMZ are attenuated to a certain extent, since mixing of groundwater with low-concentrated backwater river could only partly explain the

decrease of these compounds. A similar rate of attenuation could be presumed for CBZ and SMZ.

Several marker OMPs (e.g. bezafibrate, diclofenac and ibuprofen) were not detected in the aquifer under oxic conditions.

Unexpectedly, the results during the flood events showed that most of the OMP concentrations in the Danube were similar as during the seasonal sampling period.

The load of BTri, CBZ and SMZ in the Danube was higher, possibly due to an increase in TSS in the river or to the inflow of the Donaukanal in this section of the Danube. Groundwater concentrations of BTri, CBZ and SMZ during the flood events were higher than in the Danube and reached further into the aquifer, in comparison with seasonal sampling. During the flood in 2016, highly degradable compounds such as diclofenac and bezafibrate could enter the aquifer up to a distance of 24 m from the river and BTri was significantly less attenuated than during the seasonal sampling period.

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5. Overall conclusions and future challenges

This thesis investigated the effect of surface water infiltration of the river Danube on the flow and transport of groundwater and on the contaminants introduced into the aquifer in a riverbank filtration (RBF) system. To quantify this effect, I sought to analyse both chemical and microbial parameters to give a complete overview of contaminants introduced into the aquifer due to RBF. An extensive monitoring campaign was conducted which captured changes in water quality of the infiltrating river (using the combined set of parameters), seasonal changes and changes in water levels (including two flood events). This was combined with previously conducted modelling approaches to get a better insight in the hydrological processes influencing the transport of the contaminants. Due to the high efficiency of the hyporheic zone to remove contaminants, the sampling locations were designed to achieve higher spatial resolution.

Due to the combination of different parameters and the highly dynamic nature of the investigated RBF system along the Danube, it was of paramount importance to obtain representative samples throughout the whole system. Groundwater fluctuations close to the river were higher than further inland and could have an influence on the representativeness of the samples.

Chapter 2 showed that samples taken for the analysis of micropollutants and standard chemical parameters were stable during pumping, even when they were taken close the river. Both the watertable fluctuations and the fluctuations in contaminant concentrations did not affect the stability of the obtained chemical samples. These fluctuations did however have an impact on the stability of some microbial parameters such as leucine incorporation. The samples drawn directly after the start of pumping were not representative for the microbiological quality of the aquifer, since biofilms were present during pumping of the first three well volumes. Samples representative for the microbiological quality of the aquifer could only be drawn after pumping of 15 well volumes. This showed the challenges when taking samples for the analysis of a combined set of parameters.

Chapter 3 discussed the vulnerability of the microbial water quality in the RBF system due to the dynamic nature (fluctuation in water levels) of the river Danube. It showed that bacterial abundance, biomass and carbon

production decreased significantly on their way from the river towards further inland. Where the river contained a higher proportion of large cells due to the availability of nutrients, the groundwater contained more small cells. Overall, biomass and bacterial abundance were higher in the river and correlated with several chemical parameters as well as with temperature. In the groundwater however, these correlations were absent. Under regular discharge conditions, it was shown that the temporal changes in the microbial metrics monitored were more closely aligned with fluctuations in groundwater flow velocities. This correlation extended till 10 m away from the riverbank under regular conditions, whereas during flood events, it extended up to 24 m away from the riverbank. Furthermore, the surface water introduced a higher amount of bacterial cells in the aquifer during these flood events, which could not be explained by calculated turnover times. The amount of large cells in the groundwater close to the river increased dramatically during HQ2016 and was most likely caused by the infiltrating surface water, which almost solely contained large cells. The groundwater showed a lower response during HQ2015, due to the lower potential difference between river water and groundwater level and therefore a smaller volume of surface water introduced to the aquifer during this event.

As chapter 3 identified the impact of surface water infiltration on the groundwater microbial community, **chapter 4** studied the effect of this infiltration on organic micropollutant (OMP) removal in the aquifer. Using a total of 7 OMPs, the influence of the river on the groundwater quality was also identified for these parameters. The three WWTP markers BTri, CBZ and SMZ were all found in the groundwater close to the river during seasonal sampling. It was shown that in contrast to previous studies performed under oxic conditions, BTri was almost fully removed at the drinking water abstraction well. CBZ and SMZ however were attenuated to a certain extent. Mixing with groundwater coming from the less chemically contaminated backwater river could not fully explain the decrease in concentration of CBZ and SMZ at the drinking water abstraction well. Therefore, some degradation was assumed along the way through the aquifer for these parameters. Several other marker OMPs were not detected in the aquifer during seasonal sampling. During flood events, the concentrations of BTri, CBZ and SMZ were higher in the groundwater than in the Danube and higher concentrations could reach up to 24 m into the aquifer. Similar as to the microbial community, the influence of HQ2016 was higher than HQ2015 due to the potential difference between river water and groundwater level and the volume of surface water introduced to the aquifer. During and directly after HQ2016, even highly degradable

compounds like diclofenac (which was absent under normal conditions), were found up to a distance of 24 m in the aquifer.

This thesis showed that obtaining samples for a combination of chemical and microbial parameters was not an easy task. This information however could be of great benefit for drinking water utilities. The results showed that drinking water abstraction wells in particular close to a river and under oxic conditions can quickly react on changing hydraulic conditions. The influence of the river reached further into the aquifer and affected the microbiological characteristics and the extent of chemical contamination in a similar manner. It was shown that relatively small flood events (annual average floods) did not have an impact on the quality of the groundwater reaching the drinking water abstraction well in our study area but abstraction wells closer to a river in other areas could very well be impacted. Bacterial abundance might be able to act as an indicator for surface water – groundwater interactions. Drinking water utilities might therefore have the possibility to monitor this interaction using this specific parameter. The monitoring of bacterial abundance can be done using flow cytometry, which is much more cost-effective than the analysis of OMPs, and might be used near-real time. Furthermore, the potential difference between river water and groundwater level and, as a result of this potential difference, the volume of newly introduced surface water have shown to be very important parameters for the extent of surface water - groundwater interaction. This applies to both the microbiological characteristics and the chemical contamination. Therefore, a combination of the hydrogeological parameters of the RBF system and the microbiological characteristics of the groundwater measured near-real time at the abstraction well might be able to help drinking water utilities to optimize their water abstraction strategies and react quicker to changing hydrological conditions.

As this study focused on surface water – groundwater interactions through the use of the microbial community and identified the influence of the interaction on chemical contamination, future work should focus on the contamination with microbiological contaminants like viruses, bacteria and protozoa. The EU drinking water directive obligates Member States to ensure safe drinking water, free of any micro-organisms which constitute a potential danger to human health. Since groundwater contains much less micro-organisms than surface water, current sampling techniques are not sufficient to ensure an appropriate decrease in infection risk. The removal mechanisms for micro-organisms during groundwater passage are very different than for chemicals and results found in this work can therefore only give an indication of the extent of surface water influence in the

groundwater and cannot be used as a surrogate. Once the removal of the micro-organisms can be attributed to certain mechanisms, models could help the drinking water utilities to obtain more information about the infection risk of the actual drinking water. In these models, concentrations found in the infiltrating surface water can than serve as boundary conditions. Concentrations in the surface water are much higher than in the aquifer and are measured more easily.

The results can also be used in a 3D model to understand OMP behavior in more depth. So far, only calculations based on mixing of groundwater coming from the river and groundwater coming from the backwater river could give an indication whether CBZ and SMZ were degraded during aquifer passage. As CBZ is believed to be a substance with a conservative behavior, it is of profound importance to elucidate the processes responsible for the decrease in concentration observed in this RBF system. Modelling could gain more insight into the different processes responsible for the attenuation of the OMPs and the changes caused by smaller flood events. So far it was not absolutely clear why OMP concentrations in the groundwater were higher than in the river during floods. Furthermore, future work should also focus on the influence of bigger flood events. Since some climate change scenarios suggest an increase in extreme events such as intense rainfall, the severity of floods might increase in the future. More severe flood events could inundate the floodplain, which could have an extensive impact on the groundwater quality. This did not occur during this study and is therefore a suggestion for future work. Modelling could help to give an indication of the extent of contamination during bigger flood events in which sampling might not be possible. This information is very helpful for the development of operating procedures during severe flooding of RBF systems used by drinking water utilities.

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7. Appendix A

7.1 External contaminants

The external contaminants analyzed in this study consisted of two parameters of microbial faecal pollution (*E. coli* and enterococci), eight different micropollutants (benzotriazole, bezafibrate, bisphenol A, carbamazepine, diclofenac, estrone, ibuprofen and sulfamethoxazole) and several inorganic parameters.

7.1.1 Micropollutant sample preparation

One-liter samples were filled in glass bottles and transported to the lab in a cooling box of 4 °C for the analysis of micropollutants. Two samples were taken after 15 min and 75 or 105 min of pumping. The samples were stored at 4 °C before analysis. Two times 400 g of sample was carefully weighed and in a third sample additional standards were added (resulting in an extra 50 ng/L of all analytes). The samples were then extracted using a Strata-X 33u 200 mg polymeric reversed phase SPE cartridge (Phenomenex, Germany). The samples were passed through the preconditioned cartridge with a vacuum manifold at a rate of approximately two drops per second. SPE catridges were preconditioned using 3x1 mL methanol and 3x1 mL deionized water. After loading, the cartridges were dried under vacuum using a drying apparatus. The compounds were eluted with 6x1 mL methanol and were evaporated to approximately 0.5 mL. 0.5 mL deionized water was added to a total volume of 1 mL in amber vials.

7.1.2 Analytical method

For the quantitative instrumental analysis of the micropollutants, a high performance liquid chromatograph (Primaide 1210 Auto Sampler, Hitachi High Technologies, USA) combined with a hybrid triple quadrupole linear trap ion trap tandem mass spectrometer Q Trap 3200 (Applied Biosystems, Foster City, CA, USA) equipped with electrospray ionization (ESI) source operated in negative- and positive-ion mode was used. Three compounds (benzotriazole, carbamazepine and sulfamethoxazole) were measured with ESI source operating in positive mode. All other compounds were measured with ESI source operating in negative mode. A C18 column (Phenomenex Luna 5u, 150 x 3.0mm) with precolumn (Phenomenex Purospher, 5 µm) was used for LC separation with a flow rate of 800 µL/min. The mobile phase consisted of 60% deionized water (inhouse production) and 40% ChromaSolv acetonitrile (Sigma-Aldrich, Austria) with 0.1% acetic acid (Sigma-Aldrich,

Austria). The injection volume was set to 50 µL, with an injection speed of 10 µL/s. All other parameters can be found in Table 10.

7.1.3 Quality control

All samples were extracted in duplicate. Some samples were extracted in triplicate, where one of the samples had extra analytes spiked in an end concentration of 50 ng/L. Additionally, during every extraction, two deionized water samples spiked with 50 ng/L of the analytes were extracted simultaneously. Limits of quantification (LOQ) were calculated using a signal to noise ratio of 10. The LOQ ranged from 0.35 ng/L for carbamazepine to 70.5 ng/L for bisphenol A (values for LOQ and LOD can be found in Table 11). Recoveries of all compounds can be found in Table 12. Quantification of all samples was performed with a linear 9 point calibration curve (with r^2>0.99). The concentrations ranged from 50 ng/L up to 10 µg/L.

7.1.4 Cultivation based methods

Bacteriological analysis included E. coli and intestinal enterococci as faecal indicators. Four-litre samples were filled in sterilized plastic containers and transported to the lab in a cooling box of 4°C. Samples were taken every 15 min to a total of 75 or 105 min (5 to 7 sampling time points). For each parameter 1 L water sample was processed in sub portions of 10mL, 100mL and 900mL. The microbiological analyses were performed on the day of sampling. The enumeration and confirmation were performed according to ISO standards using membrane filtration methods. For the determination of *E. coli* Trypton-Bile-X-Glucuronide (TBX) medium (Oxoid, Hampshire, UK) was used, the incubation conditions were 44 ± 0,5 °C for 44 ± 4 h (ISO 16649-1, 2001). Enterococci were cultivated on Slanetz–Bartley medium (Oxoid) at 44 ± 0,5 °C for 44 ± 4 h followed by confirmation by transferring the membranes to Bile-Esculin agar (Oxoid) for 2 h (ISO 7899-2, 2000). The results were expressed as colony forming units (cfu) per liter.

7.2 Intrinsic parameters

The intrinsic parameters analyzed in this study consisted of spores of aerobic spore formers (bacterial spores), total bacterial abundance (including large and small cells), bacterial 3H-leucine incorporation, physicochemical parameters and several inorganic parameters.

7.2.1 Cultivation based methods

Sampling was carried out every 15 min to a total of 75 or 105 min (5/7 sampling time points). Four-litre samples were filled in sterilized plastic containers and transported to the lab in a cooling box of 4 °C. The microbiological analysis was performed on the same day as the sampling. Bacterial spores from aerobic spore formers representing microorganisms in their permanent stage were measured according to ISO standards using membrane filtration methods. They were cultivated after pasteurization of the water sample (60 °C; 15 min) at 22 ± 2 °C for 7 d on yeast extract agar (Scharlab, Barcelona, Spain) (ISO 6222, 1999). The results were expressed as colony forming units (cfu) per liter.

7.2.2 Cell count methods

Total bacterial abundance were measured using the slightly modified protocol of (Riepl et al., 2011). In short, depending on the type of water, between 1 mL and 100 mL of sample was fixed for 1 - 2 hours at room temperature using sterile filtered formaldehyde (final concentration 1.8 %). 500 µL to 40 mL was filtered on a 0.2 µm membrane filter (Anodisc 25, Whatman, Germany). The filter was mounted on a drop of SYBR-Gold (Invitrogen, Lofer, Austria), diluted to a final concentration of 1:400 of the stock solution. The filter membrane was incubated at room temperature (22 ± 2 °C) in the dark for 15 ± 3 minutes. The filter was rinsed three times with 1 mL sterile, filtrated autoclaved water and dried in the dark. After drying, the dry filter membrane was mounted between a microscope slide and cover slip, with a drop of anti-fading solution (Citifluor, Groepl, Austria) on both sides. The samples were either stored at -20 °C or analysed immediately with a Nikon epifluorescence microscope (Nikon Eclipse 50i). Cells were classified in large cells (rod shaped cells and coccoid cells with diameter > 1.0 µm) and small cells (coccoid cells with a diameter < 1.0 µm). Whenever cells were clumped together and embedded in a visible background matrix, they were classified as particles coming from biofilm.

7.2.3 Bacterial ^{3}H-leucine incorporation

Bacterial 3H-leucine incorporation (LI) was measured based on protocols of (Kirschner and Velimirov, 1999; Simon and Azam, 1989). Briefly, 3H-leucine was added to triplicate 10 mL samples at a final concentration of 10 nM. Duplicate control samples were stopped with trichloroacetic acid (TCA, 5 % final conc., Sigma-Aldrich, Germany) directly after the addition of 3H-leucine. Both controls and samples were incubated for 30 min in the dark at the measured temperature of the aquifer. At the end of the incubation samples were also stopped by adding TCA. One-hundred µL of 35% NaCl was added to enhance precipitation of macromolecules inclusive proteins

and all samples were incubated for 30 min at 18 °C. After incubation, the samples were filtered through a cellulose nitrate filter (0.45 µm) which was subsequently washed with 5 mL of 5% TCA, 80% ethanol and distilled water each. Filters were dried overnight in scintillation tubes. After adding 5 mL of scintillation cocktail, radioactivity was measured in a Perkin Elmer, TriCarb 2300 TR scintillation counter.

7.2.4 Inorganic parameters

Two-hundred fifty mL samples were filled in plastic bottles and transported to the lab in a cooling box of 4 °C for the analysis of inorganic parameters. Two samples were taken after 15 and 75 min of pumping. The samples were stored at 4 °C before analysis. Samples were analyzed for a large set of inorganic parameters (see Table 13). Anion and cation analysis was performed using ion chromatography. Absorption photometry was used to measure ammonium and nitrite (see Table 13).

Table 10 Analyte data

Analyte	Formula	Supplier and purity (%)	Precursor/ Product ion	CAS
Benzotriazole	$C_6H_5N_3$	Sigma-Aldrich (≥98)	120.1/65.1	95-14-7
Bezafibrate	$C_{19}H_{20}ClNO_4$	Sigma-Aldrich (≥98)	360.0/274.1	41859-67-0
Bisphenol A	$C_{15}H_{16}O_2$	Sigma-Aldrich (≥99)	227.0/212.1	80-05-7
Carbamazepine	$C_{15}H_{12}N_2O$	Sigma-Aldrich (≥99)	237.2/194.3	298-46-4
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	Sigma-Aldrich (>98.5)	293.8/250.1	15307-79-6
Estron	$C_{18}H_{22}O_2$	Sigma-Aldrich (≥99)	269.1/144.9	53-16-7
Ibuprofen	$C_{13}H_{18}O_2$	Sigma-Aldrich (≥98)	205.0/161.0	31121-93-4
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	Sigma-Aldrich	254.2/92.2	723-46-6

Table 11 LOQ and LOD values (in ng/L) for all samples. LOQs and LODs for all compounds with an asterisk were derived from samples which were spiked with an extra 50 ng/L of analyte

LOQ/LOD (ng/L)	LSG39 August	LSN28 August	LSN28 September	LSG30 September
Benzotriazole	4.42/1.47	6.53/2.18	4.40/1.47	5.62/1.87
Bezafibrate	2.01/0.67	2.41/0.80	2.94/0.98	2.08/0.69
Bisphenol A*	48.6/16.2	30.3/10.1	46.5/15.5	51.4/17.1
Carbamazepine	0.35/0.12	0.54/0.18	0.54/0.18	0.59/0.20
Diclofenac*	5.23/1.74	5.22/1.74	4.60/1.53	7.39/2.46
Estrone*	26.4/8.80	30.1/10.0	33.8/11.3	26.0/8.67
Ibuprofen*	17.1/5.71	29.2/9.73	17.8/5.92	23.0/7.68
Sulfamethoxazole	1.01/0.34	1.25/0.42	1.17/0.39	0.83/0.28

Table 12 Recoveries of all analytes during all time series analyses. Recoveries for all compounds with an asterisk were derived from samples which were spiked with an extra 50 ng/L of analyte

Recoveries (%)	LSG39 August	LSN28 August	LSN28 September	LSG30 September
Benzotriazole	48	38.64	45.86	46.5
Bezafibrate	81.6	78.96	92	83.2
Bisphenol A*	76.96	71.36	84.8	75.76
Carbamazepine	82.52	75.14	92.13	78.64
Diclofenac*	79.04	77.84	94.4	80.8
Estrone*	46.24	59.92	80.8	66.96
Ibuprofen*	85.6	83.2	96.8	86.4
Sulfamethoxazole	59.08	40.99	46.32	54.28

Table 13 Inorganic and organic parameter analysis. All untis are given in mg/L

Parameter	**Standard method**	**Measuring principle**	**Instrumentation used**	**LOQ (mg/L)**
TOC	EN1484	Oxidation to carbon dioxide by UV radiation, persulfate	Phoenix 8000, Tekmar Dohrmann, Mason, Ohio, USA	0.1
NH_4^+	ISO 7150-1	Adsorption photometry	Lambda 25, PerkinElmer, Walthman, Massachusetts, USA	0.02
NO_2^-	EN26777	Adsorption photometry	" "	0.01
NO_3^-	ISO 10304-1	Ion chromatography	DIONEX, ICS-1100, Thermo Scientific, Walthman, USA	1.0
Ca^{2+}	ISO 14911	Ion chromatography	" "	2.0
Mg^{2+}	ISO 14911	Ion chromatography	" "	0.5
Cl^-	ISO 10304-1	Ion chromatography	" "	0.5
SO_4^{2-}	ISO 10304-1	Ion chromatography	" "	1.0
Na^{2+}	ISO 14911	Ion chromatography	" "	0.5
K^+	ISO 14911	Ion chromatography	" "	0.5

Table 14 Statistical analysis using the Mann Whitney test

			Non-normalized			
	μ_c (t=15 min)	μ_c (t=75 min)	n1	n2	Mann-Whitney *U*	*P*
BTri (ng/L)	68.5	74.55	4	4	11	>0.10
CBZ (ng/L)	9.54	8.83	8	8	51	0.05>*P*>0.025
SMZ (ng/L)	4.36	3.30	4	4	14	0.10>*P*>0.05
Bacterial spores (#/L)	670	535	4	4	10	>0.10
Bacterial abundance (cells/mL)	2.26E+5	2.40E+5	4	4	7	>0.10
Leucin incorporation (pmol/L/h)	0.12	0.16	12	11	110	0.005>*P*>0.001[1]

			Normalized			
	μ_c (t=15 min)	μ_c (t=75 min)	n1	n2	Mann-Whitney *U*	*P*
BTri (ng/L)	-0.25	0.25	4	4	7	>0.10
CBZ (ng/L)	0.34	-0.34	8	8	47	0.10>*P*>0.05
SMZ (ng/L)	0.68	-0.68	4	4	15	0.10>*P*>0.05
Bacterial spores (#/L)	0.38	-0.43	4	4	13	0.10>*P*>0.05
Bacterial abundance (cells/mL)	-0.47	0.49	4	4	10	>0.10
Leucin incorporation (pmol/L/h)	0.57	0.001	12	11	110	0.005>*P*>0.001[1]

BTri = benzotriazole; CBZ = carbamazepine; SMZ = sulfamethoxazole.

[1]Statistically significant (at $P \leq 0.05$, Bonferroni corrected).

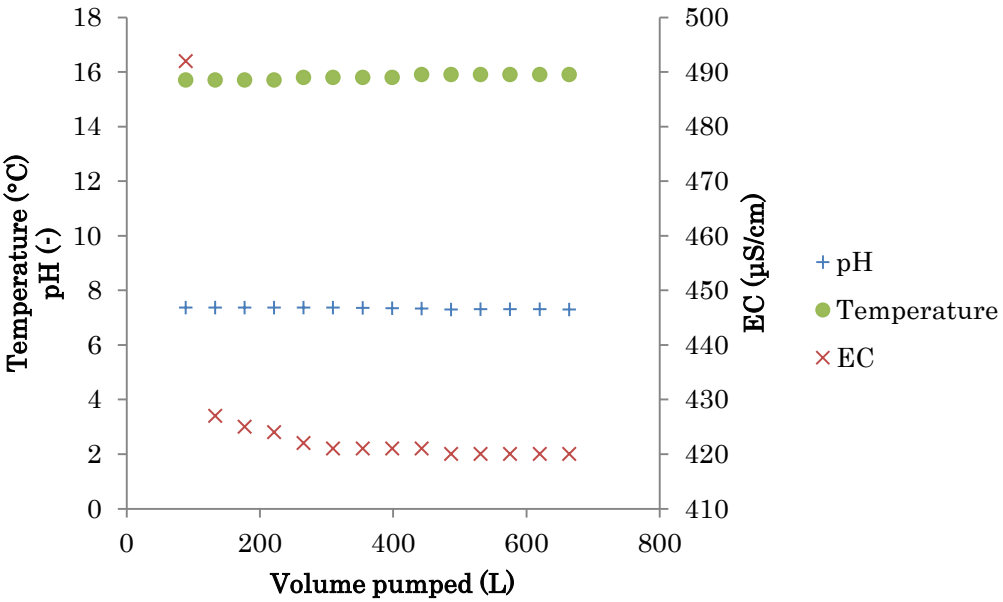

Figure 11 Temperature, pH and EC values after the unset of pumping

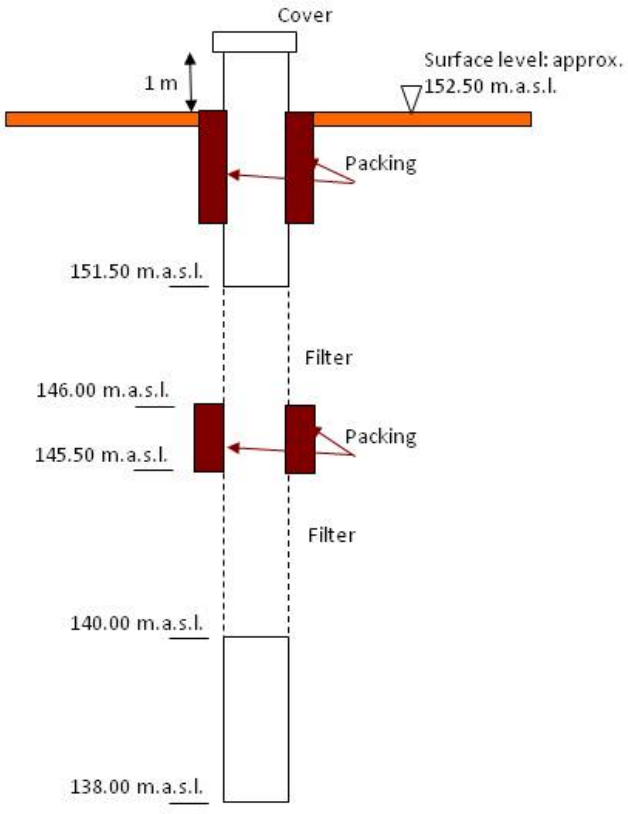

Figure 12 Construction details of LSG39

8. Appendix B

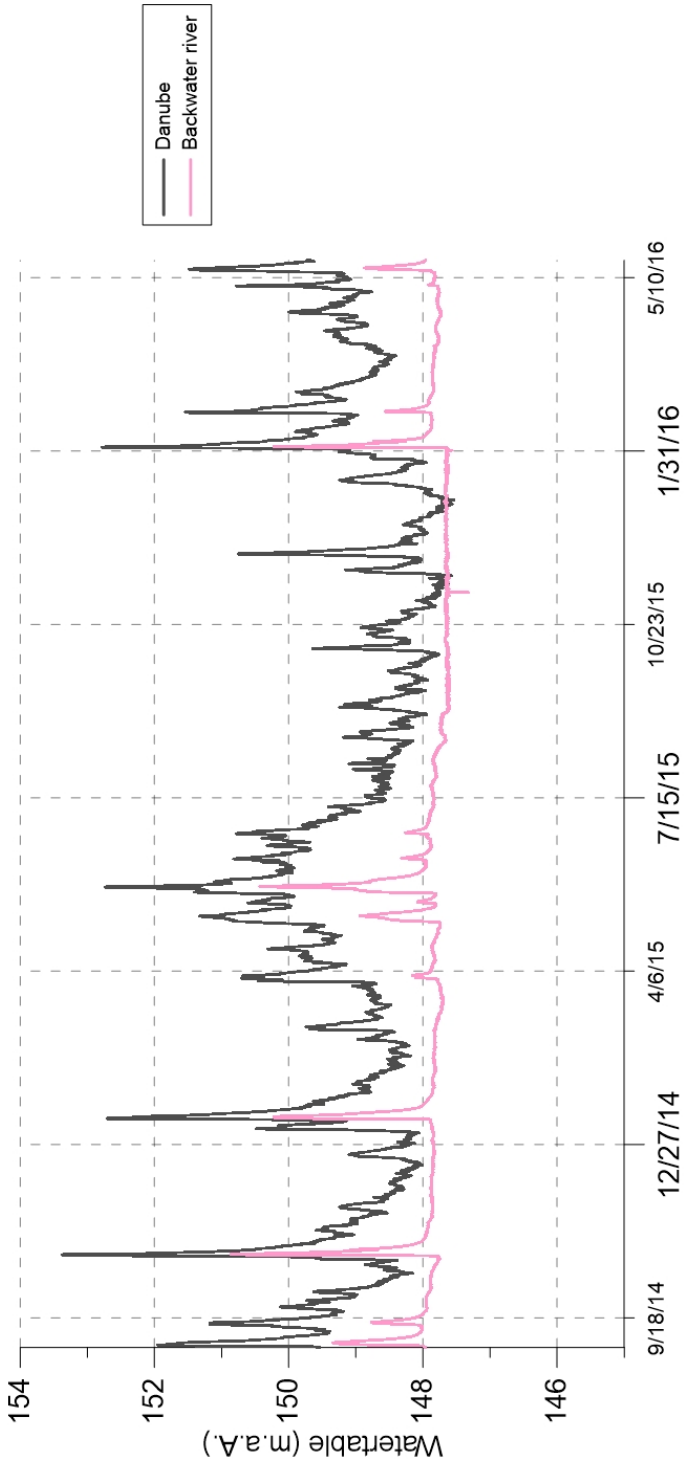

Figure 13 Waterlevel (in m.a.A.) of the Danube and the backwater river. It can be clearly seen that above a certain level in the Danube, the backwater river was connected to the Danube

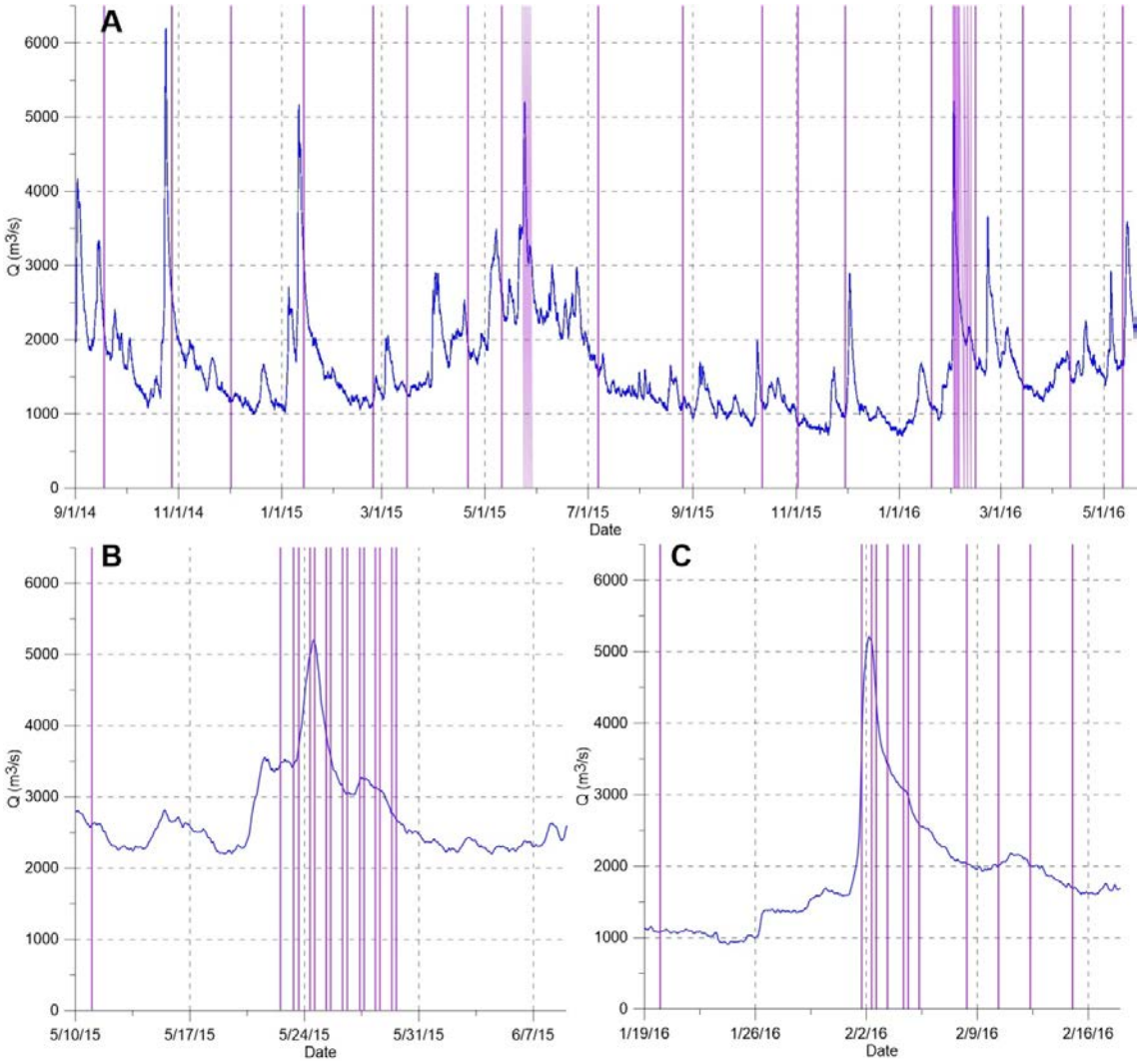

Figure 14 Sampling dates (pink vertical lines) a) from October 2014 until May 2016, b) during HQ2015 and c) during HQ2016

Table 15 Distance from the river to the monitoring wells

	Distance (m)	Cumulative distance from river (m)
Danube-LSG41	10	10
LSG41-LSG40	3.2	13.2
LSG40-LSG30	11.1	24.3
LSG30-LSG2	258.4	282.7
LSG2-PGAW3	268.4	551.1
PGAW3-LSN28	152.5	703.6
LSN28-LSG11	78.6	782.2
LSG11-Backwater river	100	882.2

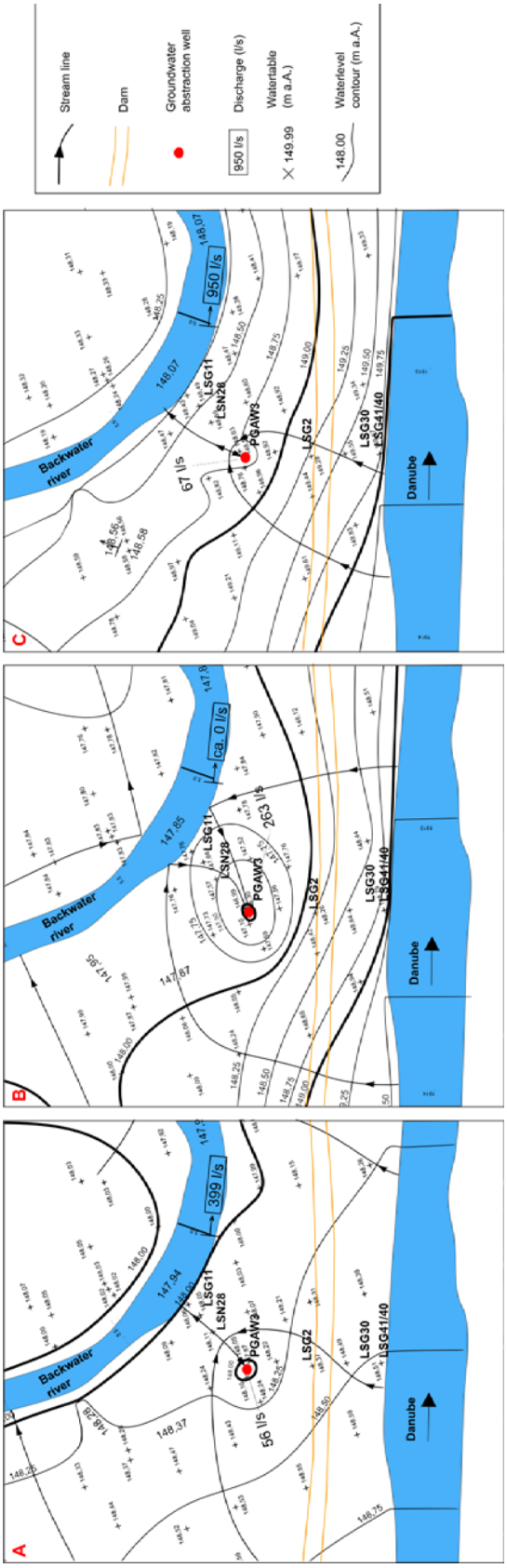

Figure 15 Groundwater contour maps during **a)** low discharges, **b)** average discharges and **c)** high discharge of the Danube

		Flow velocity m/s	DOC mg/L	NH_4 mg/L	NO_3 mg/L	T °C	pH	EC µS/cm
LSG41	BCP	0.693	0.315	-0.216	-0.254	0.042	-0.097	0.427
	Biomass	0.442	-0.038	-0.233	-0.269	0.161	0.106	0.213
	Large cells	0.473	-0.102	-0.224	-0.127	0.010	0.016	0.186
LSG40	BCP	-0.130	0.152	-0.022	-0.051	-0.222	0.246	0.683
	Biomass	-0.126	0.115	-0.188	-0.158	-0.025	0.247	0.329
	Large cells	-0.156	0.088	-0.116	0.029	-0.196	0.159	0.326
LSG30	BCP	0.122	-0.379	-0.002	-0.317	0.319	-0.161	-0.194
	Biomass	0.157	0.070	-0.019	-0.220	0.151	0.130	-0.144
	Large cells	0.109	0.096	0.065	0.005	-0.077	-0.104	-0.092
LSG2	BCP	0.629	-0.186	0.020	-0.465	0.232	0.079	-0.304
	Biomass	0.283	-0.026	-0.410	-0.407	0.159	0.452	-0.059
	Large cells	0.467	0.000	-0.273	-0.411	0.272	0.218	-0.265
PGAW3	BCP	-0.060	-0.179	-0.548	-0.114	0.141	0.392	-0.428
	Biomass	-0.018	-0.358	-0.007	-0.203	-0.123	0.443	0.026
	Large cells	0.476	-0.436	-0.083	-0.102	-0.268	0.421	0.191
LSN28	BCP	0.145	0.073	-0.304	0.126	0.376	-0.025	-0.242
	Biomass	-0.126	0.196	0.013	-0.259	0.422	-0.175	-0.180
	Large cells	0.370	0.369	0.201	0.139	0.352	-0.162	-0.289
LSG11	BCP	-0.234	0.048	-0.465	0.201	0.301	0.041	-0.412
	Biomass	-0.441	0.177	-0.476	0.014	0.193	-0.199	-0.499
	Large cells	-0.281	0.077	-0.443	0.224	0.348	-0.108	-0.552

Table 16 Heatmap of the Pearson correlation between BCP/Biomass/Large cells and flow velocity/selected standard parameters. The correlations were calculated for all groundwater monitoring wells. Flow velocities were based on the average value of the gradient measured between 3 and 6 days before the sampling date

HQ2015		Flow velocity m/s	TOC mg/L	SO_4 mg/L	NO_3 mg/L	T °C	pH	EC µS/cm
LSG41	Biomass	0.149	-0.182	0.273	-0.255	-0.146	0.059	0.183
	Large cells	-0.029	-0.276	0.515	-0.434	-0.011	-0.140	0.513
LSG30	Biomass	-0.423	NA	-0.007	-0.153	-0.195	0.151	0.267
	Large cells	-0.312	NA	0.302	0.267	-0.139	-0.119	0.326

HQ2016		Flow velocity m/s	TOC mg/L	SO_4 mg/L	NO_3 mg/L	T °C	pH	EC µS/cm
LSG41	Biomass	0.853	0.147	0.780	0.220	0.370	0.549	0.129
	Large cells	-0.402	0.527	-0.128	-0.173	-0.125	-0.272	0.322
LSG30	Biomass	0.664	-0.335	0.757	0.195	-0.423	0.207	0.860
	Large cells	-0.259	0.527	-0.447	-0.429	0.421	-0.180	-0.343

P
<0.05
0.05<p<0.10
0.10<p<0.20
0.20<p<0.50
0.50<p<0.70
0.70<p<1.0

Table 17 Heatmap of the Pearson correlations between Biomass/Large cells and flow velocity/selected standard parameters during the flood events of May 2015 and February 2016. Correlations were calculated for wells LSG41 and LSG30. Flow velocities were based on the average value of the gradient measured between 1 and 5 hours before the point of sampling

Table 18 Total cell counts (TTC) and Total viable active cell counts (TVAC)

	24/04/2017			09/05/2017			29/05/2017		
Sampling point	TTC cells/ml	TVAC cells/ml	% of total cells	TTC cells/ml	TVAC cells/ml	% of total cells	TTC cells/ml	TVAC cells/ml	% of total cells
LSG 41	150350	490	0.33	131543	696	0.53	171263	870	0.51
LSG 40	128797	340	0.26	124778	658	0.53	138279	985	0.71
LSG 30	87239	230	0.26	90554	131	0.14	93189	188	0.20
LSG 2	62632	140	0.22	57786	170	0.29	56883	258	0.45
PGAW3	50341	120	0.24	48910	106	0.22	47253	160	0.34
LSN 28	106850	90	0.08	102032	189	0.19	100637	258	0.26
LSG 11	95021	280	0.29	109009	210	0.19	97152	366	0.38

Table 19 Turnover times based on the TVAC measurements

Sampling point	minimum turnover time days	maximum turnover time days
LSG 41	0.3	9
LSG 40	0.2	18.3
LSG 30	0.3	6.5
LSG 2	2.1	17.4
PGAW3	2.5	35.7
LSN 28	0.3	15.7
LSG 11	0.2	17.8

9. Appendix C

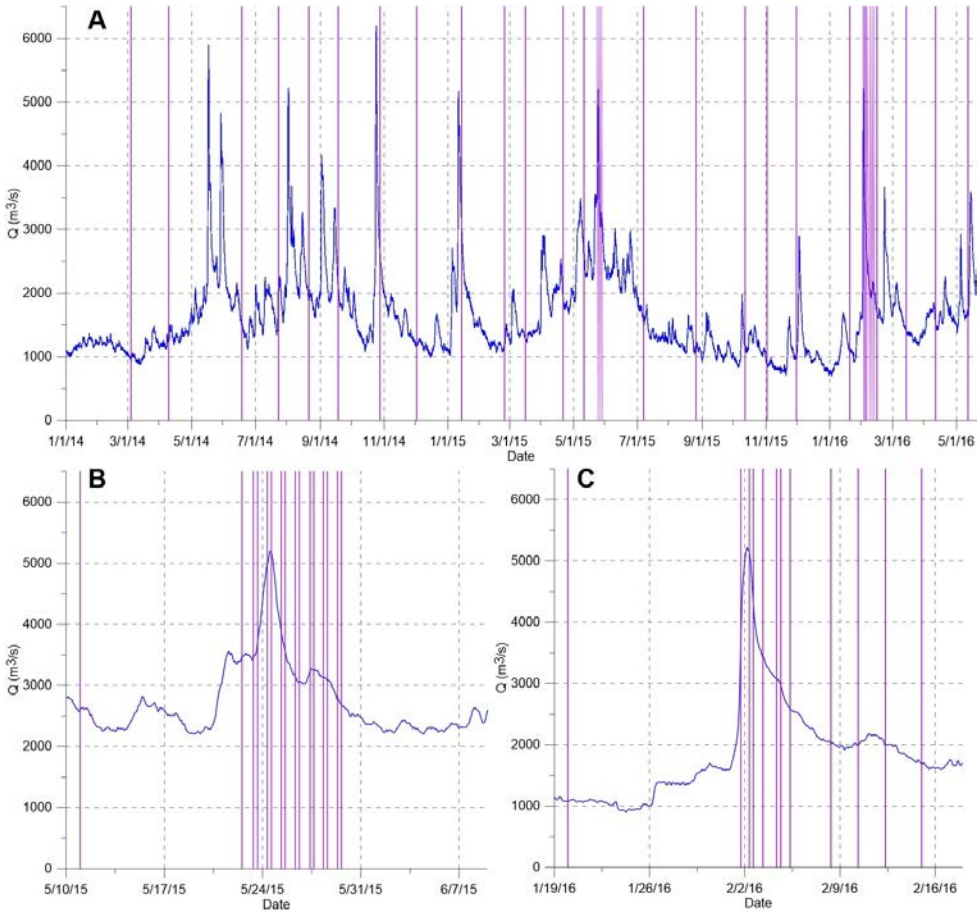

Figure 16 Sampling dates (pink vertical lines) a) from March 2014 until May 2016, b) during HQ2015 and c) during HQ2016

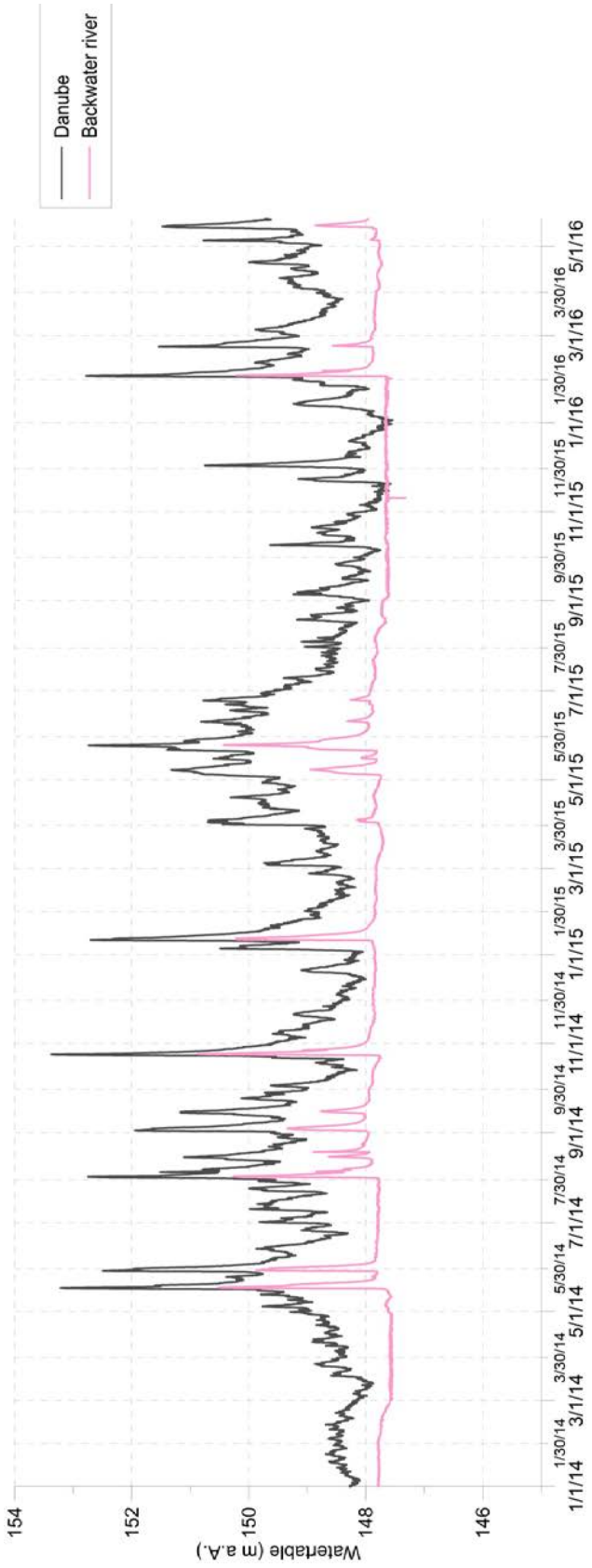

Figure 17 Water level (in m.a.A.) of the Danube and the backwater river. It can be clearly seen that above a certain water level in the Danube, the backwater river was connected to the Danube

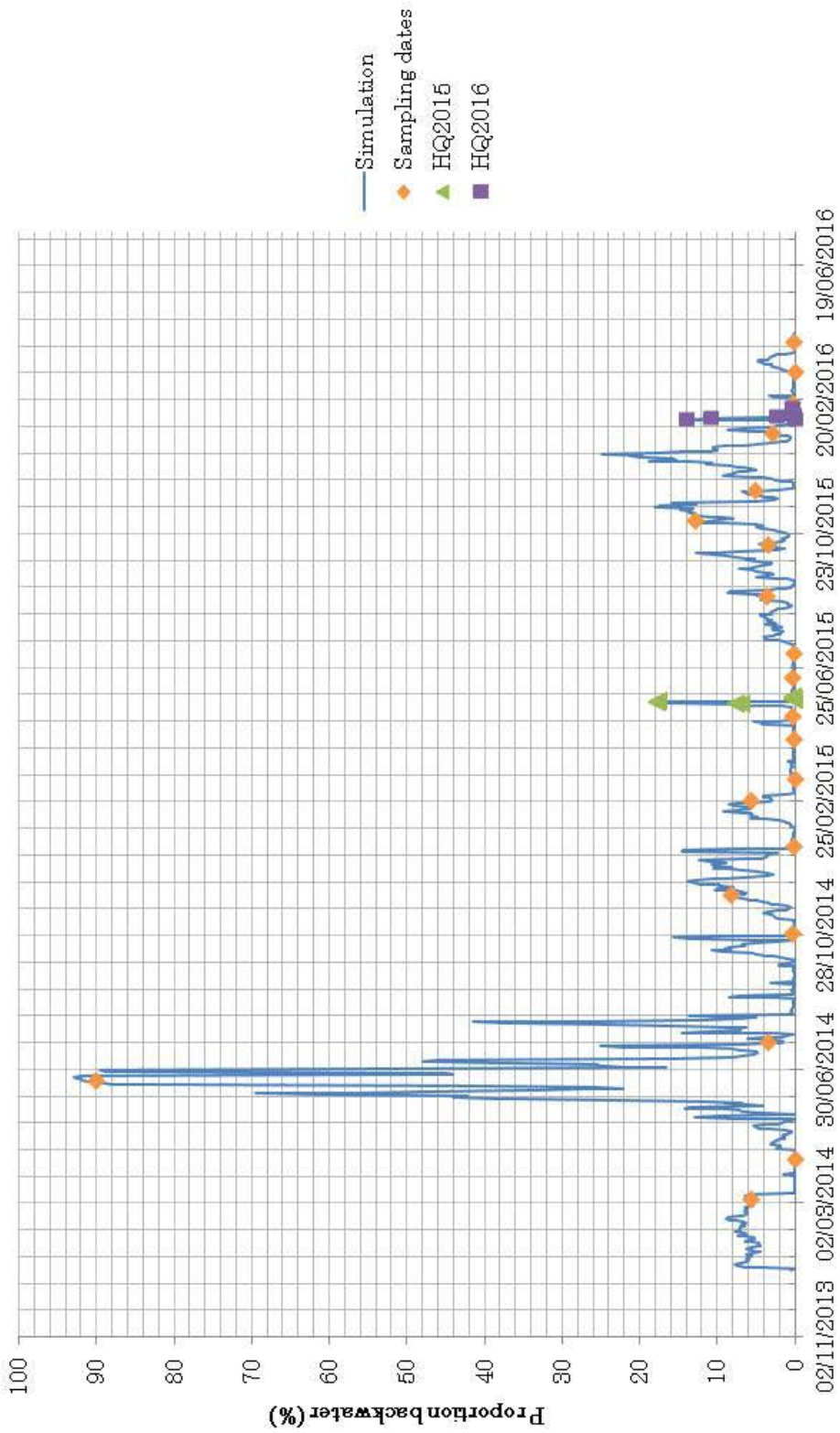

Figure 18 Proportion of backwater river water in PGAW3 including seasonal sampling dates in orange, HQ2015 in green and HQ2016 in purple

Table 20 Analyte data

Analyte	Formula	Supplier and purity (%)	Precursor/Product ion	CAS
Benzotriazole	$C_6H_5N_3$	Sigma-Aldrich (≥98)	120.1/65.1	95-14-7
Bezafibrate	$C_{19}H_{20}ClNO_4$	Sigma-Aldrich (≥98)	360.0/274.1	41859-67-0
Bisphenol A	$C_{15}H_{16}O_2$	Sigma-Aldrich (≥99)	227.0/212.1	80-05-7
Carbamazepine	$C_{15}H_{12}N_2O$	Sigma-Aldrich (≥99)	237.2/194.3	298-46-4
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	Sigma-Aldrich (>98.5)	293.8/250.1	15307-79-6
Ibuprofen	$C_{13}H_{18}O_2$	Sigma-Aldrich (≥98)	205.0/161.0	31121-93-4
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	Sigma-Aldrich (≥98)	254.2/92.2	723-46-6

Table 21 Validation data for groundwater (GW) and surface water (SW)

	LOQ (ng/L)		Relative recovery (%)		Inter-day precision (RSD)	
	GW	SW	GW	SW	GW	SW
Benzotriazole	2.5	4.5	54.2	47.8	29.8	25.2
Carbamazepine	0.5	1.5	100.0	82.0	27.0	23.4
Sulfamethoxazole	1.0	1.7	67.2	50.2	18.9	13.9
Bezafibrate	0.6	0.6	84.2	80.0	19.9	14.8
Bisphenol A	50	50	71.3	64.4	14.3	17.6
Diclofenac	2.8	3.5	91.8	91.7	19.6	20.1
Ibuprofen	7.0	7.0	94.2	94.2	21.5	18.9

Table 22 Mixing scenarios of Danube and backwater river influenced water abstracted by PGAW3. Calculated concentrations were based on no attenuation taking place (Danube and backwater river) and on a certain attenuation (LSG2 and backwater river)

	Proportion BW (%)	Calculated concentration in PGAW3 (Danube + BW)	Calculated concentration in PGAW3 (LSG2 + BW)	Average measured conc. In PGAW3
BTri	0	183.24	1.42	5.01
	10	166.39	2.75	
	30	132.70	5.43	
	60	82.16	9.43	
	90	31.62	13.44	
	100	14.78	14.78	
CBZ	0	17.97	12.54	9.27
	10	16.57	11.69	
	30	13.78	9.98	
	60	9.58	7.41	
	90	5.39	4.84	
	100	3.99	3.99	
SMZ	0	5.44	3.83	2.38
	10	5.07	3.62	
	30	4.34	3.21	
	60	3.24	2.59	
	90	2.14	1.98	
	100	1.77	1.77	

seasonal sampling	Danube	LSG41	LSG40	LSG30	LSG2	PGAW3	LSN28	LSG11	Backwater
Parameter	*0 m*	*10 m*	*13 m*	*24 m*	*283 m*	*551 m*	*704 m*	*782 m*	*862 m*
T (°C)	11.4 (3.5-22)	11.6 (7.9-19.4)	11.6 (8.3-18.2)	11.6 (3-17.1)	12.5 (8.1-14.8)	12.1 (11.2-14.4)	12.1 (10.6-14.8)	12.3 (11.0-14)	13.2 (4-29.1)
EC (µS/cm)	436 (317-882)	472 (356-575)	481 (394-582)	451 (384-518)	477 (401-549)	512 (430-544)	601 (538-729)	599 (529-689)	520 (414-639)
pH	8.1 (7.7-8.8)	7.5 (7.1-8.2)	7.4 (6.9-8.2)	7.5 (7.1-8.2)	7.4 (7.0-8.0)	7.5 (7.0-8.1)	7.3 (6.9-7.8)	7.3 (7.1-7.9)	8.1 (7.5-9.0)
DOC (mg/L)	2.2 (1.1-3.9)	0.8 (0.4-1.1)	0.8 (0.4-1.3)	0.8 (0.4-1.3)	0.6 (0.2-1.0)	0.6 (0.2-1.7)	0.7 (0.4-1.6)	0.8 (0.5-2.0)	2.3 (1.4-3.7)
NH_4-N (mg/L)	0.024 (n.d.-0.08)	0.009 (n.d.-0.031)	0.008 (n.d.-0.023)	0.006 (n.d.-0.019)	0.009 (n.d.-0.030)	0.011 (n.d.-0.041)	0.011 (n.d.-0.024)	0.009 (n.d.-0.019)	0.025 (0.004-0.069)
NO_3^- (mg/L)	8.0 (3.6-13.0)	6.5 (2.5-10.9)	6.8 (2.5-11.0)	6.9 (3.1-10.7)	7.2 (4.2-9.7)	5.4 (3.9-8.3)	1.3 (n.d.-4.2)	1.3 (n.d.-5.1)	0.7 (n.d.-7.3)
NO_2^- (mg/L)	0.01 (n.d.-0.05)	0 (n.d.-0.04)	0 (n.d.-0.03)	0 (n.d.-0.01)	0 (n.d.-0.01)	0 (n.d.-0.2)	0 (n.d.-0.01)	0 (n.d.-0.01)	0 (n.d.-0.02)
HCO_3^- (mg/L)	185 (99.3-228)	221 (120-305)	226 (115-306)	209 (113-250)	228 (131-259)	248 (139-274)	310 (173-347)	309 (172-356)	255 (136-334)
Cl^- (mg/L)	20 12-35)	22 (13-21)	21 (14-30)	20 (11-30)	20 (13-28)	19 (15-25)	17 (14-22)	18 (14-24)	21 (16-31)
K^+ (mg/L)	2.3 (0.7-8.6)	1.8 (0.7-4.0)	1.9 (0.9-4.5)	2.2 (0.7-6.1)	2.0 (0.6-6.7)	2.1 (1.0-3.8)	2.0 (0.2-4.9)	1.9 (1.1-4.9)	2.2 (0.8-11)
Ca^{2+} (mg/L)	54 (41-69)	58 (30-85)	61 (47-75)	57 (37-71)	58 (30-73)	60 (38-78)	62 (28-104)	59 (23-108)	57 (29-92)

Table 23 Average values of the standard parameters in the surface- and groundwater samples during the seasonal sampling. Values in brackets represent to minimum and maximum values. Values in meters are the distances to the Danube

Groundwater Monitoring&Remediation

Technical Note

Does Pumping Volume Affect the Concentration of Micropollutants in Groundwater Samples?

by Inge H. van Driezum, Julia Derx, Ernis Saracevic, Alexander K.T. Kirschner, Regina Sommer, Andreas H. Farnleitner, and Alfred Paul Blaschke

Abstract

Information on concentrations of micropollutants (such as pharmaceuticals, pesticides, and industrial chemicals) in most highly dynamic riverbank filtration (RBF) systems is lacking, in contrast to data on standard chemical parameters. Sampling protocols have thus far been based on the stabilization of standard chemical parameters in relatively pristine environments. To determine whether groundwater samples for micropollutant analysis can be taken at a similar pumping volume as samples for testing standard chemical parameters in both environments, three groundwater monitoring wells in an RBF system were sampled at two points in time (after pumping of 3 well volumes and after pumping of 15 well volumes). Micropollutant concentrations were not significantly different between the two sampling points; therefore, appropriate samples can be drawn after pumping 3 well volumes. For a specific microbiological parameter (leucin incorporation), a statistically significant difference was found.

Introduction

Particularly in aquifers that are used for drinking water, such as riverbank filtration (RBF) systems (Hiscock and Grischek 2002; Derx et al. 2013), groundwater quality is of great importance, and appropriate water-protection measures should be applied (European Parliament 2006). An important task for hydrogeologists and water hygienists is to obtain representative groundwater samples when exploring the groundwater quality. A key environmental problem expected in the near future is the increasing contamination of surface- and groundwater bodies with thousands of chemical compounds. Many long-term effects of, for example, micropollutants on aquatic life and on human health remain unknown (Schwarzenbach et al. 2006). Groundwater originating from aquifers which are influenced by surface waters, such as RBF systems frequently contain micropollutants (Heberer 2002; Heberer et al. 2004; Kreuzinger et al. 2004; Hoppe-Jones et al. 2010; Huntscha et al. 2013). It is therefore of paramount importance that groundwater samples are representative of the part of the aquifer surrounding the monitoring well. To adequately determine micropollutant concentrations, adequate sampling procedures that can tackle changes caused in the RBF system by high variability in river water levels are needed. The current standard procedure for sampling groundwater is to pump the monitoring well for three to five well volumes (USEPA 1986; DVWK 1992; Nielsen and Nielsen 2007) or until various physicochemical parameters, such as pH, temperature, electrical conductivity (EC), and dissolved oxygen, stabilize (Robin and Gillham 1987; BMLFUW 2015). In several studies, field tests for obtaining representative groundwater samples were performed (Robin and Gillham 1987; Gibs and Imbrigiotta 1990; Barcelona et al. 1994; Puls and Paul 1995; Novak and Watts 1998; Barcelona et al. 2005; Kwon et al. 2008; Kozuskanich et al. 2011; Shani et al. 2012; Harter et al. 2014; Roudnew et al. 2014). Most focused on standard chemical parameters or microbiological constituents. The study by Novak and Watts (1998) tested pesticide concentrations in shallow coastal plain aquifers which were not influenced by large water table fluctuations. In aquifers with low hydraulic conductivities (ranging from 2.9×10^{-5} to 2.1×10^{-4} m/s), pesticides were shown to stabilize after pumping two well volumes. Gibs and Imbrigiotta (1990) showed that organic compounds such as benzene stabilized in 55% of the cases after purging three well volumes in unconfined sand and gravel aquifers. Barcelona et al. (1994), on the other hand, showed that pumping only a fraction of a wellbore volume (<50%) was sufficient to achieve stabilization of volatile organic compounds such as trichloroethylene. These studies showed the number of well volumes pumped before stabilization was reached differ between the type of compound and the type of aquifer. The common features of these studies was that the aquifers were moderately permeable (hydraulic conductivity values $<1 \times 10^{-4}$ m/s) and not under the direct influence of surface water. Due to the high variability in water levels and input concentrations from a river, it was expected that a low number of pumping volumes as determined in previous studies would not be sufficient for micropollutants to stabilize similar to standard chemical parameters.

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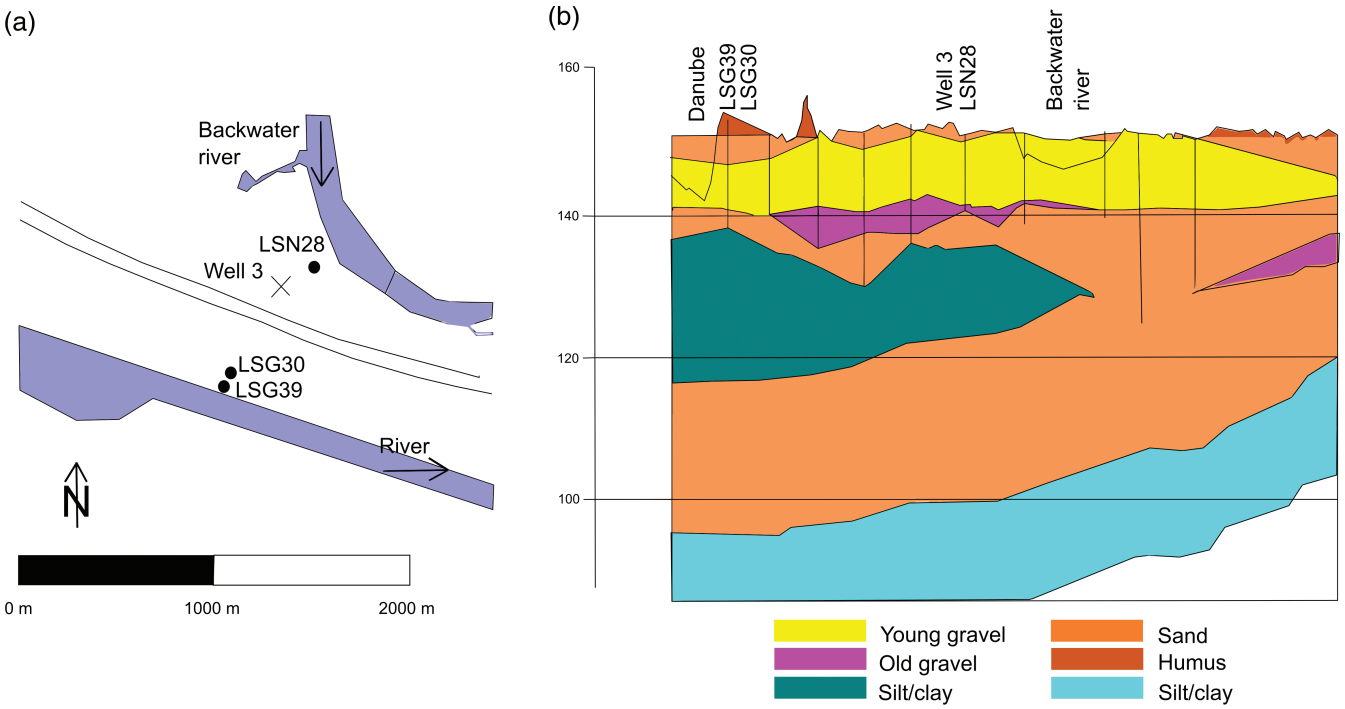

Figure 1. (a) Situation of the three sampled groundwater monitoring wells LSG39, LSG30, and LSN28. The groundwater abstraction well is depicted as well 3 and (b) schematic cross section of the transect with the hydrogeological layers.

We therefore investigated whether pumping volume affects micropollutant concentrations in a highly dynamic RBF environment and whether samples can be obtained simultaneously with those collected for standard chemical parameters. To test the hypothesis that micropollutant concentrations after 3 well volumes are not statistically different than those found after 15 well volumes, three representative groundwater monitoring wells were sampled. Several other chemical and microbiological parameters were measured to allow cross-comparison and to support the interpretation of results.

Research Method

The site investigated was the porous groundwater aquifer (PGA) study site (see Figure 1). It is an alluvial backwater and floodplain area, extending on the left bank of a river downstream of the city of Vienna. A total of five groundwater abstraction wells are located in the PGA. When water levels in the river rise, water flows from the river into the backwater river of the floodplain, causing regular flooding events. The main river always infiltrates into the aquifer, which is part of one of the main groundwater bodies in Austria. Groundwater quality in the area is therefore potentially influenced by a combination of anthropogenic activities, industry, wastewater treatment plants (WWTPs) further upstream, and flooding events. Surface waters in the PGA have been extensively studied since they are regularly situated in the well capture zones of the groundwater abstraction wells. The PGA is monitored with a high temporal and spatial resolution with more than 200 hydraulic pressure data loggers distributed over the study area. Furthermore, a calibrated 3D groundwater flow and transport model was available which was used to study the transient well capture zones and the impact of river water level fluctuations on the microbiological groundwater quality (Farnleitner et al. 2014). However, groundwater data related to the behavior of chemical contaminants has been scarce. The riverbank in this area consists of riprap. Due to clogging between these boulders, no or almost no infiltration directly through the riverbank occurred. River water can infiltrate into the groundwater only through the riverbed (Blaschke et al. 2003). The upper layer of the PGA consists of silt and has a thickness from 1 to 10 m. The underlying confined aquifer consists of sand and gravel and has a thickness in between 3 and 15 m. Hydraulic conductivities of the PGA range from 5×10^{-2} to 5×10^{-4} m/s as depicted by a 3D groundwater flow model after calibration to both steady flow conditions during high pumping rates of the well and to transient flow conditions during a flood event (Farnleitner et al. 2014). These values were also confirmed by pumping tests conducted in the area. Underneath the aquifer are alternating sand and clay/silt layers. Conditions in the PGA are predominantly oxic. Dissolved organic carbon concentrations in the aquifer ranged from 0.5 to 4.0 mg/L, with most of the concentrations below 2 mg/L (data from 2005 to 2013, Mayr et al. 2014).

Two sampling campaigns were performed at wells which were situated in a transect along the groundwater flow direction toward groundwater abstraction well 3 (PGAW3) and the backwater river as described below. The monitoring wells, with a diameter of 5.1–12.7 cm, extended from the surface to the clay layer, which was at a maximum depth of 14 m. The construction details of one of the wells are given in Figure S2 in Appendix S1 (Supporting information). The wells were situated in an area with high variation in

groundwater levels, which represented the highly dynamic nature of the system. Two wells were situated close to the river, one (LSG39) 10 m from the river and one (LSG30) 19 m from the river. The other well (LSN28) was located 705 m from the river, between PGAW3 and the backwater river. Particle tracking simulations performed with the calibrated 3D model revealed that the travel times from the Danube toward LSG39 ranged from 1.5 to 18.5 d during mean flow conditions and to a maximum of 1 to 10 d during high flow conditions (Derx et al. 2013). Travel times toward LSG30 were in a similar range as LSG39. Travel times from LSN28 toward PGAW3 were influenced by both the backwater and the pumping rate of PGAW3. Travel times increased from 62 to >100 d during low flow conditions. As a measure for groundwater level dynamics, the sum of the absolute differences of hourly groundwater levels over the course of a year was calculated (the higher the sum, the higher the dynamics). In order to allow for comparability, we chose a time period in which continuous data was available for all wells. Therefore, the period between February 2014 and December 2014 was chosen.

Samples were taken in August and September 2014, where sampling was performed at an abstraction rate of 0.77 L/s for a total of 15 well volumes. Groundwater levels were measured with pressure transducers during sampling in order to quantify whether a drawdown occurred in the monitoring wells. Water levels in the river and groundwater fluctuated considerably between the two campaigns. During the second sampling campaign, PGAW3 was pumping, which caused a difference in groundwater travel time from either the river or the backwater river toward PGAW3.

After pumping of 3 well volumes and again after pumping of 15 well volumes, 1-L samples were obtained and stored in glass bottles in the dark until analyses for a set of eight micropollutants. Immediately afterwards, 250-mL samples were obtained and stored in plastic bottles for analysis of several organic parameters. In addition, 4-L samples were obtained at intervals of three well volumes and stored in sterilized plastic containers for bacteriological analysis per ISO standards and published protocols (Simon and Azam 1989; Kirschner and Velimirov 1999; Farnleitner et al. 2010; Riepl et al. 2011).

Temperature, pH, and EC were measured in the field using a portable Sension+MM150 sensor system (Hach-Lange, Austria). To show whether there was a contamination due to the river, carbamazepine (CBZ; Drewes et al. 2003; Clara et al. 2004; Huntscha et al. 2013), benzotriazole (BZT; Kahle et al. 2009; Huntscha et al. 2013), and sulfamethoxazole (SMZ; Kolpin et al. 2002; Miao et al. 2004) were analyzed. All micropollutants were determined using solid phase extraction (SPE)-combined with HPLC-MS/MS (see Appendix S1 for a detailed chemical and microbial analysis description and definition of parameters). Tested microbiological parameters included *Escherichia coli*, intestinal enterococci, bacterial spores from aerobic spore formers representing microorganisms in their resting stage, total bacterial abundance (including presence or absence of biofilm particles; Riepl et al. 2011), and bacterial ^{3}H–leucine incorporation (LI; Simon and Azam 1989; Kirschner and Velimirov 1997; Kirschner and Velimirov 1999).

A normalization procedure (z-transformation) was performed to enable pooling and comparison of parameters between 3 and 15 well volumes for all wells. This normalized deviate was calculated using

$$C_{st}^{n} = \frac{c^{i} - m_{c}}{s_{c}} \quad (1)$$

where C_{st}^{n} is the normalized deviate, c^{i} is the original concentration, m_{c} is the sample mean of c, and s_{c} is the sample standard deviation of c. Using these standardized numbers, a Mann–Whitney test (Sokal and Rohlf 1997) was performed to assess potential differences between samples taken after 3 and 15 well volumes.

Results

As seen in Table 1, the technical duplicates deviated by less than 20% from the mean.

Table 1

Micropollutants Found in Duplicate Samples after Pumping 3 and 15 Well Volumes

	LSG39[1]	LSG39[1]	LSN28[1]	LSN28[1]	LSN28[2]	LSN28[2]	LSG30[2]	LSG30[2]
Parameter	**3 vol**	**15 vol**	**3 vol**	**15 vol**	**3 vol**	**15 vol**	**3 vol**	**15 vol**
Benzotriazole (ng/L)	60.0	62.5	<LOQ	<LOQ	<LOQ	<LOQ	95.2	84.8
	52.8	82.0					66.0	69.3
	56.4 (6%)	**72.3 (13%)**	<LOQ	<LOQ	<LOQ	4.50	**80.6 (18%)**	**77.1 (10%)**
Carbamazepine (ng/L)	14.6	11.4	3.85	3.28	4.36	3.70	18.4	17.6
	13.2	16.4	3.40	2.83	3.51	3.72	15.0	11.7
	13.9 (5%)	**13.9 (18%)**	**3.63 (6%)**	**3.06 (8%)**	**3.93 (11%)**	**3.71 (1%)**	**16.7 (10%)**	**14.6 (20%)**
Sulfamethoxazole (ng/L)	3.27	3.08	LOD	LOD	LOD	LOD	6.00	3.78
	3.17	2.11					5.00	4.23
	3.22 (2%)	**2.59 (19%)**	LOD	LOD	LOD	LOD	**5.50 (9%)**	**4.00 (5%)**

Note: Values in bold are arithmetic means; values in parentheses are deviations from the mean.
[1]August 2014 sampling campaign.
[2]September 2014 sampling campaign.

Table 2

Standard Chemical and Microbiological Parameters Found after Pumping 3 and 15 Well Volumes

Parameter	LSG39[1] 3 vol	LSG39[1] 15 vol	LSN28[1] 3 vol	LSN28[1] 15 vol	LSN28[2] 3 vol	LSN28[2] 15 vol	LSG30[2] 3 vol	LSG30[2] 15 vol
pH	7.3	7.44	7.47	7.44	7.44	7.49	7.71	7.66
EC (µS/cm)	457	433	555	559	558	547	367	374
Temp (°C)	14.4	15.3	14	13.5	14.6	15.8	17.7	16.7
$CaCO_3$ (mg/L)	138	136	175	177	175	177	112	113
TOC (mg/L)	0.85	0.84	0.88	0.88	0.80	0.80	0.90	0.90
NH_4^+ (mg/L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
NO_2^- (mg/L)	0.01	0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
NO_3^- (mg/L)	1.7	1.80	<LOQ	<LOQ	<LOQ	<LOQ	3.30	3.20
Ca^{2+} (mg/L)	71.0	69.0	82.0	82.0	82.0	82.0	55.0	56.0
Mg^{2+} (mg/L)	13.0	13.0	22.0	22.0	22.0	22.0	12.0	12.0
Cl^- (mg/L)	12.0	12.0	16.0	16.0	16.0	17.0	12.0	12.0
SO_4^{2-} (mg/L)	26.0	26.0	36.0	37.0	35.0	35.0	23.0	23.0
Na^{2+} (mg/L)	9.20	9.20	12.0	12.0	12.0	12.0	8.20	8.20
K^+ (mg/L)	1.90	1.90	2.10	2.10	2.20	2.20	1.90	1.90
Bacterial spores (#/L)	180	70	1100	530	900	1400	500	140
Bacterial abundance (cells/mL)	2.54E+5	2.79E+5	2.96E+5	3.28E+5	2.03E+5	2.22E+5	1.51E+5	1.30E+5
Leucin incorporation (pmol/L/h)[3]	0.275 (0.049)	0.181 (0.013)	0.062 (0.002)	0.041 (0.012)	0.065 (0.003)	0.369 (0.150)	0.064 (0.009)	0.056 (0.003)

Note: Values in parentheses are deviations from the mean.
[1]August 2014 sampling campaign.
[2]September 2014 sampling campaign.
[3]Average (standard deviation) of technical replicates.

Supported by high recovery values of the SPE method (48–92%, Ternes and Joss 2007), the analytical method was deemed appropriate for the purpose of this investigation. A Mann–Whitney test performed using normalized concentrations revealed no statistically significant difference in micropollutant concentration after 3 vs. 15 pumping volumes (see Table S5 in Appendix S1).

The standard chemical parameters and the physicochemical parameters also showed no statistically significant difference in concentrations (see Table 2).

The micropollutant with the highest groundwater concentration was BZT. It was below the limit of quantification (<LOQ) in well LSN28 but averaged 80.6 ng/L in LSG30. On the other hand, SMZ was below the limit of detection (LOD) in LSN28 but was found in all other wells, averaging up to 6.00 ng/L. CBZ was found in all wells, averaging as little as 3.34 ng/L in LSN28 and as much as 15.7 ng/L in LSG30. All other micropollutants were below the LOD (see Table 1). Micropollutant concentrations were higher closer to the river and decreased significantly toward the backwater river.

Physicochemical and standard chemical parameters were used to describe general water quality. Temperature, pH, and EC stabilized after pumping only one well volume (approximately 4 min, see Figure S1 in Appendix S1). Of the standard chemical parameters, none were significantly different at any point in time (see Table 2). Temperature and EC varied through the system. *E. coli* was not detected in any of the wells after pumping of 3–15 well volumes. Enterococci were not detected in LSG39 or LSG30, but were detected in low numbers after pumping of 15 well volumes in LSN28 in September (data not shown). In addition, bacterial spores, LI, and total bacterial abundance were used to describe general microbiological characteristics. Bacterial spores were found in all samples and ranged from 20 cfu/L in LSG39 in August to 1500 cfu/L in LSN28 in September. A Mann–Whitney test performed with normalized concentrations revealed statistically significant evidence that pumping time influences LI (see Table S5 in Appendix S1). The concentration of bacterial spores was generally highest in LSN28. Bacterial LI was different between LSG39 and wells further from the river, falling as distance from the river increased. Samples taken after pumping of three well volumes and sometimes up to six well volumes contained biofilm (see Appendix S1), especially in wells closer to the river.

The dynamics of the system was shown by the calculation of the absolute differences in groundwater levels. LSN28 had the lowest dynamics, of 35.5 m in an 11-month period. Further toward the river, the dynamics increased to 45.7 m for LSG30 and 45.8 m for LSG39. Measurements of groundwater levels taken during ampling showed the groundwater level decreased only 5 cm during pumping.

Discussion

It has been shown that human pharmaceuticals in rivers can vary on a daily basis due to the fluctuations in WWTP effluent concentrations (Kreuzinger 2007; Weigelhofer et al. 2015; Zoboli et al. 2015). Furthermore, the discharge fluctuations in the river can also have an impact on micropollutant concentrations in the river. Despite these fluctuations, the concentrations in the PGA were demonstrated to be the same whether a monitoring well was pumped for 3 or 15 well volumes. No detectable difference between stabilization of micropollutant concentrations and standard chemical parameter concentrations was found, as suggested by Gibs and Imbrigiotta (1990). Hydraulic conductivities from the study of Gibs and Imbrigiotta (1990) were lower than in the PGA (a maximum value of 1×10^{-3} m/s vs. 5×10^{-2} m/s in this study). Furthermore, no change in stabilization of micropollutant concentrations between the monitoring wells was found, although a difference in stabilization between monitoring wells was suggested by Novak and Watts (1998). Because hydraulic conductivities at the study site were high, the standard chemical parameters stabilized earlier than in a chalk environment, studied by Sorensen et al. (2013) and by Kwon et al. (2008). Differences in micropollutant concentrations from well to well can be explained by different travel times, dilution of the infiltrating river water and the effectiveness of removing contaminants in an RBF system by for example biodegradation or sorption (Henzler et al. 2014; Hamann et al. 2016). Therefore, concentrations in wells LSG39 and LSG30 were higher than those in LSN28.

Microbiological parameters were more variable, particularly LI, as was shown by Kwon et al. (2008) and Roudnew et al. (2014). In this paper, the maximum increase in bacterial population was much lower than was found by Kwon et al. (2008). This can be explained by the high hydraulic conductivity values of the PGA and the presence of major external influences, as opposed to a relatively pristine environment lacking such influences. Although Roudnew et al. (2014) suggested more stability of microbial parameters due to a constant recharge by a river, this can only partly be concluded from this study. Harter et al. (2014) suggested that field water quality parameters were sufficient indicators to screen wellbore and near-well microbiological contamination. However, with microscopic analysis we found high numbers of biofilm particles (faintly stained fluffs with intensive bacterial colonization) present once the micropollutants and physicochemical parameters stabilized. Because *E. coli* could be present and could detach from biofilm (LeChevallier et al. 1987; Banning et al. 2003), these samples are unlikely representative for the aquifer, even if *E. coli* concentrations were below the LOD. The high number of biofilm particles observed during the initial pumping period was most likely caused by the growth of biofilm on the surfaces of the boulders and its gradual release during pumping, until all loose biofilm particles have been removed.

The pumping rate caused a minor drawdown of 5 cm during sampling. Vandenberg and Varljen (2000) and Barcelona et al. (2005) showed that stabilization (and not the degree of drawdown) was important in collecting representative samples. Pumping rates, however, could influence the sampled microbiological community. In coarse gravel, like in the PGA, variations in the microbiological community induced by pumping could not be distinguished from natural temporal variations (Shani et al. 2012). Because of the high hydraulic conductivity of the PGA aquifer and the low drawdown, we do not propose use of a low-flow pumping procedure.

Conclusions

Results of the studied alluvial PGA clearly demonstrate that samples for determining micropollutant concentrations, can be taken at the same time as those taken for determining standard chemical parameters (e.g., after 3 well volumes). This might also apply to similar sites where the aquifer is strongly influenced by surface waters and where the hydraulic conductivity of the aquifer is in a similar range. No statistical significant evidence was present that suggested micropollutant concentrations were not stable during pumping, neither was there for standard chemical parameters. The fluctuations of the watertable and the fluctuation of contaminant concentrations in the river did not affect the stabilization of the chemical parameters. Leucine incorporation however did show a statistically significant difference between the samples taken at the two different points in time. Furthermore, samples taken after pumping of three well volumes from wells close to the river do not represent the microbiological quality of the study site due to the presence of biofilm. Stabilization of standard chemical parameters and micropollutants is insufficient for measuring microbiological parameters.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Additional details to experimental and results including Supplementary Tables S1-5 and Figures S1 and S2

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Spatiotemporal analysis of bacterial biomass and activity to understand surface and groundwater interactions in a highly dynamic riverbank filtration system

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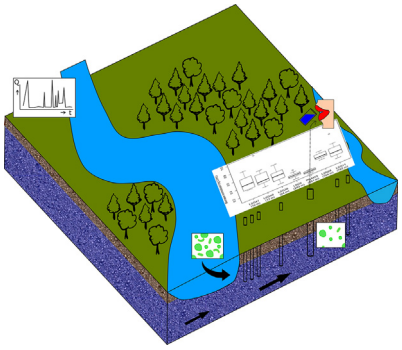
HIGHLIGHTS

- Bacteria in groundwater in RBF systems are influenced by the infiltrating river.
- Fluctuations in bacterial variables are linked to the hydrological dynamics.
- An increased influence was observed during flood events.
- During flood events the infiltration extends further into the aquifer.
- Increases in bacterial numbers and activity are not caused by a nutrient input.

GRAPHICAL ABSTRACT



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ABSTRACT

Characterization of surface water – groundwater interaction in riverbank filtration (RBF) systems is of decisive importance to drinking water utilities due to the increasing microbial and chemical contamination of surface waters. These interactions are commonly assessed by monitoring changes in chemical water quality, but this might not be indicative for microbial contamination. The hydrological dynamics of the infiltrating river can influence these interactions, but seasonal temperature fluctuations and the supply of oxygen and nutrients from the surface water can also play a role. In order to understand the interaction between surface water and groundwater in a highly dynamic RBF system of a large river, bacterial abundance, biomass and carbon production as well as standard chemical parameters were analyzed during a 20 month period under different hydrological conditions. In the investigated RBF system, groundwater table changes exhibited striking dynamics even though flow velocities were rather low under regular discharge conditions. Bacterial abundance, biomass, and bacterial carbon

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production decreased significantly from the river towards the drinking water abstraction well. The cell size distribution changed from a higher proportion of large cells in the river, towards a higher proportion of small cells in the groundwater. Although biomass and bacterial abundance were correlated to water temperatures and several other chemical parameters in the river, such correlations were not present in the groundwater. In contrast, the dynamics of the bacterial groundwater community was predominantly governed by the hydrogeological dynamics. Especially during flood events, large riverine bacteria infiltrated further into the aquifer compared to average discharge conditions. With such information at hand, drinking water utilities are able to improve their water abstraction strategies and react quicker to changing hydrological conditions in the RBF system.

1. Introduction

Riverbank filtration (RBF) systems are important sources for drinking water abstraction in many countries (Henzler et al., 2014; Hoppe-Jones et al., 2010; Ray et al., 2002; Tufenkji et al., 2002) due to their effective removal of contaminants like bacteria (Pang et al., 2005), viruses (Schijven and Hassanizadeh, 2000) and organic micropollutants (Huntscha et al., 2013; Massmann et al., 2008). During RBF, surface water interacts with the aquifer and may pose a threat for the microbial and chemical water quality. These interactions and their exchange processes and pathways are of vital importance for the protection of water resources used by drinking water utilities. Although surface water infiltration into the aquifer can be indicated by changes in physical and chemical water quality characteristics, these may not be necessarily indicative for the transport of microorganisms and pathogens (Taylor et al., 2004). All these processes are dependent on hydrogeological, biochemical and biological factors (Hiscock and Grischek, 2002) and take place mostly in the transition zone (Kalbus et al., 2006). In this zone, hydrogeological characteristics affect flow velocity, infiltration rates and mixing proportions of river water with groundwater and impact the efficacy of the reduction or elimination of contaminants. Although the transition zone usually extends not more than a few meters away from the river bank, it can extend up to several kilometers inland in large alluvial river systems with highly porous aquifers (Boulton et al., 1998; Stanford and Ward, 1988). Due to the infiltration of oxygen-rich river water high in particulate (POC) and dissolved organic carbon (DOC), the highest biological activity, which depends particularly on bacteria (Craft et al., 2002; Findlay et al., 1993; Pusch, 1996), can be found in the hyporheic zone (Gibert and Mathieu, 1997).

The nature and extent of surface water-groundwater interaction can be determined by assessing the changes in the microbial characteristics of both water bodies, such as total bacterial abundance, biomass and activities. Changes of these parameters in the groundwater are likely to be influenced by the interaction with surface water and can be affected by the composition of the aquifer material, the hydraulic gradient, temperature fluctuations in the surface water, and the supply of oxygen and inorganic nutrients (Bott and Kaplan, 1985; Vanek, 1997). Bacterial abundance, biomass and activities are also important features of groundwater or spring water used as drinking water (Farnleitner et al., 2005). Due to their importance, several studies (Brugger et al., 2001; Ellis et al., 1998; Lin et al., 2012; Stegen et al., 2016; Velasco Ayuso et al., 2009b; Zhou et al., 2012) examined the changes in the microbial characteristics in relation to the hydrological dynamics. In addition to hydrological dynamics, groundwater quality and seasonal temperature fluctuations were also shown to have an influence on the microbial characteristics. These fluctuations impacted the microbial characteristics to the greatest extent where river water and groundwater mixing was greatest (Lin et al., 2012). It could even be that less frequent and large increases in river water levels may enhance the microbial activity due to the transport of larger quantities of labile organic carbon into the hyporheic zone (Stegen et al., 2016). An approach to study changes in microbial groundwater characteristics is the analysis of spatiotemporal patterns in bacterial biomass and activity. Some studies exist that correlate bacterial biomass and activity with hydrogeological metrics, but they were either limited to the distance between the river and the groundwater wells or to water table changes (Brugger et al., 2001; Ellis et al., 1998). Important hydrogeological factors like aquifer characteristics and the hydraulic gradient were not taken into account. Furthermore, samples in these studies were taken along relatively small rivers and large increases in river water levels during flood events were not examined. Therefore, the main goal of our study was to examine surface water-groundwater interactions by assessing bacterial biomass and activity changes in a large and highly dynamic river over an extended period of time. The following questions were therefore addressed: (i) is microbial water quality in an RBF system vulnerable to surface water infiltration, especially during flood events? If so, (ii) are these changes primarily caused by the hydrological dynamics or do temperature and geochemical changes also play an important role? As the transition zone can extend up to several kilometers inland in large alluvial systems, another objective (iii) is to quantify the extent of the river's influence on the bacterial dynamics. For this purpose, river water and groundwater samples from six monitoring wells and one drinking water abstraction well in a porous aquifer (PGA) were taken on a monthly basis from October 2014 to May 2016. The monitoring wells were located along a gradient from the river towards the drinking water abstraction well. In order to account for changes in biomass and activity under extreme flow conditions, two flood events were sampled more extensively.

2. Materials and methods

2.1. Study site

The study site is a porous aquifer (Fig. 1) along the river Danube, the second longest river in Europe and the most international river in the world with 19 countries within its catchment area.

This alluvial backwater and floodplain area with forest, meadows and surface water bodies is located on the left bank of the Danube, downstream of the Austrian capital of Vienna. The floodplain is part of a national park and a Natura 2000 protected area as well as a drinking water protection zone with an area of approximately 50 km^2 (Derx et al., 2016) situated within one of the main groundwater bodies of Austria. Five groundwater abstraction wells are located in the floodplain, making the aquifer an important drinking water resource. The local groundwater flow direction is from southwest to northeast. There is continuous infiltration of river water into the groundwater. The riverbank in this area consists of riprap. Due to clogging between these boulders, no or almost no infiltration directly through the riverbank occurs. River water can therefore only infiltrate into the groundwater through the riverbed (Blaschke et al., 2003). The backwater river is connected with the Danube above a water level of 150.5 m above the Adriatic Sea level (m a.A.) in the Danube at the station Fischamend (occurring just below a flood event with a recurrence of 1 year) (Supplementary Fig. S1).

By means of multiple borehole logs and topset bed exploration, 4 different soil layers are distinguished. Fig. 1c shows a cross section of the studied transect. The upper layer of the PGA consists of sand (orange) and humus (dark orange) and has a thickness varying from 1 to 5 m.

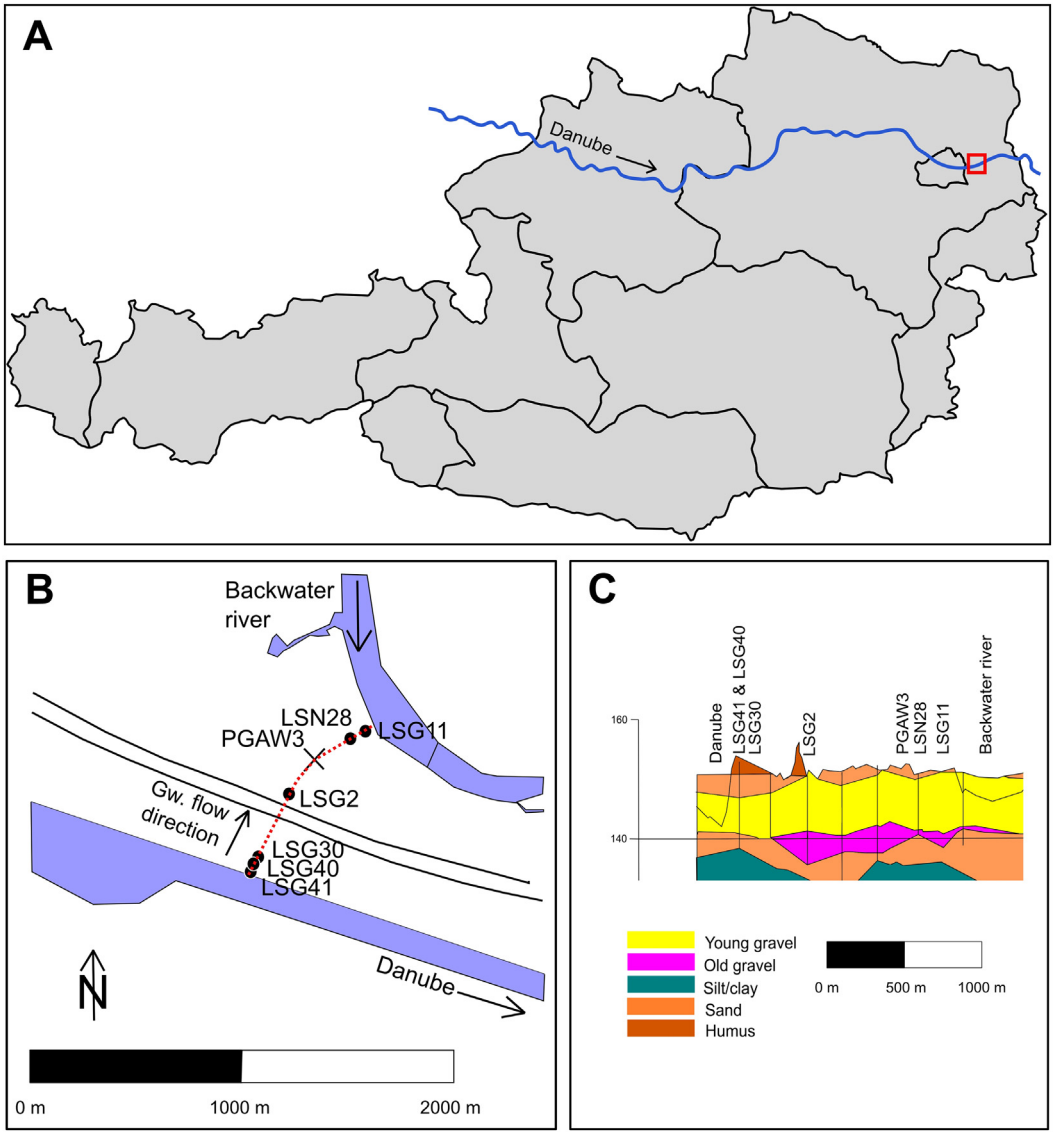

Fig. 1. a) Situation of the Natura 2000 protected area (red square) in Austria, b) the sampled transect including monitoring wells LSG41, LSG40, LSG30, LSG2, LSN28 and LSG11. The groundwater abstraction well is depicted as PGAW3 and c) Schematic cross section (dotted red line in b) of the transect with the hydrogeological layers and the groundwater monitoring wells (shown as black vertical lines). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

The main layers of the aquifer are young (yellow) and old Danube gravel (pink) and sand (orange). The aquifer has a thickness varying from 4 to 10 m along the transect. Underneath the aquifer there are alternating clay/silt (cyan) and sand layers (not shown). Hydraulic conductivities of the PGA were determined using a 3D groundwater flow and transport model that was calibrated to both steady flow conditions during high pumping rates of the wells and to transient flow conditions during a flood event (Farnleitner et al., 2014). The hydraulic conductivities in the entire PGA ranged from 5×10^{-4} m/s to 5×10^{-2} m/s and were also confirmed by pumping tests conducted in the area. In the studied transect, interpretation of the calibrated 3D model and geophysical measurements showed that the hydraulic conductivity (0.016 m/s) and the effective porosity (0.125) were constant. Mayr et al. (2014) showed conditions in the PGA were predominantly oxic. Groundwater gradients, flow velocities and travel times from the Danube towards the groundwater abstraction well were calculated using the measured water levels between the Danube and PGAW3 and Eq. (1). The water level gradients were calculated for each sampling date. The corresponding travel times ranged from a minimum of 11.5 days to a maximum of 47.4 days. These travel times correspond to the direct and thus shortest flow paths from the Danube to PGAW3.

In order to capture the dynamics of the system, groundwater gradients were estimated by calculating the differences in water levels between the wells where the measurements were taken on each sampling date. The gradient in monitoring well LSG41 was based on the water level difference between the Danube and the well. The gradient in LSG40 was based on the piezometric head difference between LSG41 and LSG40 etc. These values were then divided by the distance between the two points (Supplementary Table S1). The gradient is positive whenever the groundwater flow direction is from the Danube towards the groundwater abstraction well and further towards the backwater river. The flow velocities in the saturated zone were based on the gradients between the wells and calculated for each well pair according to the following equation:

$$v = \frac{K\Delta h}{n_e}$$

where v is the flow velocity (m/s), K is the hydraulic conductivity (with a value of 0.016 m/s), Δh is the gradient (−) and n_e is the effective porosity (with a value of 0.125).

2.2. Sampling

Monthly samples ($n = 18$, Supplementary Fig. S2) were taken from October 2014 to May 2016 in a transect extending from the Danube towards a drinking water abstraction well and the backwater river. In this period, river discharges ranged from 693 m^3/s to 6197 m^3/s (Supplementary Fig. S2). During two flood events with a one-year return period in May 2015 (HQ2015) and February 2016 (HQ2016), samples were taken at an increased frequency in order to account for differences in infiltration during increased groundwater flow velocity ($n = 25$, Supplementary Fig. S2). Two surface water locations and 6 groundwater monitoring wells as well as the drinking water abstraction well were sampled during each monthly sampling event. During the flood events, samples were collected from the Danube and two wells close to the river (LSG41 and LSG30). Three of the groundwater monitoring wells (LSG41, LSG40 and LSG30) are situated close to the river to capture the high variability in river and groundwater levels in the system (Fig. 1). LSG2 is located between these three wells and the drinking water abstraction well (PGAW3). LSN28 and LSG11 are situated between the drinking water abstraction well and the backwater river. All monitoring wells were screened from 1 m below the surface till the silt/clay layer, over a length of approximately 14 m. Groundwater levels were recorded manually during all sampling events. Additionally, hourly hydraulic pressure and temperature values were recorded continuously in all groundwater monitoring wells from October 2014 until May 2016. Furthermore, hourly recorded values for electrical conductivity were available for selected monitoring wells. Hourly river water level and discharge values from the station Fischamend (rkm 1908) between January 2014 and January 2017 were kindly provided by the Austrian federal waterway authority *viadonau*.

Groundwater samples for standard chemical parameters were taken after pumping of 3 well volumes, whereas samples for microbial parameters were taken after pumping of 15 well volumes (van Driezum et al., 2017). The samples were taken using a suction pump with an abstraction rate of 0.77 L/s. Temperature, pH, electrical conductivity and dissolved oxygen were measured in the field using a portable Sension+ MM150 sensor system (Hach-Lange, Austria) and a portable Profiline multi 3320 sensor system (WTW, Germany). 250 mL of ground- and surface water was taken in clean plastic bottles to be analyzed for standard chemical parameters, whereas autoclaved plastic gallons (4 L) were used for the microbial parameters.

2.3. Organic and inorganic parameters

After pumping of 3 well volumes, 250 mL samples were filled in plastic bottles and transported to the lab in a cooling box of 4 °C for the analysis of inorganic parameters. The samples were stored in the lab at 4 °C before analysis. Samples were analyzed for a large set of organic and inorganic parameters (Supplementary Table S2). Anion and cation analysis was performed using ion chromatography. Absorption photometry was used to measure ammonium and nitrite (Supplementary Table S2).

2.4. Bacterial cell counts

Total bacterial cell counts (TCC) was measured using the slightly modified protocol of Riepl et al. (2011). Depending on the type of water, between 1 mL (surface water) and 100 mL (groundwater) of sample was fixed with para-formaldehyde. 200 μL to 40 mL was filtered on a 0.2 μm membrane filter (Anodisc 25, Whatman, Germany) and stained with SYBR® Gold (Fisher Scientific, Austria). The slides were either stored at −20 °C or analyzed immediately with a Nikon epifluorescence microscope (Nikon Eclipse 50i). Cells were classified in large cells (rod shaped cells and coccoid cells with diameter > 1.0 μm) and small cells (coccoid cells with a diameter < 1.0 μm).

2.5. Bacterial ^{3}H–leucine incorporation

Bacterial ^{3}H–leucine incorporation (LI) was measured based on protocols of Kirschner and Velimirov (1999) and Simon and Azam (1989). Briefly, ^{3}H–leucine was added to triplicate 10 mL samples at a final concentration of 10 nM. Duplicate control samples were stopped with trichloroacetic acid (TCA, 5% final conc., Sigma-Aldrich, Germany) directly after the addition of ^{3}H–leucine. Both controls and samples were incubated for 30 min (surface water samples) to 24 h (groundwater samples) in the dark at the measured temperature of the aquifer. At the end of the incubation, samples were also stopped by adding TCA. One-hundred microliters of 35% NaCl was added to enhance precipitation of macromolecules inclusive proteins and all samples were incubated for 30 min at 18 °C. After incubation, the samples were filtered through a cellulose nitrate filter (0.45 μm) which was subsequently washed with 5 mL of 5% TCA, 80% ethanol and distilled water each for the purification of proteins. Filters were dried overnight in scintillation tubes. After adding 5 mL of scintillation cocktail, radioactivity was measured in a Perkin Elmer, TriCarb 2300 TR scintillation counter.

2.6. Bacterial carbon production, biomass and turnover time

Bacterial carbon production (BCP) was estimated according to (Simon and Azam, 1989) using the following equation:

$$BCP = LI * 131.2/(Leu\ per\ protein) * (cell\ C\ per\ protein) * ID$$

where LI is the leucine incorporation rate (mol/L/h), 131.2 is the molecular weight of leucine, Leu per protein is 0.073 (the fraction of leucine in protein), cellular carbon (C) per protein is 0.86 (Simon and Azam, 1989) and ID is the isotope dilution. Sufficiently high concentrations of leucine were added to compensate the ID. BCP values were given in ng C/mL/h. A constant value of 20 fg C per large bacterial cell and 10 fg C per small cell was used to calculate biomass (Bott and Kaplan, 1985; Lee and Fuhrman, 1987). Biomass values were given in ng C/mL. The turnover times of the bacterial biomass were calculated by dividing biomass with bacterial carbon production. Turnover time values were given in days.

2.7. Total viable counts

Total viable active counts (TVAC) were estimated according to Riepl et al. (2011). To assess the amount of cells that actively contribute to biomass production, the number of TVAC was determined in all groundwater wells during three separate sampling campaigns conducted during spring 2017. Samples were taken from all groundwater monitoring wells during this sampling campaign. Briefly, 1 mL of groundwater sample was filtered through a black, 0.4 μm pore-size polyester filter (CB04) and counterstained with 1 mL of counterstain medium CSE/2 (Biomérieux, France). After incubation of 1 h ± 5 min at 37 °C on a ChemSol A4 saturated labeling pad in a petridish, the labeling pad was transferred on a labeling pad saturated with dye (Chemchrome V6). This was incubated for another 30 min at 30 °C before transferring the pad to a membrane holder. Then, it was immediately enumerated with a solid-phase cytometer (Chemscan RDI; Biomérieux, France) using the Bioburden discrimination settings according to the manufacturer's instructions (Catala et al., 1999). Positive signals detected and discriminated as viable active cells by the system were inspected and validated visually (all signals if $n \leq 100$ or 100 representative signals if $n > 100$). All working steps were performed under laminar airflow.

2.8. Statistical analysis

Correlation analysis of microbial parameters with hydrological, physical and chemical variables was performed using the Pearson product correlation and the Spearman rank order correlation. Normality of

the data was tested by visual examination of the quantile-quantile plots. A *P*-value of 0.05 was set as a significance threshold. A multiple linear regression was performed between several chemical parameters and BCP. To determine whether there was a statistically significant difference between the proportion of large cells in the surface water samples and in the groundwater samples, an ANOVA test and its associated *post-hoc* test were used (functions aov and TukeyHSD). All statistical analyses were performed using R 3.1.1., partly using the *Hmisc* package (v. 4.1.1).

3. Results and discussion

3.1. Both the Danube and the backwater river influence groundwater quantity and quality in the study area

In order to get an insight in which parameters may have an influence on bacterial biomass and activity dynamics in the groundwater, it is of profound importance to identify the dynamics of the main hydrological parameters in the studied aquifer that can be affected by the Danube and the backwater river. Substantial water table fluctuations and gradients in temperature, pH and chemical constituents like nitrate and DOC are common characteristics of the transition zone. During the studied period, the water table change of the Danube was almost as high as 6 m (maximum value of 153.38 m a.A., minimum value of 147.54 m a.A., Table 1 and Fig. 2) with a peak in late October 2014.

Water levels within the aquifer were consistently lower than surface water levels in the Danube. In the three nearest monitoring wells (LSG41, LSG40 and LSG30), groundwater tables exhibited striking hydrological dynamics (Fig. 2), although the fluctuations were slightly lower than in the river (maximum of 3.83 m). Due to pumping, groundwater tables were decreasing closer to the groundwater abstraction well PGAW3. The dynamics in wells PGAW3, LSN28 and LSG11 was similar with a maximum water table change of 3.7 m (Table 1). Groundwater gradients were calculated for all groundwater monitoring wells in the transect (Table 1). The gradients from the groundwater monitoring wells situated between the river and PGAW3 were predominantly positive, indicating that river water was infiltrating into the aquifer and groundwater flow was towards the groundwater abstraction well.

The gradient increased further towards PGAW3, due to constant pumping of the groundwater abstraction well. As expected, the gradient from PGAW3 towards LSN28 was predominantly negative, meaning groundwater flow towards PGAW3 from the direction of the backwater river (Fig. 1). Contour maps created during different flow conditions of the Danube (Supplementary Fig. S3) showed that the well capture zone of PGAW3 does not always include LSN28 and LSG11. Under certain conditions, the backwater was fed by the Danube (Supplementary Fig. S1). This had an influence on the gradient between LSN28, LSG11 and the backwater river. During the rising limb of a flood event, water flows into the backwater river. Water levels in the backwater rise and cause an extension of the well capture zone towards LSN28 and LSG11. The gradient was negative and the groundwater flow direction was towards the groundwater abstraction well. The flow direction in the backwater reverses during the falling limb and the gradient simultaneously reversed. This was confirmed by a 3D groundwater flow and transport model (Farnleitner et al., 2014).

Temperature is another parameter frequently used to investigate the interaction between groundwater and surface water (Schmidt et al., 2006). Both the Danube and the backwater river showed pronounced seasonal changes in surface water temperature and had highest temporal variability (Table 1). A seasonal trend was also observed in wells LSG41, LSG40 and LSG30, albeit with a lag time of approximately 2 months (not shown). Less pronounced seasonality was shown in PGAW3, LSN28 and LSG11. A seasonal pattern was also observed for nitrate. Peak concentrations in the river were observed during the winter months, but were not correlated to the water table (not shown). Nitrate concentrations in the groundwater wells between the Danube and PGAW3 were only 20% to 30% less than in the Danube and seemed to be influenced by the river. Wells LSN28 and LSG11 (which were located between PGAW3 and the backwater river) in contrast, were influenced by the backwater river ($r = 0.50$, $P = 0.035$ for LSG11 and $r = 0.55$, $P = 0.019$ for LSN28). Other standard chemical parameters (NH_4^+, Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, HCO_3^-) and EC did not exhibit any seasonality.

A clear distinction between both surface waters and the groundwater could be seen for the DOC concentrations. Analysis of variance (one-way ANOVA) showed that the average concentrations and fluctuations in the Danube (2.35 ± 0.67 mg/L) and in the backwater river (2.17 ± 0.52 mg/L) were significantly higher than in the groundwater in all wells (average concentration of 0.71 ± 0.27 mg/L, Table 2). No clear seasonal DOC pattern could be observed in the surface waters nor in the groundwater, which was in contrast to other studies (Brugger et al., 2001; Ellis et al., 1998; Zhou et al., 2012). No statistically significant correlations were found between groundwater flow velocity and DOC in any of the wells.

3.2. Enhanced surface water infiltration during flood events governs the seasonal dynamics of bacterial biomass and carbon production during RBF

After tracing which river characteristics are of major influence on groundwater quality in the study area, the next step was to identify the spatiotemporal dynamics of bacterial biomass and BCP in both surface water and groundwater.

3.2.1. Total cell counts

Total bacterial cell counts (TCC) in the Danube ranged from 1.77×10^6 to 6.14×10^6 cells/mL and from 2.35×10^6 up to 2.45×10^7 cells/mL in the backwater river (Table 2), with corresponding biomass values ranging from 27.2 up to 81.7 ng C/mL and from 38.9 up to 302 ng C/mL, respectively. These values were in the same range as found during the Joint Danube Survey 2007 (Velimirov et al., 2011) and in rivers of similar discharge such as the Pearl river, the river Rhine and the river Meuse (Duan et al., 2007; Scherwass et al., 2010; Servais, 1989). TCC and bacterial biomass in the Danube were positively correlated to temperature ($r = 0.61$, $P = 0.007$, Supplementary Table S3). A similar trend, but no significant correlation, was observed in the backwater river. The variation in TCC was higher in the backwater due to the discontinuous inflow of river water (Kirschner and Velimirov, 1997). DOC concentrations in both the Danube and the backwater river were in a similar range during summer, but were not correlated to TCC. The correlations with other nutrients, which were mainly negative, were more pronounced in the Danube than in the backwater river (not shown).

Table 1
Water table, gradient, temperature and conductivity range of the surface and groundwater bodies during the studied period.

	Water table difference (in m a.A.)	Gradient (%)[a]	Temperature range (in °C)	Electrical conductivity (μS/cm)
Danube	147.54–153.38	n.a.	2.8–23.2	329–882[b]
LSG41	147.33–151.16	1.97–18.6	7.5–16.1	367–757
LSG40	147.33–151.15	−0.26 – 0.28	8.2–15.3	317–914
LSG30	147.33–151.12	0.005–0.16	7.1–18.1	360–610
LSG2	146.89–150.15	0.04–0.37	9.9–14.4	418–575
PGAW3	145.33–149.96	0.13–0.64	10.9–14.1	480–544[b]
LSN28	146.52–150.22	−0.75–0.05	9.8–14.6	533–729[b]
LSG11	146.73–150.35	−0.26–0.06	9.8–14.5	438–548
Backwater	147.32–150.86	n.a.	0[c]–31.3	414–639[b]

[a] Gradient values are given as average values, with the minimum and maximum values given in parentheses. The gradient given at LSG41 is calculated from water table differences between the Danube and LSG41, at LSG40 water table differences between LSG41 and LSG40 were used etc.
[b] Logger values for this parameter were not available. Instead, hand held measurements taken during the sampling campaigns were used.
[c] The backwater river was frequently frozen during the winter.

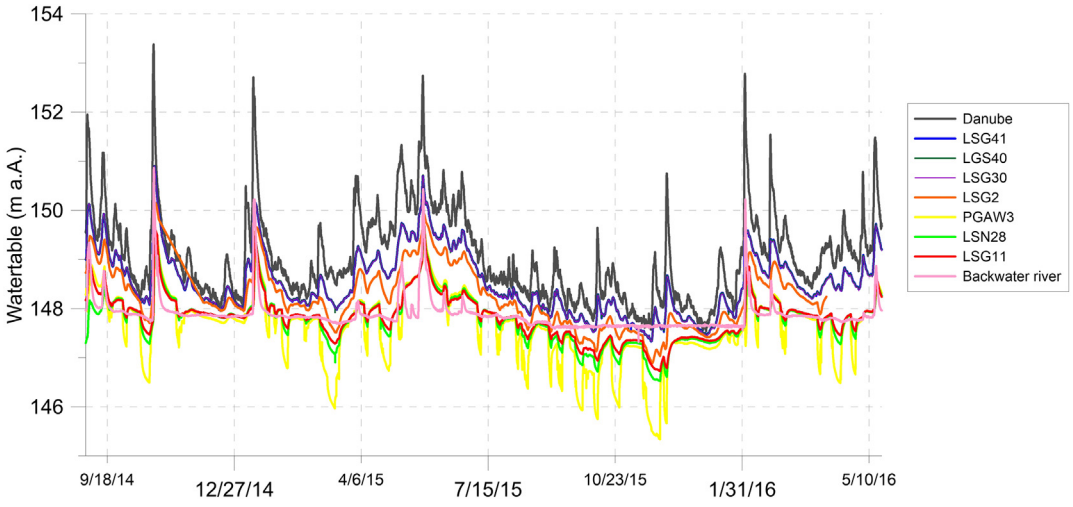

Fig. 2. Watertables of both surface waters and all monitoring wells. Values of wells LSG41, LSG40 and LSG30 are very similar, therefore only the hydrograph of LSG41 can be distinguished.

In the groundwater wells, mean TCC were significantly lower than in the surface waters, ranging from 4.64×10^4 cells/mL up to 4.04×10^5 cells/mL (Table 2 and Fig. 3).

They were in a similar range as reported by Alfreider et al. (1997), Brugger et al. (2001) and Zhou et al. (2012), even though the infiltrating rivers or lakes in those studies were significantly smaller than the Danube river. No clear seasonal patterns in TCC were observed in our study, although this has been found elsewhere (Velasco Ayuso et al., 2009a). The corresponding bacterial biomass values in the groundwater (Table 2) ranged from 0.59 ng C/mL in PGAW3 up to 5.66 ng C/mL in LSG40. Highest bacterial cell counts and biomass was found in the wells closest to the river (up to a maximum distance of 24 m). In these first meters, only 5% of TCC measured in the river was found in the groundwater and further decreased to only 2% in the groundwater abstraction well. Brugger et al. (2001) found a similar decrease in bacterial abundance along the flow path; a less pronounced decrease was shown for the Flathead river in Ellis et al. (1998), caused by 10-fold lower TCC values in this river. The absolute numbers however were in the same order of magnitude. Not only did the absolute values of TCC and biomass show a clear decrease towards the groundwater abstraction well PGAW3, the temporal variability also decreased significantly. The highest temporal variability of TCC and biomass in the groundwater was observed in wells LSG41, LSG40 and LSG30 next to the Danube, and in well LSG11 next to the backwater (Fig. 4a).

Both the river and the backwater river showed a similar temporal pattern as the wells close to the surface water bodies. This variability could therefore be attributed to the input of water from either the river or the backwater river. Lowest values and temporal variability in TCC and biomass were observed in the wells with the highest distance to the river (LSG2, PGAW3) corresponding to observations made earlier (Brugger et al., 2001; Ellis et al., 1998). This part of the aquifer is relatively pristine and the concentrations of most nutrients were lowest. In contrast to the surface waters, no correlation between bacterial abundance and standard chemical parameters and also no correlation with temperature was observed for the groundwater samples.

3.2.2. Bacterial carbon production

BCP varied from 7.87×10^{-3} up to 0.59 ng C/mL/h in the surface water samples. This was well within the range of other rivers (Bernard et al., 2000; Brugger et al., 2001; Fischer and Pusch, 2001), but slightly lower than during the Joint Danube Survey 2007 (Velimirov et al., 2011), which was a snapshot of the river Danube in autumn. BCP in the Danube coincided with peaks in water level ($r = 0.82$, $P = 3.3 \times 10^{-5}$) but did not show significant seasonality. The backwater river on the contrary showed a temperature dependence ($r = 0.64$, $P = 0.004$), but no significant correlation between BCP and water level. In both the Danube and the backwater river, BCP was positively correlated to DOC ($r = 0.59$, $P = 0.013$; $r = 0.77$, $P = 3 \times 10^{-4}$ respectively). Only few other chemical parameters (HCO_3^- and Cl^-) correlated with BCP in both surface waters. Due to the lower quantity and quality of DOC, BCP values in groundwater are typically much lower than in surface water. Indeed, BCP was much lower (up to 4 orders of magnitude) in the groundwater of the investigated PGA, ranging from 4.18×10^{-6} in PGAW3 up to 1.64×10^{-3} ng C/mL/h in LSG11 (Table 2). A broad range of BCP values for groundwater samples was found in similar studies, ranging from below the detection limit up to 1.82 ng C/mL/h (Alfreider et al., 1997; Brugger et al., 2001; Velasco Ayuso et al., 2009b). The very high values measured by Velasco Ayuso et al. (2009b) were most likely due to the high carbon production and the high amount of readily degradable DOC in the coastal environment that infiltrated into the aquifer. Similar to biomass, BCP decreased further along the flow path towards PGAW3 (Fig. 4b). The temporal variability in each well concurrently decreased and was lowest in LSG2 and PGAW3. As for bacterial numbers, no significant correlations could be found between BCP and DOC or other nutrients once the river water infiltrated into the groundwater. In addition, no correlation with water temperature was observed.

Several explanations can be proposed for the lack in correlations between these parameters. Most likely, stochastic ecological processes govern the microbial communities in groundwater aquifers (Stegen et al., 2016). Only when readily available labile organic carbon enters the aquifer, the microbial community responds. It can be hypothesized that due to a relatively low amount of readily degradable DOC in our investigated groundwater, no correlation was found between DOC and any of the microbial parameters during the monthly sampling period. A multiple regression analysis between several nutrients and the microbial parameters further confirmed the lack of correlations between these parameters in groundwater samples, as also observed by Zhou et al. (2012). Under regular discharge conditions of the Danube, when flow velocities in the groundwater body are low, the high quality DOC does not reach far into the aquifer and bacterial production values stay in a low range. Only when groundwater flow velocities increase significantly, caused by a flood event in the river and concomitant infiltration of surface water into the groundwater body, nutrients - but also bacteria - are effectively pressed into the groundwater and a correlation between bacterial production and bacterial numbers with flow velocity would occur. In well LSG41 located nearest to the river, such correlation was indeed observed ($r = 0.69$, $P = 1.4 \times 10^{-3}$ and $r = 0.47$, $P = 0.048$, for BCP and large cells, respectively; Supplementary Table S3). For the wells further towards the groundwater abstraction well

Table 2
Average values of (physico) chemical parameters and microbiological parameters during the monthly sampling campaigns. Values in brackets are min-max values. Values in meters are the distance to the Danube.

	Danube	**LSG41**	**LSG40**	**LSG30**	**LSG2**	**PGAW3**	**LSN28**	**LSG11**	**Backwater**
Parameter	*0 m*	*10 m*	*13 m*	*24 m*	*283 m*	*551 m*	*704 m*	*782 m*	*882 m*
T (°C)	10.6 (3.5–22)	11.6 (7.9–19.4)	11.7 (8.3–18.2)	11.6 (8–17.1)	12.5 (8.1–14.8)	12.1 (11.2–14.4)	12.2 (10.6–14.8)	12.4 (11.5–14)	12.1 (4–29.1)
EC (μS/cm)	445 (329–882)	473 (411–575)	481 (413–582)	448 (398–518)	475 (401–549)	509 (480–544)	598 (533–729)	601 (529–689)	521 (414–639)
pH	8.0 (7.6–8.8)	7.4 (7.1–8.2)	7.4 (6.9–8.2)	7.5 (7.1–8.2)	7.4 (7.0–8.0)	7.4 (7.0–8.1)	7.3 (6.9–7.8)	7.3 (7.1–7.9)	8.1 (7.5–9.0)
DOC (mg/L)	2.4 (1.3–3.9)	0.8 (0.4–1.1)	0.8 (0.4–1.3)	0.9 (0.4–1.3)	0.6 (0.2–0.9)	0.5 (0.2–1.7)	0.7 (0.4–1.6)	0.7 (0.5–2.0)	2.2 (1.4–3.4)
NH4-N (mg/L)	0.024 (0.003–0.049)	0.010 (n.d.-0.031)	0.008 (n.d.-0.023)	0.006 (n.d.-0.019)	0.01 (n.d.-0.030)	0.012 (n.d.-0.041)	0.011 (n.d.-0.024)	0.009 (n.d.-0.019)	0.025 (0.004–0.069)
NO_3^- (mg/L)	8.4 (4.8–13.0)	6.6 (2.5–10.9)	6.8 (2.5–11.0)	7.0 (3.1–10.7)	7.2 (4.3–9.7)	5.7 (4.0–8.3)	1.5 (0.7–4.2)	1.2 (n.d.-5.1)	0.8 (n.d.-7.3)
Bacterial abundance (cells/mL)	3.78×10^6 (1.77×10^6–6.14×10^6)	1.99×10^5 (1.35×10^5–3.08×10^5)	1.89×10^5 (9.77×10^4–4.04×10^5)	1.55×10^5 (5.83×10^4–2.94×10^5)	9.14×10^4 (4.64×10^4–1.45×10^5)	7.31×10^4 (4.80×10^4–9.55×10^4)	1.85×10^5 (1.37×10^5–2.41×10^5)	1.69×10^5 (8.21×10^4–2.81×10^5)	8.75×10^6 (2.35×10^6–2.45×10^7)
Large cells (cells/mL)	1.83×10^6 (9.5×10^5–2.68×10^6)	6.09×10^4 ($2.65 \times$ –1.64×10^5)	5.92×10^4 (2.54×10^4–1.62×10^5)	4.09×10^4 (1.73×10^4–1.02×10^5)	2.30×10^4 ($1.51 \times$ –4.10×10^4)	1.34×10^4 (9.94×10^3–2.03×10^4)	3.86×10^4 (2.54×10^4–5.18×10^4)	4.47×10^4 (2.16×10^4–1.64×10^5)	2.81×10^6 (1.53×10^6–5.76×10^6)
Proportion large cells (%)	49.9 (33–71.1)	29.0 (18.8–63.7)	30.5 (19.5–56.4)	27.2 (12.8–56.6)	25.6 (17.6–34.8)	19.3 (12.4–26.8)	21.3 (11.8–31.0)	25.8 (10.6–58.5)	41.1 (15.5–65.1)
Biomass (ng C/mL)	56.1 (27.2–81.7)	2.60 (1.67–4.22)	2.48 (1.23–5.66)	1.96 (0.83–3.48)	1.14 (0.62–1.86)	0.87 (0.59–1.08)	2.23 (1.70–2.83)	2.13 (1.06–4.45)	116 (38.9–302)
Leucine incorporation (pmol/L/h)	87.3 (5.09–384)	0.276 (0.028–0.995)	0.249 (0.025–0.99)	0.069 (0.013–0.413)	0.015 (0.007–0.033)	0.008 (0.003–0.023)	0.037 (0.011–0.211)	0.104 (0.006–1.06)	90.5 (7.00–248)
BCP (ng C/mL/h)	1.35×10^{-1} (7.87×10^{-3}–5.93×10^{-1})	4.27×10^{-4} (4.35×10^{-5}–1.54×10^{-3})	3.85×10^{-4} (3.90×10^{-5}–1.53×10^{-3})	1.06×10^{-4} ($2.03 \times$ $^{-5}$–6.39×10^{-4})	2.26×10^{-5} (1.13×10^{-5}–5.16×10^{-5})	1.26×10^{-5} (4.18×10^{-6}–3.49×10^{-5})	5.72×10^{-5} (1.65×10^{-5}–3.26×10^{-4})	1.16×10^{-4} (9.73×10^{-6}–1.64×10^{-3})	1.40×10^{-1} (1.08×10^{-2}–3.83×10^{-1})

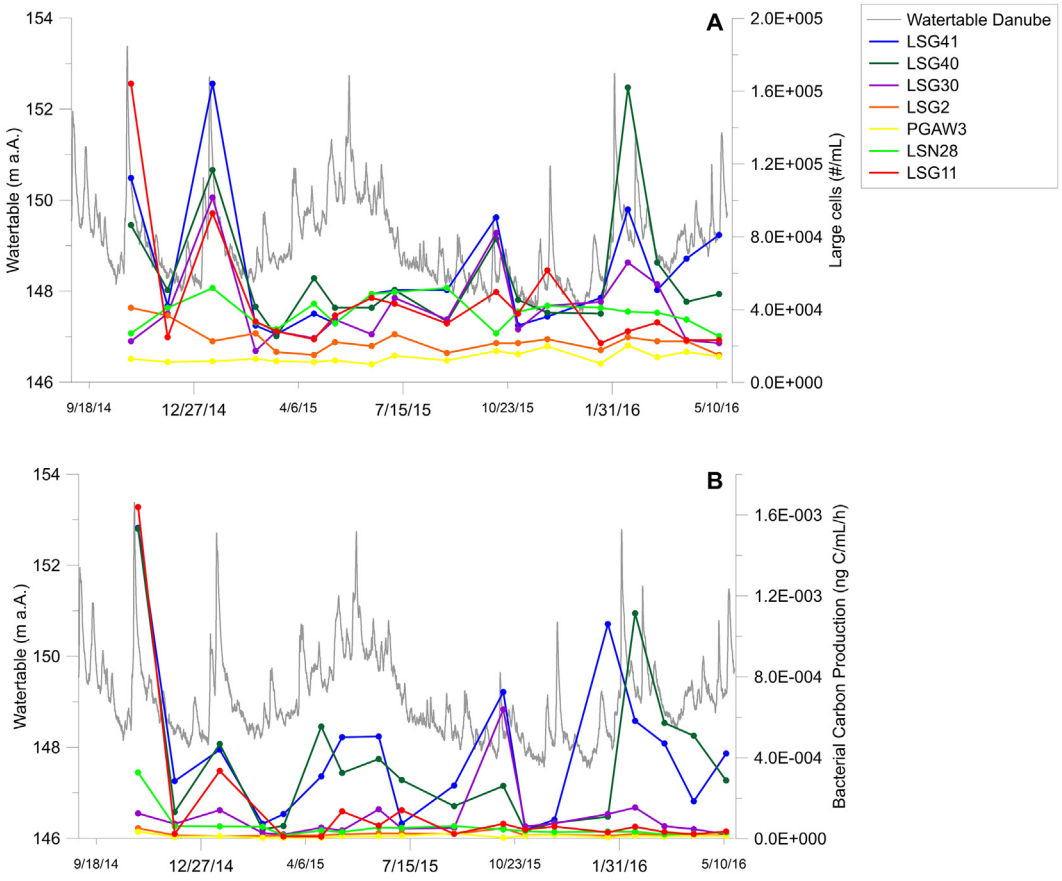

Fig. 3. a) Abundance of large bacterial cells and **b)** the bacterial carbon production in all groundwater monitoring wells versus the water table of the Danube.

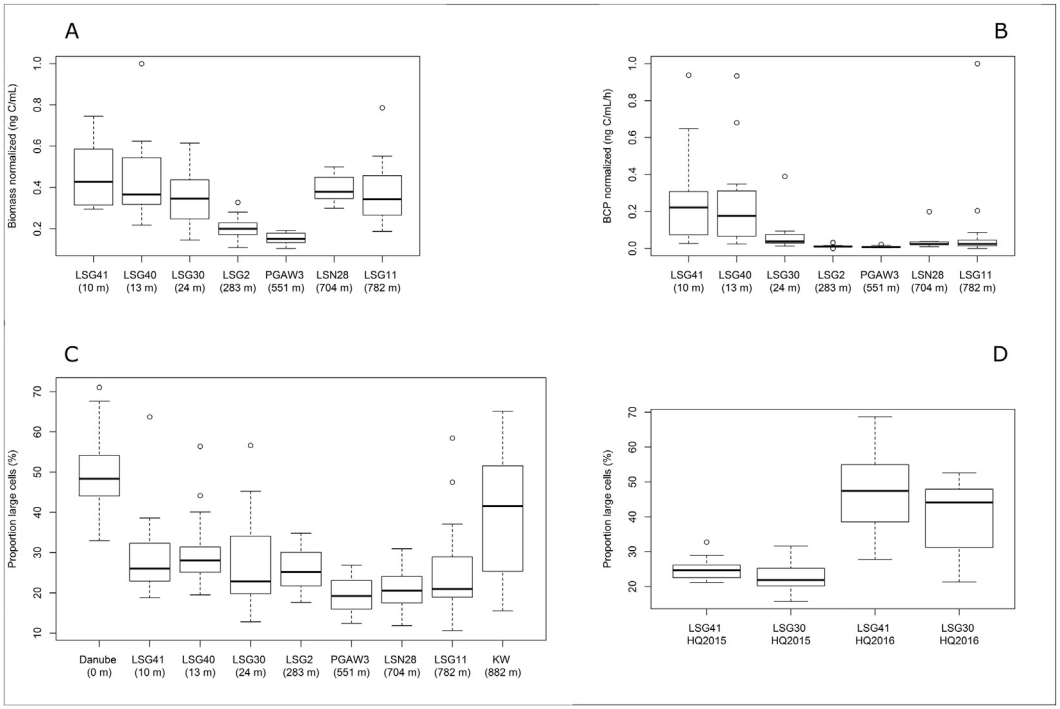

Fig. 4. Boxplots of **a)** normalized biomass in the groundwater monitoring wells during the monthly sampling campaign, **b)** normalized BCP in the groundwater monitoring wells during the monthly sampling campaign, **c)** the proportion of large cells during the monthly sampling campaign and **d)** the proportion of large cells during HQ2015 and HQ2016.

however, no correlation between flow velocity and BCP or large cells was found.

3.2.3. Cell size distribution as indicator of surface water infiltration

Besides the significant differences between TCC and BCP in surface- and groundwater, the proportion of large cells may be used as an indicator of surface water infiltration. It has been shown that size distribution of hyporheic bacteria can be very similar to river samples, but changes while moving further away from the river into the aquifer (Ellis et al., 1998). To test this hypothesis, cell counts were classified into large cells (rod shaped cells and coccoid cells with a diameter > 1.0 μm) and small cells (cocci with a diameter < 1.0 μm). In both surface water and groundwater, TCC were dominated by small cells. There was however a distinct difference in the proportion of large cells in the surface waters relative to the proportion of large cells in the groundwater (Fig. 4c). The river water consisted of a much larger proportion of large cells, which may be attributable to higher availability of nutrients. During subsurface passage, the proportion of large cells in the water matrix decreased due to the lack of nutrients and readily degradable organic carbon (Zhou et al., 2012). A one-way ANOVA showed that the difference in proportion of large cells was statistically significant between the river samples and the groundwater samples taken during the monthly sampling campaigns. Our hypothesis was that during flood events, due to the higher flow velocities and the increased amount of water entering the aquifer, the proportion of large cells in the groundwater close to the river would be much more similar to river water than under normal flow conditions.

3.3. Flood events lead to a short-time response of the bacterial groundwater community and a concomitant increase in TCC and large cells

We hypothesized that during flood events, the influence of the river on the groundwater is higher than under regular discharge conditions and this influence reaches more distant wells. In order to account for changes in TCC and size distribution of bacterial cells entering the aquifer under high flow velocities, additional samples were taken during two flood events. Although during both sampled flood events peak water levels in the Danube were similar (Table 3 and Table 4), minimum surface water levels were distinctly different, which was also seen in the dynamics of the groundwater levels (Table 3 and Table 4).

Another difference between the two flood events was seen in the gradient and flow velocities. The maximum gradient during HQ2015 between the Danube and LSG41 was 20.4%, whereas the maximum gradient between the Danube and LSG41 during HQ2016 was 31%. Flow velocities were approximately 1.5 fold higher during HQ2016 (0.040 m/s) than during HQ2015 (0.026 m/s). In contrast to the Danube, TOC concentrations in the groundwater were stable throughout both flood events and did not significantly differ between the two events. Brugger et al. (2001) showed that a peak in DOC concentration in the Enns river resulted in higher DOC concentrations only at the stations near the river (up to 6 m), but had no effect on the more distant stations, which could explain the lack of correlation in our groundwater wells. Nitrate concentrations in the groundwater during HQ2016 were very similar to those in the river and were significantly higher than under regular discharge conditions. A significant correlation between flow velocity and nitrate was however not present. None of the other nutrients showed significant changes during the flood events in the groundwater samples. During HQ2015, TCC in the groundwater (Table 3) were in the same range as the average TCC values measured during the monthly sampling campaigns (Table 2). During HQ2016, TCC in the groundwater was twice as high as during HQ2015 (Table 4). In the Danube however, average TCC values were in a similar range as during the monthly sampling campaigns (Table 2 and Table 4). TCC in the river started to increase as water levels rose and stayed fairly constant during the six following days. TCC in the nearest monitoring well, LSG41, showed a similar clear increase which was associated with the low travel times from the Danube towards LSG41. Although no clear decrease was seen in the river, the bacterial abundance in LSG41 decreased rapidly after the peak in water level (Fig. 5b), obviously linked to a decrease in the gradient. In LSG30, a similar but attenuated pattern could be found. Probably due to the lower gradient, travel times from LSG41 towards LSG30 were much longer. Therefore, the rise in TCC was less evident than in LSG41. The variability in the proportion of large cells in the groundwater during HQ2016 was much higher than during HQ2015 and was caused by the response of the bacterial community to the increased gradient and flow velocity (Fig. 5). Because the response was temporally shifted, no correlation was found between large cells and the flow velocity during HQ2016 (Fig. 5c). For biomass however, a statistical significant correlation with flow velocity was present in both wells (LSG41: $r = 0.853$, $p = 1.7 \times 10^{-3}$; LSG30: $r = 0.664$, $p = 0.036$; Supplementary Table S4). These correlations were much higher than under regular discharge conditions. During HQ2015, these correlations were not present due to the lower gradients and flow velocities. This suggests that during flood events with high gradients, an increased

Table 3
Minimum and maximum values of the water table difference and gradient and average values of (physico)chemical and microbiological parameters during HQ2015. Values in brackets are min-max values. Values in meters are the distance to the Danube.

HQ2015	**Danube**	**LSG41**	**LSG30**
	0 m	*10 m*	*24 m*
Water table difference (m a.A.)	150.10–152.74	149.63–150.70	149.62–150.71
Gradient (%)	n.a.	5.77–20.4	−0.08-0.10
T (°C)	13.0 (11.7–15.2)	11.6 (11.0–13.9)	12.7 (12.0–13.9)
EC (μS/cm)	345 (329–365)	505 (425–560)	397 (391–401)
pH	7.94 (7.52–8.05)	7.28 (7.05–7.45)	7.40 (7.27–7.52)
TOC (mg/L)	2.73 (1.60–3.60)	0.90 (0.80–0.90)	1.00 (1.00–1.00)
NO_3^- (mg/L)	7.44 (6.00–8.10)	5.40 (4.30–6.60)	6.60 (6.30–6.80)
Bacterial abundance (cells/mL)	n.a.	2.12×10^5 (1.46×10^5–2.58×10^5)	1.06×10^5 (9.56×10^4–1.20×10^5)
Large cells (cells/mL)	n.a.	5.29×10^4 (3.40×10^4–6.37×10^4)	2.44×10^4 (1.51×10^4–3.38×10^4)
Proportion large cells (%)	n.a.	25.1 (21.2–32.7)	23.1 (15.7–31.6)
Biomass (ng C/mL)	n.a.	2.65 (1.80–3.22)	1.30 (1.11–1.46)

Table 4
Minimum and maximum values of the water table difference and gradient and average values of (physico)chemical and microbiological parameters during HQ2016. Values in brackets are min-max values. Values in meters are the distance to the Danube.

HQ2016	**Danube**	**LSG41**	**LSG30**
	0 m	*10 m*	*24 m*
Water table difference (m a.A.)	147.95–152.78	147.80–149.74	147.80–149.71
Gradient (%)	n.a.	1.50–31.0	0.01–0.19
T (°C)	6.18 (5.40–7.00)	8.41 (7.80–9.20)	7.53 (7.00–8.30)
EC (μS/cm)	384 (359–440)	440 (432–453)	426 (420–435)
pH	7.78 (7.60–8.00)	7.64 (7.60–7.70)	7.65 (7.40–7.80)
TOC (mg/L)	4.40 (2.70–6.40)	4.40 (2.70–6.40)	1.30 (1.20–1.40)
NO_3^- (mg/L)	11.0 (9.80–12.0)	9.97 (8.80–11.0)	10.9 (10.0–12.0)
Bacterial abundance (cells/mL)	3.80×10^6 (1.87×10^6–4.97×10^6)	4.48×10^5 (2.46×10^5–7.32×10^5)	2.63×10^5 (1.88×10^5–3.46×10^5)
Large cells (cells/mL)	3.30×10^6 (1.26×10^6–4.48×10^6)	2.28×10^5 (9.50×10^4–4.32×10^5)	1.08×10^5 (6.26×10^4–1.66×10^5)
Proportion large cells (%)	85.0 (67.6–94.0)	49.1 (31.7–68.7)	41.0 (21.3–52.6)
Biomass (ng C/mL)	71.0 (31.3–94.4)	7.22 (3.41–11.4)	3.86 (2.54–5.12)

and extended influence (up to a distance of 24 m) of the river can be observed. The proportion of large cells in the monthly samples (Fig. 4c) was significantly different between the surface water and groundwater samples. During HQ2016, the proportion of large cells in the Danube increased significantly (Fig. 5c) and was much higher than during the monthly sampling campaigns (Table 2) or during HQ2015. Peak values in both wells were reached one day after the peak in gradient (which corresponded to the travel time from LSG41 to LSG30; Fig. 5c).

3.4. Turnover times of the bacterial biomass are too long to explain the observed increase in TCC in the groundwater wells during flood events

Lin et al. (2012) showed the influence of the temporal dynamics in water level on the community composition of the Hanford aquifer. During higher water levels two groups of Actinobacteria were found which were not present under lower water levels. A distinction between inflow of riverine bacteria, elution from the lower vadose zone, or environmental selection of aquifer bacteria by the riverine nutrients could however not be made since the study did not analyze the riverine microbial population. We hypothesize that only when large amounts of surface water flow into the aquifer and when flow velocities are high, riverine bacteria enter the aquifer. It is less likely that bacteria detach from the subsurface sediments of the lower vadose zone, since this would have also meant an increase in abundance during the HQ2015 flood. This was, however, not observed. Due to the lack of correlations between the chemical parameters and the microbiological parameters, it was unlikely that the riverine nutrients were the source of the increasing abundances. Moreover, turnover times of the bacterial biomass are too long to explain the observed increase in bacterial numbers in the groundwater wells during flood events. Turnover times varied from 3.72 up to 201 days in the Danube to 12.7 up to 200 days in the backwater river. Lowest values measured in the Danube were measured following peaks in discharge ($r = -0.64$, $P = 0.004$) and were in a similar range as during peak discharges in other rivers (Bernard et al., 2000; Billen et al., 1990; Brugger et al., 2001). Turnover times in the groundwater (84–10,514 days) were much longer than in both surface waters. They were shortest in the wells close to the river (LSG41 and LSG40) and increased significantly towards PGAW3 (Table 2). On average they were generally one order of magnitude higher than in similar studies (Brugger et al., 2001; Ellis et al., 1998). The calculated turnover times were based on total cell counts. These however, do not only include viable cells, but also a mixture of dormant and dead cells. TVAC counts (Supplementary Table S5) constituted only a low percentage (below 1%) of total cell counts. In the wells next to the river (LSG41 and LSG40), the percentage of TVAC was highest, whereas it was lowest in the most distant wells (LSG11, LSN28). When turnover times were calculated on the basis of the viable active cells, they were in the range of only a couple of days (see Supplementary Table S6). The highest turnover times were measured in well LSG2 and in PGAW3. The lowest turnover times were measured in the wells closest to the Danube.

With the calculated turnover times, based on the total bacterial biomass, the observed increase in bacterial numbers/biomass in the wells in close proximity to the river during a flood event cannot be explained. During HQ2016 a 4.4 fold increase in TCC from 1.67×10^5 to a maximum of 7.32×10^5 cells was observed within a period of 4 days. Minimum turnover times observed within the monthly sampling campaign (including flood events) was around 100 days for the groundwater samples and it would thus need >400 days to achieve a 4.4 fold increase in bacterial numbers by the growth of the bacterial community from an additional nutrient input. Thus the observed increase has to be caused by the input of bacterial cells from the river or from detachment of bacterial cells from subsurface biofilms from the lower vadose zone due to water table changes. As the percentage of large cells during HQ2016 was similar in the groundwater and the surface water we assume that surface water infiltration is the responsible factor. Community composition profiling could prove this hypothesis.

4. Summary and conclusions

During a 20 month sampling campaign considerable spatiotemporal fluctuations were observed in bacterial cell numbers, biomass and carbon production in a porous aquifer. Under regular discharge conditions, bacterial abundance, the percentage of large cells, bacterial biomass and bacterial carbon production decreased significantly from the river and the backwater river towards the groundwater abstraction well due to processes like filtration or die-off. Despite the tendency of many environmental biota to exhibit seasonal responses and responses to nutrient stimuli, temporal changes in microbial metrics monitored in this study were more closely aligned with fluctuations in groundwater flow velocities. The observed increase in bacterial cell numbers during flood events was most likely attributable to the infiltration of surface water bacteria. Calculated turnover times of the bacterial biomass were too long to explain the observed increase in bacterial numbers in the groundwater wells. Moreover, during flood events, the percentage of

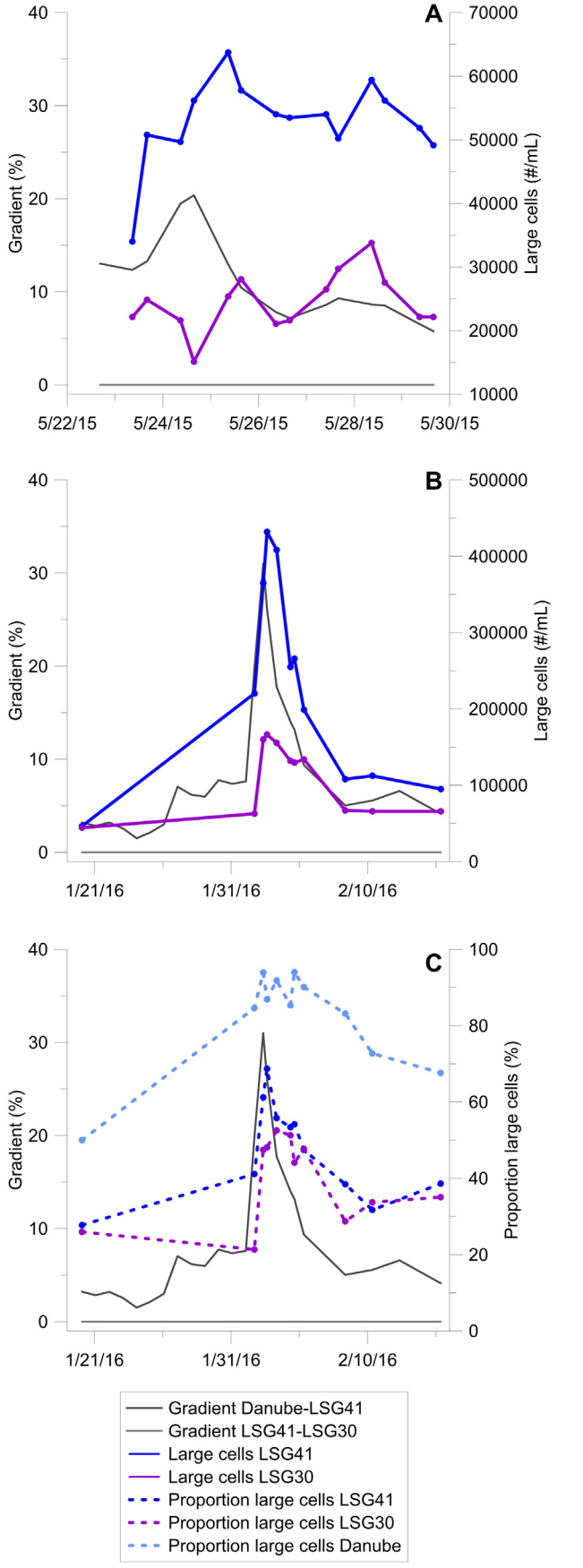

Fig. 5. Large cells versus gradient during **a)** HQ2015 and **b)** HQ2016 and **c)** the proportion of large cells versus gradient during HQ2016.

large cells in the groundwater wells was similar to the surface water. This infiltration was markedly visible in the well 10 m away from the riverbank at several occasions during the investigation period, and was extended in an attenuated way towards the well situated 24 m away from the riverbank during flood events. The drinking water abstraction well situated at a distance of approx. 550 m was never significantly affected. In contrast, the two wells close to the backwater river also showed considerable variability in microbiological parameters over the year. This was related to the influence from the backwater river that showed pronounced hydrological variability in relation to its connectivity to the main river.

The use of the bacterial abundance, biomass and activity as indicators for surface water – groundwater interaction is of high relevance for drinking water management. Bacterial cell numbers and biomass can be measured near-real time using (for example) flow cytometry. Together with information on hydrogeological characteristics of the aquifer, such as hydraulic conductivity and porosity, water utilities can use the microbiological data to improve their water abstraction strategies and react quicker to changing hydrological conditions in the RBF system.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.226.

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Spatiotemporal resolved sampling for the interpretation of micropollutant removal during riverbank filtration

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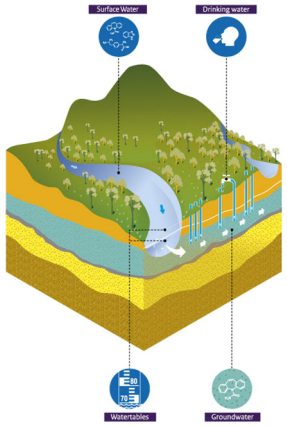
HIGHLIGHTS

- OMP fate was studied along an RBF system under normal and elevated conditions
- Benzotriazole was almost fully removed during RBF under oxic conditions
- Carbamazepine and sulfamethoxazole showed a relatively persistent behavior
- Increase in load of several OMPs in the river observed during flood events
- OMP concentrations in the groundwater were far below drinking water guideline values

GRAPHICAL ABSTRACT



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ABSTRACT

Riverbank filtration (RBF) systems along rivers are widely used as public water supplies. In these systems, many organic micropollutants (OMPs) are attenuated, but some compounds have shown to be rather persistent. Their fate and transport has been studied in RBF sites along lakes and small rivers, but not extensively along large and dynamic rivers. Therefore, the influence of flood events on OMP behavior in these large and dynamic RBF sites was investigated. Monthly samples were taken from surface- and groundwater up to a distance of 900 m from the riverbank of the Danube from March 2014 till May 2016. Two flood events were sampled more extensively nearby the river. Results showed that changes in flow conditions in the river not only caused changes in OMP concentrations, but also in their load. It was seen that the load of benzotriazole, carbamazepine and

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sulfamethoxazole in the river increased with increasing river discharges. After a relatively long, oxic groundwater passage, several OMPs were reduced. In contrast to previous work, we found that benzotriazole was almost fully removed under oxic conditions. When entering the aquifer, benzotriazole concentrations were significantly reduced and at a distance of 550 m from the river, >97% was degraded. Carbamazepine and sulfamethoxazole showed relatively persistent behavior in the aquifer. The concentrations measured during flood events were in the same range as seasonal sampling. Furthermore concentrations in the groundwater were higher during these events than in the Danube and can reach further into the aquifer. During flood events some highly degradable compounds (i.e. diclofenac) were found up to a distance of 24 m from the river. These results implied that drinking water utilities with RBF wells in oxic, alluvial aquifers located close to highly dynamic rivers need to consider a potential reduction in groundwater quality during and directly after flood events.

1. Introduction

Along large rivers such as the Danube, millions of people use drinking water from riverbank filtration (RBF). RBF systems are used in many countries (Heberer et al., 2001; Hiscock and Grischek, 2002; Ray et al., 2002; Tufenkji et al., 2002) due to the availability of large quantities of potential drinking water. They are however under much more anthropogenic stress than other, pristine groundwater sources. Due to the infiltration of low quality river water, the pristine groundwater can get contaminated with chemical and microbial substances. Substances that have been receiving increased attention are the organic micropollutants (OMPs) (Schwarzenbach et al., 2006). These pollutants comprise many different substances, such as industrial chemicals, pharmaceuticals and personal care products, but also pesticides or herbicides. Most of the pharmaceuticals and personal care products enter the environment through wastewater treatment plants (WWTPs) where they – depending on their persistence – are removed or remain in the effluent to a certain extent (Joss et al., 2005; Radjenović et al., 2009). Due to their persistence during treatment and their widespread presence in wastewater as main pathway to the aquatic environment, certain compounds have been suggested as indicators for impacts from waste water (Jekel et al., 2015), such as the corrosion inhibitor benzotriazole (BTri), the antiepileptic drug carbamazepine (CBZ) and the antibiotic sulfamethoxazole (SMZ).

Several studies have examined the behavior of these and other OMPs during RBF or similar systems (Heberer et al., 2008; Kahle et al., 2009; Rauch-Williams et al., 2010; Reemtsma et al., 2010; Regnery et al., 2015; Scheurer et al., 2011). Many of these studies however were conducted in RBF systems connected to small rivers or lakes. The range of water level fluctuations in these systems was much lower than along dynamic rivers such as the Danube (with water level fluctuations of up to 8 m).

Several factors such as groundwater residence times, redox conditions and mixing with pristine groundwater have shown their importance for the attenuation of OMPs (Burke et al., 2014b; Epting et al., 2018; Massmann et al., 2008, 2006; Storck et al., 2012; Wiese et al., 2011). Changing redox conditions and groundwater residence times for example can have an effect on removal rates of OMPs in the groundwater (Bertelkamp et al., 2016b) due to their effect on the biodegradation processes taking place in the aquifer. Not only the seasonal dynamics can influence the transport of the OMPs in the groundwater, flood events can also have an effect on their behavior. During a flood event, groundwater residence times can be shortened due to increased flow velocities (Derx et al., 2013; Sprenger et al., 2011). Furthermore, the composition of the infiltrating surface water can change the redox conditions in the aquifer and simultaneously have an influence on the micropollutant removal. Electron acceptors or donors can react abiotically with OMPs in the environment. The feasibility of these reactions is dependent on the prevailing environmental (redox) conditions (Schwarzenbach et al., 2017). Under oxic conditions for example, aerobic respiration can take place and OMPs can be oxidized. Especially oxic RBF systems are highly vulnerable to flood events due to a possible shift in redox conditions (Sprenger et al., 2011). Unfortunately, little is known so far on this removal in large and dynamic RBF systems. Therefore it is of paramount importance to gain more insight in the behavior of OMPs in these RBF systems. The aim of this paper was therefore to investigate the influence of flood events on the behavior of OMPs along a large and dynamic river. This was done by addressing the following questions: (i) What is the behavior of OMPs in an alluvial porous aquifer during RBF along a large and highly dynamic river? and (ii) Do flood events change the presence and behavior of OMPs in surface- and groundwater along this large and dynamic river? For this purpose, river and groundwater samples were taken from two surface water locations, six groundwater monitoring wells and a drinking water abstraction well in an alluvial porous aquifer (PGA). Seasonal samples were taken monthly between March 2014 and May 2016 and were analyzed for a mixture of 7 OMPs and standard chemical parameters. To account for changes during extreme river level fluctuations, two flood events with water level fluctuations of up to 5 m (with a recurrence of 1 year) were sampled at a higher temporal resolution.

2. Materials and methods

2.1. Study area and instrumentation

The study was conducted at an RBF system on the left bank of the Danube, downstream of the Austrian capital of Vienna, as previously described by van Driezum et al. (2018) (Fig. 1). The water quality in the studied section of the Danube is impacted by upstream wastewater treatment plant discharges (Frick et al., 2017). The total amount of wastewater discharges is based on 13 million inhabitants and a corresponding PE of 20 million inhabitants (Zessner and Lindtner, 2005). It thus contributes to 2.5% of the discharge of the Danube under mean flow conditions. Discharges of the Danube in Vienna can range from 700 m^3/s during low flow conditions up to 11,000 m^3/s, such as during the 2013 flood (Blöschl et al., 2013). The discharge regime of the river at this point was classified as alpine influenced (Wimmer et al., 2012). The RBF system is part of an alluvial backwater and floodplain area containing five groundwater abstraction wells used for drinking water. The daily extraction capacity of all five wells is 109,000 m^3. A transect containing several monitoring wells and a groundwater abstraction well was chosen which was continuously fed by the infiltrating Danube, resulting in predominantly oxic conditions (Mayr et al., 2014). The main layers of the unconfined aquifer consist of gravel and sand and have a thickness varying from 3 to 15 m. Hydraulic conductivities in the transect ranged from 5×10^{-4} m/s to 5×10^{-2} m/s, determined by pumping tests conducted in the area and a 3D groundwater flow and transport model (for calibration details refer to Farnleitner et al. (2014)). Local groundwater flow is directed from the southwest to the northeast. Underneath the aquifer are alternating sand and clay/silt layers with hydraulic conductivities of at least 2 orders of magnitude lower.

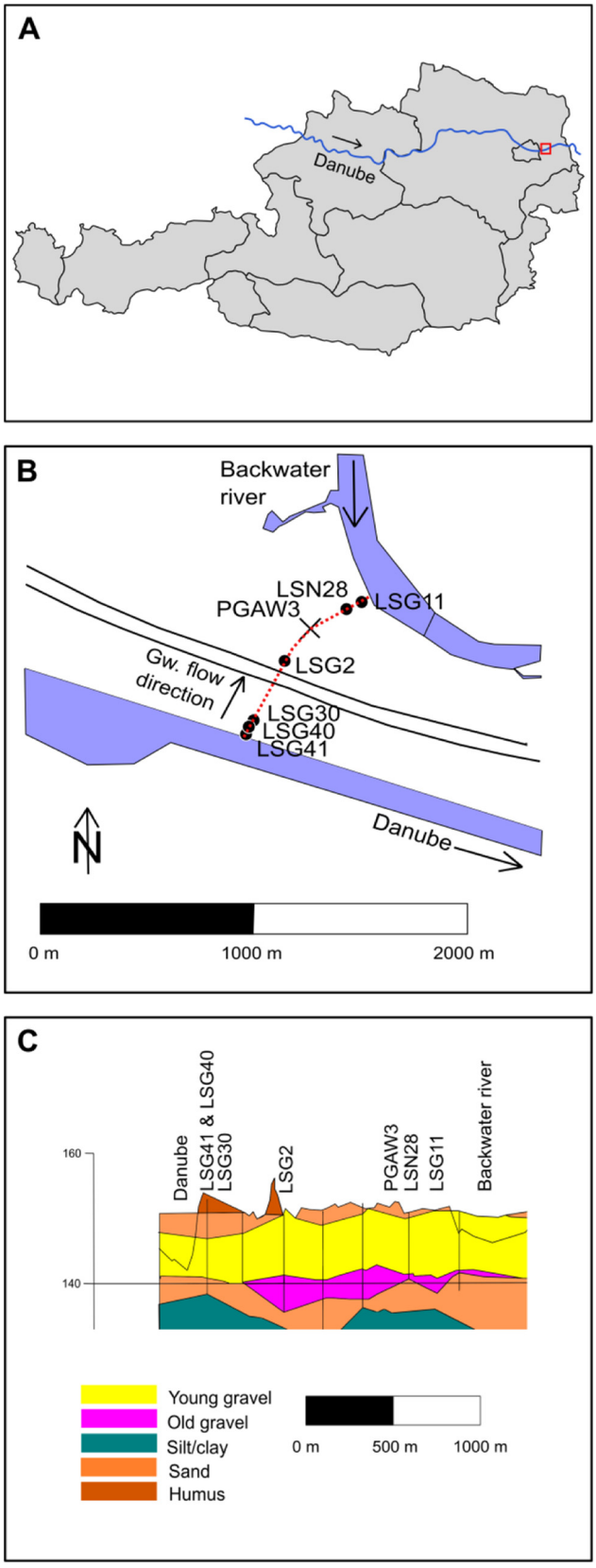

Fig. 1. a) Situation of the Natura 2000 protected area (red square) in Austria, b) the sampled transect including monitoring wells LSG41, LSG40, LSG30, LSG2, LSN28 and LSG11. The groundwater abstraction well is depicted as PGAW3and c) Schematic cross section (dotted red line in b) of the transect with the hydrogeological layers and the groundwater monitoring wells (shown as black vertical lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The transect with the sampled wells extends from the Danube towards the backwater river. It consists of 2 surface water locations, 6 groundwater monitoring wells and one groundwater abstraction well with a maximum extraction capacity of 0.28 m^3/s (PGAW3, Fig. 1). Three wells (LSG41, LSG40 and LSG30) are located close to the river and are subjected to high variability in river and groundwater levels. One well (LSG2) is located between these three wells and PGAW3. Wells LSN28 and LSG11 are located between PGAW3 and the backwater river. All wells are screened over the full length of the saturated aquifer. Travel times from the Danube towards PGAW3 based on the hydraulic gradient can be found in van Driezum et al. (2018) and range between 11.5 days to 47.4 days. Travel times towards the three nearest monitoring wells (LSG41, LSG40 and LSG30) ranged from 1 h to LSG41 (10 m away from the Danube) to 5.4 days to LSG30 (24 m away from the Danube) during the monitoring period from March 2014 to May 2016.

The backwater river is a sequence of connected ponds which is connected to the Danube when water levels in the river exceed 150.5 m a.A. (meter above the Adriatic Sea) at the river gauge station *Fischamend* (river kilometer 1908, occurring just below a flood event with a recurrence of 1 year).

Hourly hydraulic pressures and water temperatures were recorded continuously during the monitoring period in all groundwater monitoring wells. Hourly Danube water level and discharge values were measured at the station *Fischamend*.

2.2. Sampling strategy

Monthly samples were taken at all sampling locations from March 2014 to May 2016 ($n = 22$, Supplementary Fig. S1). During this period, discharges in the Danube ranged from 693 m^3/s to 6197 m^3/s. In addition to the monthly samples, two flood events with a one-year return period (HQ2015 and HQ2016) were sampled with an increased sampling frequency ($n = 25$) in the Danube and in wells LGS41 and LSG30.

Groundwater samples for micropollutants and standard chemical parameters were taken after pumping 3 well volumes at an abstraction rate of 0.77×10^{-3} m^3/s (van Driezum et al., 2017). A portable Sension + MM150 sensor system (Hach-Lange, Austria) and a portable Profiline multi 3320 sensor system (WTW, Germany) were used in the field to measure temperature, pH, electrical conductivity and dissolved oxygen.

2.3. Chemical analysis

2.3.1. Inorganic and organic parameter analysis

A volume of 250 mL of ground- and surface water was taken in clean plastic bottles which were cooled at 4 °C and immediately transported to the lab. Anion and cation analyses were performed using ion chromatography. Absorption photometry was used to measure ammonium and nitrite (Supplementary Table S1).

2.3.2. OMP analysis and quantification

For this study, seven OMPs were selected based on their potential to serve as indicator substances for wastewater sources (Jekel et al., 2015). One-liter samples were filled in cleaned, clear glass bottles and transported to the lab in cooling boxes at 4 °C immediately. All samples were stored at 4 °C until analysis. Analysis of OMPs by solid phase extraction (SPE) followed by high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was performed using the method described in van Driezum et al. (2017). The LC-MS/MS system consisted of a Primaide HPLC with 1210 Autosampler (Hitachi High Technologies, USA) coupled to a hybrid triple quadrupole linear trap ion trap tandem mass spectrometer Q Trap 3200 (Applied Biosystems, Foster City, CA, USA) equipped with electrospray ionization (ESI) source operated in negative- and positive-ion mode. Details on analyte data and precursor-product ion can be found in Supplementary Table S2. The compounds were identified by retention time match and their specific HPLC-MS/MS transitions. The recoveries and LOQs of the

different compounds can be found in the supporting information (Supplementary Table S3).

2.4. Mixing ratios of Danube and backwater river in PGAW3

In order to give an indication of the behavior of the OMPs, mixing ratios of the Danube and backwater river in PGAW3 were calculated based on daily 2-D groundwater flow simulations during the study period. As stated previously, river water enters the backwater when water levels exceed a certain threshold value. When water levels in the backwater rise, groundwater flow paths towards PGAW3 might change and water of a different composition can be extracted in PGAW3. The 2-D variable saturated groundwater flow model was previously developed for the studied transect (Naus, 2015). The mean deviation between measured and simulated groundwater levels was 0.2 m at maximum after calibration. For calculating the daily mixing ratios the simulated inflow rates were summed along river beds of the Danube and the backwater, respectively, over the full simulation time. With the results from the mixing ratio procedure, OMP concentrations were calculated in PGAW3. Calculations were made for 6 mixing scenarios. Two scenarios with solely Danube water and solely backwater river water were taken as extremes, whereas the scenarios with a mixture of both sources were more likely to occur (Supplementary Table S4, van Driezum et al., 2018). For CBZ, no degradation is assumed (Clara et al., 2004). SMZ removal under oxic conditions is slow and only partial (Table 4). For simplification, no degradation was assumed. Danube and backwater river concentrations were taken for the calculation of CBZ and SMZ. Calculated BTri concentrations were based on concentrations measured in LSG2 and in the backwater river.

2.5. Flow intervals and load calculation

The dataset was divided in classes depending on flow intervals for comparison of OMP loads in the Danube during the studied period. The flows were categorized according to the percentage of exceedance, as follows (US Environmental Protection Agency, 2007): *flood events* (0–2.5%), *high flows* (2.5–5%), *moist conditions* (5–10%), *mid-range flows* (10–40%), *dry conditions* (40–70%) and *low flows* (70–100%). The calculation of the cumulative frequency was based on hourly mean discharges measured at the gauging station *Fischamend* from March 2014 till May 2016. The discharges corresponding to these intervals are shown in Table 1.

The hourly loads *L* were calculated based on the method using flow intervals, as described by Zoboli et al. (2015).

2.6. Data analysis and statistics

Correlation analyses of micropollutants with hydrological, physical and chemical variables were performed using the Pearson product correlation and the Spearman rank order correlation. A *P*-value of 0.05 was set as a significance threshold for all parameters. One-way analysis of variance (ANOVA) tests and the associated *post-hoc* Tukey's range test were used (function aov and TukeyHSD) to determine if any significant difference existed between the OMP concentrations in the surface water samples and in the groundwater samples. All statistical analyses were performed using R 3.1.1., partly using the *Hmisc* package (v. 4.1.1). All graphs were prepared using Grapher 10.5 (Golden Software, Colorado, USA).

3. Results

3.1. Hydrological and chemical characterization of surface water and groundwater

The Danube showed strong fluctuations during the studied period with changes in water levels as high as 6 m (Supplementary Fig. S2). Continuously low flow periods were observed early 2014 and from July 2015 till January 2016. Discharges in these periods were mostly below 1500 m^3/s. Higher discharges were observed during spring and summer 2014 and in spring 2015 and 2016. The discharges on the days when seasonal sampling took place ranged from 862 m^3/s to 2960 m^3/s. Water level fluctuations during HQ2015 were 2.6 m and discharges ranged from 2500 m^3/s to 5200 m^3/s. Water level fluctuations during HQ2016 (4.8 m) were almost twice as high and discharges ranged from 1000 m^3/s to 5200 m^3/s.

The backwater river was only connected to the Danube during flood events. During these events, water levels increased up to 3 m, much less than in both the Danube and the groundwater (Supplementary Fig. S2).

Water level fluctuations in all groundwater monitoring wells were nearly 4 m over the entire study period. During both flood events, groundwater levels close to the river fluctuated >1 m during HQ2015 and >2 m during HQ2016. Long-term oxygen concentrations of PGAW3 (minimum of 1.9 mg/L, maximum of 4.6 mg/L, data not shown) and the measured oxygen concentrations during sampling showed conditions in the aquifer were oxic. Average nitrate concentrations (Supplementary Table S5) in the wells between the Danube and PGAW3 were well above 5 mg/L, further confirming oxic conditions. Manganese and iron concentrations taken in the PGA were predominantly below 0.1 mg/L (Mayr et al., 2014).

The portion of groundwater at well PGAW3 coming from the backwater river showed large variations from January 2014 till May 2016 (Supplementary Fig. S3). By the end of June and beginning of July 2014, the water flowing into the aquifer was almost solely coming from the backwater river. From March to July 2015 on the contrary, most of the groundwater originated from the Danube except during HQ2015 when the proportion of backwater river increased shortly to 20% (Supplementary Fig. S3).

3.2. OMP occurrence in surface waters

3.2.1. Seasonal sampling

All seven OMPs were found in both the Danube and the backwater river (Table 2) with substantial higher detection frequencies for all compounds in the Danube. Highest concentrations in the Danube were found for BTri, ranging from 58 ng/L up to 402 ng/L. The concentrations of CBZ and SMZ were 1 order of magnitude lower, ranging from 7.48 ng/L to 42.0 ng/L and from 1.86 ng/L to 15.1 ng/L respectively. Although the detection frequencies of BTri and CBZ in the backwater river were high, the concentrations were substantially lower than in the Danube (Fig. 2). Although a negative correlation with water levels was present for these compounds in the Danube ($r = -0.58$, $P = 0.005$ for BTri and $r = -0.62$, $P < 0.005$ for CBZ), the backwater showed a positive correlation between water levels and the compounds ($r = 0.87$, $P < 0.005$ for BTri and $r = 0.87$, $P < 0.005$ for CBZ, Pearson correlation). SMZ had a much lower detection frequency in the backwater river than BTri and CBZ. No clear seasonal patterns could be seen for BTri, CBZ and SMZ (Fig. 2).

Bezafibrate and diclofenac were frequently measured in the Danube, but had much lower detection frequencies in the backwater river.

Table 1
Range in discharge at Fischamend for the flow intervals. *n* is the amount of water quality samples per flow interval.

	Low flows (Q_L)	Dry conditions (Q_d)	Mid-range flows (Q_m)	Moist conditions (Q_{mo})	High flows (Q_h)	Flood events (Q_{FL})
Discharge (m^3/s)	<1250	1250–1700	1700–2500	2500–3000	3000–3500	>3500
N	*10*	*6*	*6*	*7*	*10*	*8*

Table 2
Min-max concentration values and min-max values of the ratios between the OMPs in the surface- and groundwater samples during the seasonal sampling. The values in brackets for the concentrations represent the detection frequencies; the italic values in brackets for the ratios represent the average values. Ratios were only given between values above LOQ and when >30% of the concentrations could be determined. A statistically significant correlation ($P < 0.05$, based on the Pearson correlation) between the compounds was indicated by an asterisk. Values in meters are the distances to the Danube.

Seasonal sampling	Danube 0 m	LSG41 10 m	LSG40 13 m	LSG30 24 m	LSG2 283 m	PGAW3 551 m	LSN28 704 m	LSG11 782 m	Backwater 882 m
Concentration in ng/L									
Benzotriazole	58.0–402 (22/22)	31.6–201 (22/22)	22.0–171 (22/22)	19.4–150 (22/22)	n.d.-4.65 (10/22)	<LOQ-12.9 (22/22)	LOD-10.9 (19/22)	<LOQ-31.3 (22/22)	LOD-83.4 (21/22)
Carbamazepine	7.48–42.0 (22/22)	8.05–27.9 (22/22)	7.66–26.1 (22/22)	9.58–26.1 (22/22)	7.79–19.8 (22/22)	5.70–13.7 (22/22)	2.38–6.05 (22/22)	1.82–14.4 (22/22)	<LOQ-10.8 (22/22)
Sulfamethoxazole	1.86–15.1 (22/22)	2.30–11.0 (22/22)	1.76–11.9 (22/22)	2.23–13.3 (22/22)	1.21–6.51 (22/22)	<LOQ-3.58 (22/22)	n.d.-1.42 (17/22)	n.d.-2.79 (7/22)	n.d.-3.31 (4/22)
Bezafibrate	LOD-10.1 (21/22)	n.d.-<LOQ (3/22)	n.d.-<LOQ (1/22)	n.d.-<LOQ (2/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-3.94 (3/22)
Bisphenol A	n.d.-155 (4/22)	n.d. (0/22)	n.d.-32.2 (1/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-<LOQ (1/22)
Diclofenac	<LOQ-88.2 (22/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-LOD (1/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-22.6 (3/22)
Ibuprofen	n.d.-7.29 (7/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d. (0/22)	n.d.-LOD (1/22)
OMP ratios (–)									
Benzotriazole/carbamazepine	3.49–15.7 (10.5) *(r=0.85)	3.21–9.31 (6.42)	2.43–9.98 (6.28) *(r=0.78)	1.97–8.93 (5.89) *(r=0.75)	N/A	0.07–1.35 (0.79)	0.33–2.21 (1.21)	0.18–6.00 (1.71) *r=0.57)	2.28–7.74 (4.88) *(r=0.96)
Benzotriazole/sulfamethoxazole	20.7–75.6 (36.7) *(r=0.80)	9.49–33.9 (18.9) *(r=0.65)	9.84–34.2 (18.5) *(r=0.68)	7.84–32.6 (15.6) *(r=0.50)	N/A	0.75–12.4 (4.59)	N/A	N/A	N/A
Carbamazepine/sulfamethoxazole	2.47–6.00 (3.54) *(r=0.93)	1.75–4.28 (3.00)	1.74–5.17 (3.06) *(r=0.69)	1.30–4.51 (2.74) *(r=0.66)	1.84–7.21 (3.60) *(r=0.56)	2.39–9.63 (4.28)	N/A	N/A	N/A

Similarly to BTri, CBZ and SMZ, no seasonal patterns were observed. Peak concentrations were encountered at the same time as the previously mentioned compounds. Bisphenol A and ibuprofen were only sporadically measured in both surface waters.

3.2.2. Flood event sampling

The same detection frequencies of BTri, CMZ and SMZ in the Danube were observed during both flood events as during seasonal sampling. Concentrations in the Danube were slightly lower during HQ2015 than during seasonal sampling (Fig. 2). The concentrations of BTri, CBZ and SMZ in the Danube measured during HQ2016 were in a similar range as the seasonal samples (Table 3).

Bezafibrate, bisphenol A, diclofenac and ibuprofen were sporadically detected in the Danube during HQ2015. During HQ2016, detection frequencies of these compounds were similar as during the seasonal sampling campaign, although their concentrations were clearly lower during the flood events than during seasonal sampling.

3.2.3. Load calculations

The load of these compounds was calculated in order to gain more understanding of OMP dynamics under different discharges in the Danube. A pearson correlation performed using log-transformed loads of BTri, CBZ and SMZ indeed showed all loads significantly correlated with discharges ($r = 0.58$, 0.77 and 0.66 for BTri, CBZ and SMZ, respectively, with $P < 0.005$) and during HQ2015, they even doubled (Fig. 3).

3.3. OMP attenuation during RBF

3.3.1. Seasonal sampling

BTri, CBZ and SMZ had similar detection frequencies in the groundwater as in the surface water (Table 2). Bezafibrate was only sporadically detected in the groundwater close to the river, with values around the LOQ. Bisphenol A, diclofenac and ibuprofen were not detected in any of the groundwater samples during the seasonal sampling campaigns.

Fig. 4 shows the results of the seasonal sampling for BTri, CBZ and SMZ. As can be seen, the highest OMP concentrations were found for BTri. During the first 24 m of aquifer passage (wells LSG41 (10 m), LSG40 (13 m) and LSG30 (24 m)), BTri concentrations decreased from an average value of 183 ng/L in the Danube to 103 ng/L in LSG30, which was an average removal of 44%. After another 260 m of aquifer passage (LSG2), BTri dropped to an average concentration of 1.42 ng/L and was only detected 10 out of 22 times. In PGAW3, after another 268 m of aquifer passage, BTri remained at a similarly low level and detection frequencies increased simultaneously. The removal in PGAW3 was up to 97%. Concentrations in LSN28 and LSG11 were slightly higher than in PGAW3 and seemed to be influenced by the backwater river. The temporal variations in BTri concentrations seen after 260 m of aquifer passage (LSG2) were substantially lower than in the first 24 m. Fig. 4 shows that the removal of BTri is not constant throughout the year. During an extended period of higher discharges (for example from April 2015 till the end of June 2015), the groundwater in the first meters of aquifer passage had a higher BTri concentration than the Danube.

CBZ was found in all groundwater samples and reached a maximum concentration of 27.9 ng/L in well LSG41, which was closest to the river. Concentrations were stable during the first 24 m of aquifer passage, but a decrease of up to 48% was observed towards PGAW3 (Fig. 2). The results of an ANOVA test further indicated that CBZ was not fully persistent in the PGA. According to these results, LSG41, LSG40 and LSG30 group together ($P = 0.98$), just as LSG2 and PGAW3 ($P = 0.28$). The concentrations and temporal variation in LSG2 and PGAW3 were higher than in wells LSN28 and LSG11.

A similar pattern can be seen for SMZ. The concentrations in the first 24 m of the aquifer passage stayed stable (1.76 ng/L–15.1 ng/L) and decreased towards PGAW3 (up to 56% attenuation). The temporal variability in SMZ concentrations simultaneously decreased with longer groundwater residence times. In LSN28 and LSG11, SMZ was only sporadically above the LOQ.

3.3.2. Flood event sampling

Samples were taken with a higher frequency during two flood events (HQ2015 and HQ2016) and analyzed for the studied OMPs (Table 3). It can be seen that the detection frequencies of BTri, CBZ and SMZ in the groundwater were similar during both events and comparable with the seasonal sampling campaign. No statistically significant difference between the seasonal and flood sampling events (ANOVA, $P > 0.05$) was observed for all three compounds (Fig. 2). Concentrations of the three compounds at LSG41 (10 m from the riverbank) and LSG30 (24 m from the riverbank) were even higher than in the

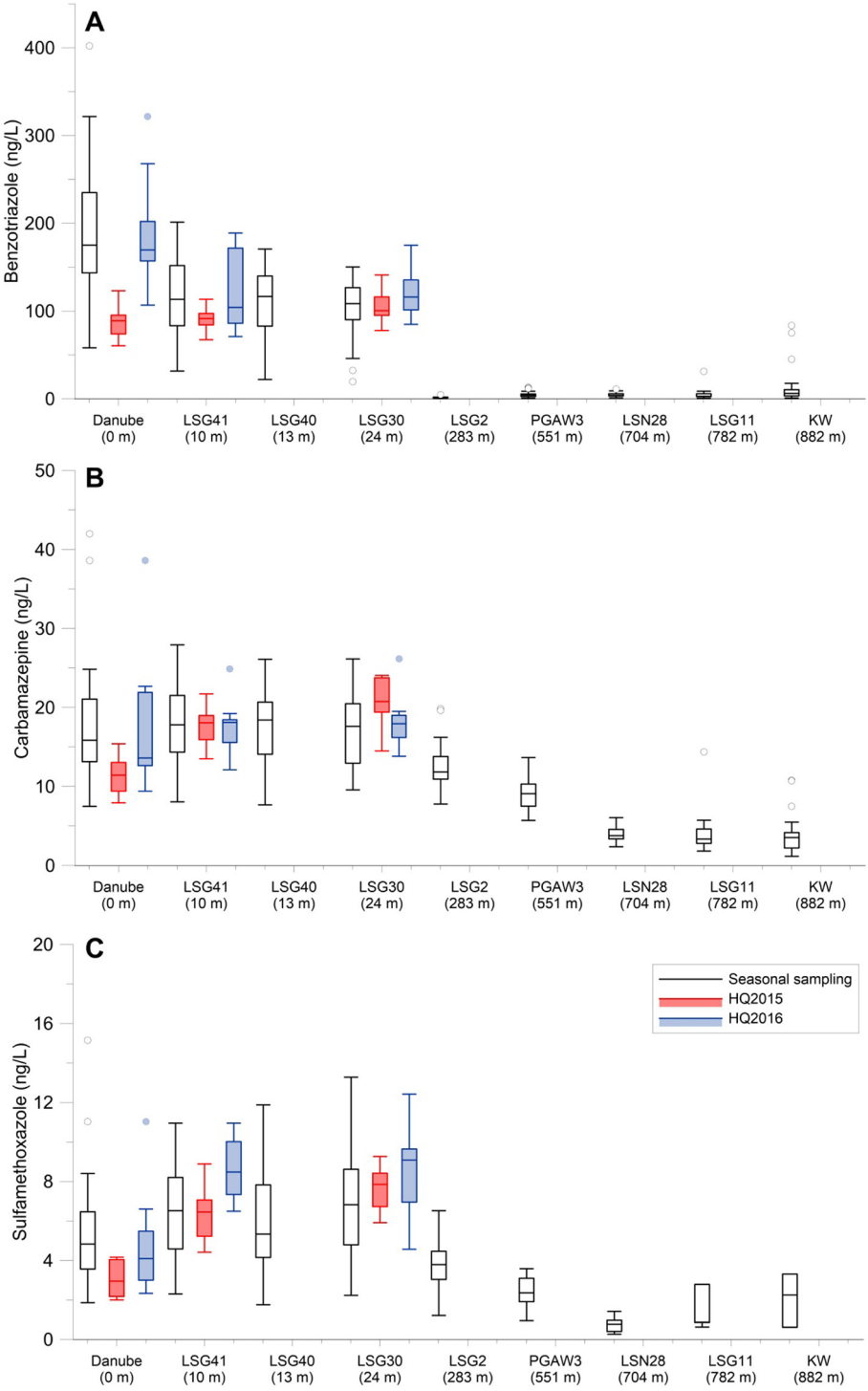

Fig. 2. Boxplots of A) benzotriazole, B) carbamazepine and C) sulfamethoxazole. White boxes represent the seasonal samples, red boxes the HQ2015 samples and blue boxes the HQ2016 samples. The boxes cover the 25th to 75th percentile, the line within the boxes the median and whiskers the 10th to 90th percentile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Danube during both events. This was especially seen for the more conservative compounds CBZ and SMZ and was most evident during HQ2015. A much lower attenuation of BTri was observed during HQ2015 (30%) and HQ2016 (0%) than during seasonal sampling (44%) after 24 m of groundwater passage. In contrast to the seasonal sampling and HQ2016, where BTri significantly correlated with CBZ and SMZ in both the Danube and the groundwater, this was only the case in the Danube during HQ2015. CBZ and SMZ significantly correlated with each other during all events in both surface- and groundwater.

Of the other measured OMPs, only bezafibrate and diclofenac were detected in the groundwater up to a distance of 24 m (LSG30) during HQ2016.

3.4. OMP ratios

In order to assess the fate of biodegradable compounds, their ratios can be calculated (Scheurer et al., 2011). The ratios between BTri, CBZ and SMZ concentrations were calculated for all samples taken in the

Table 3
Min-max values of the water table difference and gradient and of the OMPs in the surface- and groundwater samples during HQ2015 and HQ2016. The values in brackets represent the detection frequency. Values in meters are the distances to the Danube.

	Danube		LSG41		LSG30	
	0 m		10 m		24 m	
	HQ2015	HQ2016	HQ2015	HQ2016	HQ2015	HQ2016
Water table difference (m a.A.)	150.10–152.74	147.95–152.78	149.63–150.70	147.80–149.74	149.62–150.71	147.80–149.71
Gradient (%)	n.a.	n.a.	5.77–20.4	1.50–31.0	−0.08-0.10	0.01–0.19
Benzotriazole (ng/L)	60.4–123 (15/15)	107–268 (9/9)	67.3–118 (15/15)	70.9–189 (9/9)	77.8–141 (15/15)	85.1–175 (9/9)
Carbamazepine (ng/L)	7.93–15.4 (15/15)	9.40–22.7 (9/9)	13.5–21.7 (15/15)	12.1–19.2 (9/9)	14.5–30.6 (15/15)	13.8–19.5 (9/9)
Sulfamethoxazole (ng/L)	2.00–4.16 (15/15)	2.33–6.60 (9/9)	4.41–8.88 (15/15)	6.49–10.3 (9/9)	5.91–9.26 (15/15)	4.56–9.95 (9/9)
Bezafibrate (ng/L)	n.d.-0.58 (5/15)	3.58–5.92 (9/9)	n.d.	n.d.-<LOQ (2/9)	n.d.	n.d.-<LOQ (3/9)
Bisphenol A (ng/L)	n.d.-93.2 (1/15)	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac (ng/L)	6.90–21.6 (15/15)	31.0–50.2 (9/9)	n.d.	n.d.-LOQ (4/9)	n.d.	n.d.-8.60 (5/9)
Ibuprofen (ng/L)	n.d.	LOD-<LOQ (9/9)	n.d.	n.d.	n.d.	n.d.

river and the groundwater wells for both the seasonal sampling (Table 2) and the two flood events (not shown). For BTri, it can be seen that the ratios of this compound with either CBZ or SMZ decrease from the river towards the groundwater. During the first 24 m of aquifer passage, the ratios between BTri and CBZ or SMZ stayed stable and the compounds correlated significantly with each other. When moving towards PGAW3, the ratios decrease and a relatively higher amount of CBZ and SMZ is found. In contrast, the ratios between CBZ and SMZ stayed stable from the river towards PGAW3, which was confirmed by ANOVA. The compounds also correlate significantly with each other in both the groundwater and the surface water samples.

The ratios calculated for the flood events were not consistently different than during seasonal sampling. OMP concentrations and the corresponding ratios in the Danube during HQ2015 were substantially different from HQ2016 and the seasonal sampling while the difference in concentrations of the compounds was not similar. During groundwater infiltration, the OMP ratios only differed significantly ($P < 0.05$, ANOVA) between HQ2015 and HQ2016, with higher ratios during HQ2016.

4. Discussion

4.1. The behavior of OMPs during RBF along a large dynamic river

Concentrations of BTri, SMZ, bezafibrate, diclofenac, bisphenol A and ibuprofen found in the Danube samples were consistent with previous measurements in the Danube (Loos et al., 2017, 2010b) and other large European rivers (Ruff et al., 2015; Sjerps et al., 2017; Wolschke et al., 2011). The median concentration of CBZ was also consistent with previous measurements in the Danube (Loos et al., 2010b) but slightly lower than in the Rhine (Ruff et al., 2015) and much lower than in the river Thames (Nakada et al., 2017). OMP concentrations and detection frequencies were generally much lower in the backwater river than in the Danube. Since the backwater river can be seen as a series of connected ponds fed by groundwater and precipitation rather than as a river, an increase in OMP concentrations was only detected during irregular inflows of Danube water.

Bezafibrate, bisphenol A, diclofenac and ibuprofen were not found in the oxic groundwater. These compounds have been known to be fully removed during RBF under oxic conditions (Burke et al., 2014b;

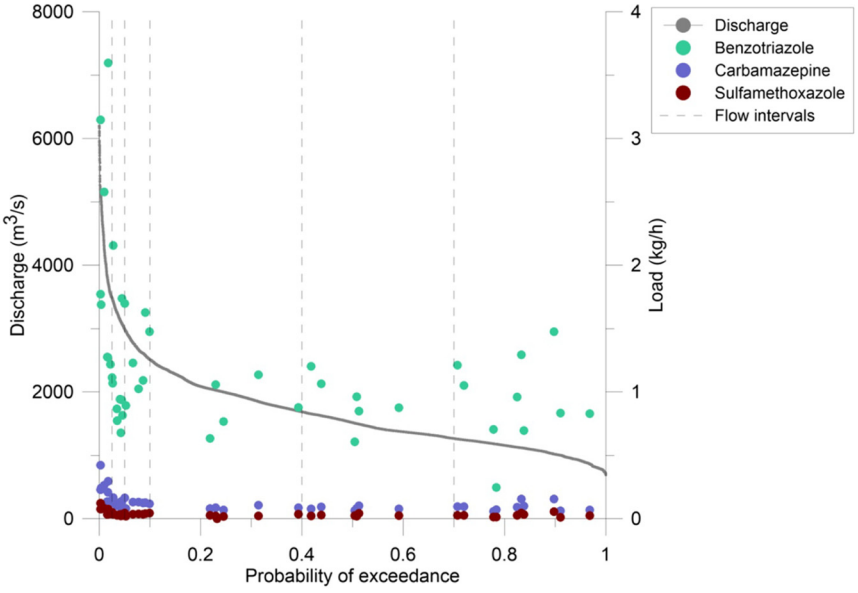

Fig. 3. Flow duration curve showing the flow intervals and the corresponding loads during these days. BTri is shown in green, CBZ in blue and SMZ in dark red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

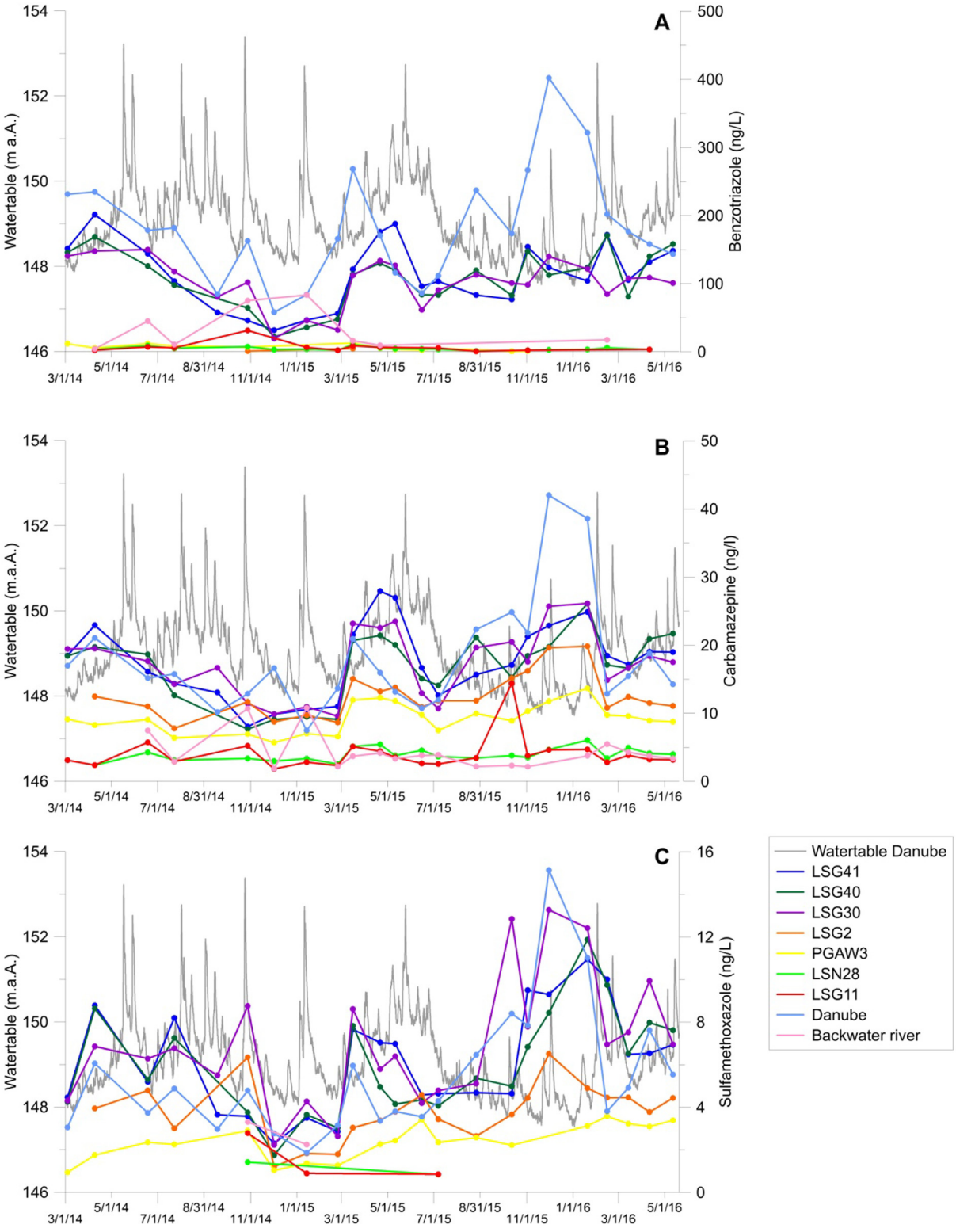

Fig. 4. Seasonal sampling results for **A)** BTri, **B)** CBZ and **C)** SMZ. Concentrations were given for both surface waters and all monitoring wells.

Heberer et al., 2004; Rauch-Williams et al., 2010; Wiese et al., 2011). Concentrations of BTri, CBZ and SMZ in the groundwater were in a similar range as in other studies (Huntscha et al., 2013; Loos et al., 2010a; Scheurer et al., 2011), but they were attenuated differently. Previously, they were known to be either fully or mostly persistent under different hydrogeological conditions (Table 4). Concentrations of these compounds in the drinking water abstraction well were far below (provisional) drinking water guideline values derived in several EU countries (Baken et al., 2018).

As for BTri, most of the degradation was found to take place in the first few meters of the aquifer. In contrast to our results, BTri was previously found to be never fully removed except for the study of Reemtsma et al. (2010), which had unstable redox conditions in the aquifer. We found an average removal of 44% after 24 m of aquifer passage. A similar

Table 4
Literature values for attenuation of BTri, CBZ and SMZ.

Compound	Removal	Aquifer properties		Travel time	Hydrological characteristics	Comments
		Hydrogeology	Redox conditions			
Benzotriazole	No removal [b), d), h)]	Unconsolidated sandy gravels [b)], predominantly sand [h)], undisturbed sandy core [d)],	Oxic [b), h)], fluctuation between oxic and anoxic [d)]	67–113 h [b)], 1–3 months [h)]	Q_{max} 160 m^3/s [b)], undisturbed core [d)], river Ijssel [h)]	Redox dependent removal, probably only full removal under anoxic conditions
	Partial removal [a), e), f), g), i)]	Unconsolidated sandy gravels [a)], microcosms with aquifer sediment [f)], alluvial aquifer[i)]	Oxic [a)], fluctuation between oxic and anoxic [e)]	7 h–3d 4 h transect A; 0–35 h transect B [a)],	Q_{max} 160 m^3/s [a)], microcosm [f)], river Rhine [g)], Q_{max} 100 m^3/s [i)]	
	90% [c)]	Predominantly sand [c)]	Fluctuation between oxic and anoxic [c)]	4–5 months [c)]	Lake [c)]	
Carbamazepine	Persistent [a), b), e), g), h), k), i), m), n), o), p)]	Unconsolidated sandy gravels [a), b)], predominantly sand [h), k), p)], sand and gravel [n), o)]	Oxic [a), b), h), n), o)], fluctuation between oxic and anoxic [e), m), o), p)], anoxic [k), o)]	7 h–3d 4 h transect A; 0–35 h transect B [a)], 67–113 h [b)], 1–3 months [h)], 1.65–3.65 years [k)], 7–30 days [n)], 25 days [o)]	Q_{max} 160 m3/s [a), b)], lake [m)], river Rhine [g)], river Ijssel [h)], Q_{max} 100 m^3/s [i)], river Lek [k)], Q_{max} 120 m^3/s [o)], $Q_{average}$ 3300 m^3/s [o)], $Q_{average}$ 106 m^3/s [o)], infiltration ponds [p)]	Overall low removal, probably due to retardation; no removal at high discharges
	Slight attenuation [d), i), l), m)]	Predominantly sand [l), m)], undisturbed sandy core [d)], alluvial aquifer[i)]	Fluctuation between oxic and anoxic [d), l)]	4–5 months [l), m)]	Lake [l), m)], undisturbed core [d)], Q_{max} 100 m^3/s [i)],	
	Degradation [j)]	Predominantly sand [j)]	Fluctuation between oxic and anoxic [j)]	4–5 months [j)]	Lake [j)]	
Sulfamethoxazole	Rather persistent [a), b), n)]	Unconsolidated sandy gravels [a), b)], sand and gravel [n)]	Oxic [a), b), n)],	7 h–3d 4 h transect A; 0–35 h transect B [a)], 67–113 h [b)], 7–30 days [n)]	Q_{max} 160 m3/s [a), b)],	Redox dependent removal; slow and only partial under oxic conditions, up to full removal under anoxic conditions
	Partial removal [h), j), l), q), r)]	Predominantly sand [h), j), l), q), r)]	Oxic [h)], fluctuation between oxic and anoxic [j), l), q), r)]	4–5 months [j), l), q), r)], 1–3 months [h)]	Lake [j), l), q), r)], river Ijssel [h)],	
	Full removal [j), k), q), r)]	Predominantly sand [j), k), q), r)]	Fluctuation between oxic and anoxic [j), q), r)], anoxic [k)]	1.65–3.65 years [k)], 4–5 months [j), q), r)],	Lake [j), q), r)], river Lek [k)]	

a) (Huntscha et al., 2013), b) (Huntscha et al., 2012), c) (Reemtsma et al., 2010), d) (Burke et al., 2014a), e) (Kahle et al., 2009), f) (Liu et al., 2013), g) (Scheurer et al., 2011), h) (Bertelkamp et al., 2016a), i) (Epting et al., 2018), j) (Henzler et al., 2014), k) (Hamann et al., 2016), l) (Wiese et al., 2011), m) (Heberer et al., 2004), n) (Storck et al., 2012), o) (Hoppe-Jones et al., 2010), p) (Massmann et al., 2006), q) (Heberer et al., 2008), r) (Grünheid et al., 2005).

removal was found under similar hydrogeological characteristics as at our site at the Thur river (Huntscha et al., 2013). In contrast to Huntscha et al. (2013), BTri was almost fully removed at the drinking water abstraction well at our site. This difference can be explained because we sampled at wider distances (and higher residence times) from the river and therefore observed significantly more BTri removal. Other studies showed no significant removal under oxic conditions (Table 4), e.g., as shown by Bertelkamp et al. (2016a) in a low conductive aquifer, even after up to 4 months of groundwater travel times. This suggests that travel time combined with oxic conditions alone does not explain BTri removal. Reported literature found no or very little removal of BTri in the environment. Under conditions with a highly active microbial community, like a WWTP, partial removal was shown (e.g. Mazioti et al., 2015). Sorption was found to be negligible (Yu et al., 2009). In our study, a high degree of river-groundwater interaction was apparent due to the high conductivity of the aquifer. Furthermore, the microbial activity was found to be relatively high (van Driezum et al., 2018). Based on previous studies, we therefore conclude that biodegradation was the main mechanism responsible for the high BTri removal. Highly conductive RBF systems, such as the PGA and along the river Thur (Huntscha et al., 2013), are more favorable to biodegradation of compounds like BTri.

CBZ has generally been classified as persistent (Table 4). Some attenuation was sporadically found, e.g. studies from lake Tegel and lake Wannsee in Berlin showed that some degradation of CBZ can occur during aquifer passage (Burke et al., 2014a; Henzler et al., 2014; Wiese et al., 2011). Bertelkamp et al. (2016a) did not find attenuation of CBZ directly in the field, but column tests indicated some removal of the compound. Our study showed that CBZ concentrations are stable during the first 24 m of aquifer passage but then slightly decreased during an extra 527 m of aquifer passage towards PGAW3. A possible explanation for this decrease in CBZ could have been mixing of the Danube and backwater river at PGAW3. This was only partially confirmed by the mixing ratio calculations (Supplementary Table S4). The proportion of backwater river must be between 30 and 60% assuming a conservative behavior of CBZ, but this is not very likely for our system (Supplementary Fig. S3).

Also SMZ was only partially removed under oxic conditions. A similar behavior was shown along the river Rhine (Storck et al., 2012), although concentrations were slightly lower in the PGA. A full removal of SMZ during RBF was previously found only under anoxic conditions (Table 4). Mixing with backwater river water could again only account for part of the decrease in concentration of SMZ as shown for CBZ.

Based on the marker ratios during seasonal sampling, the difference in attenuation between BTri on one hand and CBZ and SMZ on the other hand was clearly visible, with the latter two being similar. Several studies have shown differences in biodegradation and retardation of CBZ and SMZ (Bertelkamp et al., 2016b; Hamann et al., 2016; Henzler et al., 2014; Nham et al., 2015). Since no distinction was made between retardation and biodegradation in this study only an indication of a similar rate of attenuation between CBZ and SMZ can be given.

4.2. Do flood events change the presence and behavior of OMPs in surface and groundwater?

As was seen in Van Driezum et al. (2018), the flood events had an influence on the microbial activity and increased cell counts in the Danube. It was expected, that OMP concentrations in the Danube, on the contrary, were lower during the flood events than during seasonal sampling due to dilution. CBZ for example, is not removed during wastewater treatment (Joss et al., 2005; Radjenović et al., 2009; Zhang et al., 2008), so its load into the river is expected to be stable even when processes like combined sewer overflow (CSO) occur. BTri and SMZ are partly removed during wastewater treatment (Huntscha et al., 2014; Radjenović et al., 2009) and their loads might therefore slightly increase during flood events due to CSOs. A stable load, especially of CBZ, was however not seen in our study. Since CSOs could not be primarily responsible for the increase in OMP loads, another explanation was proposed.

During flood events, total suspended solids (TSS) can be mobilized. The TSS concentration, and also in stream phosphorus (P) concentrations can therefore increase significantly, as was seen previously in the Danube (Zessner et al., 2005; Zoboli et al., 2015). A significant trend was shown between discharge of the Danube and the TSS concentration (Nachtnebel et al., 1998). Some OMPs are partly sorbed to TSS and can desorb under conditions like flooding (da Silva et al., 2011). Consequently, the amount that can desorb is higher during flood events and can lead to an increase in OMP loads (Rivetti et al., 2015). The positive relationship of CBZ concentration (but also other pharmaceuticals) to phosphorus dynamics and TSS was also shown by Acuna et al. (2015). An increased and extended influence of the Danube on the microbial compartment of the groundwater was observed during HQ2016 as compared to HQ2015 (van Driezum et al., 2018), due to the higher increase in river water levels during the event. Because of the influence of flood events on the microbial compartment, we expect that OMP concentrations could be similarly influenced by the infiltrating surface water. It was shown that groundwater concentrations of BTri, CBZ and SMZ during the flood events slightly increased and were even higher than in the surface water. A similar increase in OMP concentrations in groundwater was also observed by Huntscha et al. (2013) along the river Thur after flood events. We observed higher OMP concentrations in the Danube and the groundwater prior to HQ2016 and to a lesser extent prior to HQ2015 relative to periods without flood events. During these events, more surface water can enter the aquifer, i.e. during HQ2015 and HQ2016 over 3 and 24 times more “fresh” water respectively can enter the aquifer during the flood peak than during the days prior to the peak as can be calculated following the procedure of Ubell (1987). This “fresh” surface water with lower concentrations mixes with older groundwater with higher OMP concentrations. Mixing occurs at a slower pace than the flow velocities during these events. This can explain why OMP concentrations reached further into the aquifer and were higher in groundwater than in the Danube during the flood events, even more so during HQ2016 than during HQ2015.

Similar to bacterial abundance (van Driezum et al., 2018), an increase of several OMPs was found in the groundwater up to 24 m away from the river during HQ2016. Bezafibrate and diclofenac were observed in the groundwater, although no correlation was found with groundwater flow velocity. Although no measurements were taken in the drinking water abstraction well during the flood events, we expect a negligible impact of the river on the groundwater quality in the abstraction well at 550 m from the river. This was supported by the lack of substantial variations in OMP concentrations in the drinking water abstraction well throughout the entire study period. The observation wells closer to the river however did show an extended impact of the river on groundwater quality. Drinking water abstraction wells that would be located closer to the river in highly conductive RBF systems can therefore be under direct stress during flood events. In these cases, more intensive monitoring of OMPs is proposed during flood events.

5. Conclusion

The results show that drinking water abstraction wells in particular close to the river and under oxic conditions can be vulnerable to an extended contamination during flood events, even from highly degradable compounds.

In contrast to previous studies, this study showed that BTri is almost fully removed by the time it reaches the drinking water abstraction well. CBZ and SMZ are attenuated to a certain extent, since mixing of groundwater with low-concentrated backwater river could only partly explain the decrease of these compounds. A similar rate of attenuation could be presumed for CBZ and SMZ. Several marker OMPs (e.g. bezafibrate,

diclofenac and ibuprofen) were not detected in the aquifer under oxic conditions.

Unexpectedly, the results during the flood events showed that most of the OMP concentrations in the Danube were similar as during the seasonal sampling period.

The load of BTri, CBZ and SMZ in the Danube was higher, possibly due to an increase in TSS in the river or to the inflow of the Donaukanal in this section of the Danube. Groundwater concentrations of BTri, CBZ and SMZ during the flood events were higher than in the Danube and reached further into the aquifer, in comparison with seasonal sampling. During the flood in 2016, highly degradable compounds such as diclofenac and bezafibrate could enter the aquifer up to a distance of 24 m from the river and BTri was significantly less attenuated than during the seasonal sampling period.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.08.300.

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