



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

Inge H. van Driezum<sup>a,b,1</sup>, Alex H.S. Chik<sup>c</sup>, Stefan Jakwerth<sup>d,1</sup>, Gerhard Lindner<sup>a,d,1</sup>, Andreas H. Farnleitner<sup>b,e,f,1</sup>, Regina Sommer<sup>d,1</sup>, Alfred Paul Blaschke<sup>a,b,1</sup>, Alexander K.T. Kirschner<sup>d,\*,1</sup>

<sup>a</sup> Institute of Hydraulic Engineering and Water Resources Management, Technische Universität Wien, E222/2, Karlsplatz 13, 1040 Vienna, Austria

<sup>b</sup> Centre for Water Resource Systems, Technische Universität Wien, Karlsplatz 13, 1040 Vienna, Austria

<sup>c</sup> Department of Civil and Environmental Engineering, University of Waterloo, 200 University Avenue West, N2L 3G1 Waterloo, ON, Canada

<sup>d</sup> Institute for Hygiene and Applied Immunology, Water Hygiene, Medical University Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria

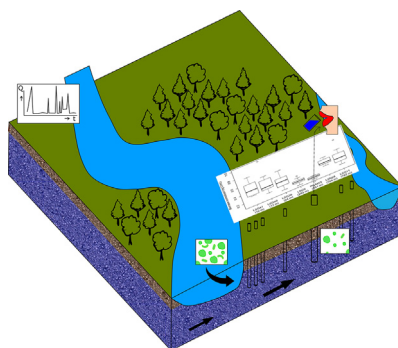
<sup>e</sup> Research Group Environmental Microbiology and Molecular Diagnostics, Institute for Chemical, Environmental and Biological Engineering, 166/5/3, Technische Universität Wien, Gumpendorferstrasse 1a, 1060 Vienna, Austria

<sup>f</sup> Department for Water Quality and Health, Karl Landsteiner University for Health Sciences, Dr. Karl Dorrek Straße 30, 3500 Krems, Austria

### 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

\* Corresponding author.

E-mail addresses: [driezum@hydro.tuwien.ac.at](mailto:driezum@hydro.tuwien.ac.at) (I.H. van Driezum), [hchik@uwaterloo.ca](mailto:hchik@uwaterloo.ca) (A.H.S. Chik), [stefan.jakwerth@meduniwien.ac.at](mailto:stefan.jakwerth@meduniwien.ac.at) (S. Jakwerth), [lindner@hydro.tuwien.ac.at](mailto:lindner@hydro.tuwien.ac.at) (G. Lindner), [andreas.farnleitner@kl.ac.at](mailto:andreas.farnleitner@kl.ac.at) (A.H. Farnleitner), [regina.sommer@meduniwien.ac.at](mailto:regina.sommer@meduniwien.ac.at) (R. Sommer), [blaschke@hydro.tuwien.ac.at](mailto:blaschke@hydro.tuwien.ac.at) (A.P. Blaschke), [Alexander.kirschner@meduniwien.ac.at](mailto:Alexander.kirschner@meduniwien.ac.at) (A.K.T. Kirschner).

<sup>1</sup> Interuniversity Cooperation Centre Water and Health, [www.waterandhealth.at](http://www.waterandhealth.at), Vienna, Austria.

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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.

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## 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.

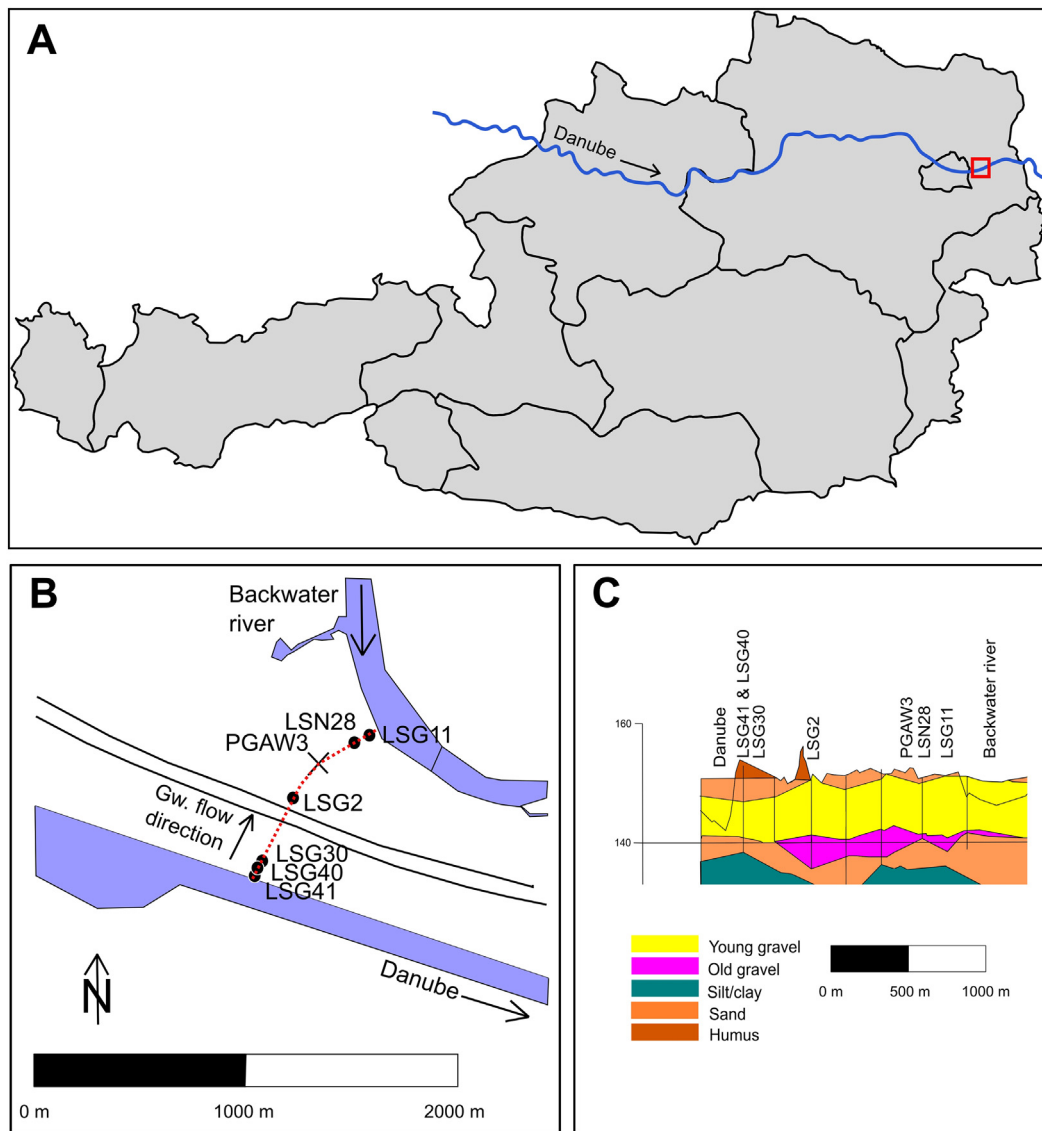
## 2. Materials and methods

### 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<sup>2</sup> (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.s.l.) 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).

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  $5 \times 10^{-4}$  m/s to  $5 \times 10^{-2}$  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),  $\Delta h$  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<sup>3</sup>/s to 6197 m<sup>3</sup>/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 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 <sup>3</sup>H-leucine incorporation

Bacterial <sup>3</sup>H-leucine incorporation (LI) was measured based on protocols of Kirschner and Velimirov (1999) and Simon and Azam (1989). Briefly, <sup>3</sup>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 <sup>3</sup>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:

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

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 (Chemscon 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 ( $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ) 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 ( $2.35 \pm 0.67$  mg/L) and in the backwater river ( $2.17 \pm 0.52$  mg/L) were significantly higher than in the groundwater in all wells (average concentration of  $0.71 \pm 0.27$  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  $1.77 \times 10^6$  to  $6.14 \times 10^6$  cells/mL and from  $2.35 \times 10^6$  up to  $2.45 \times 10^7$  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**

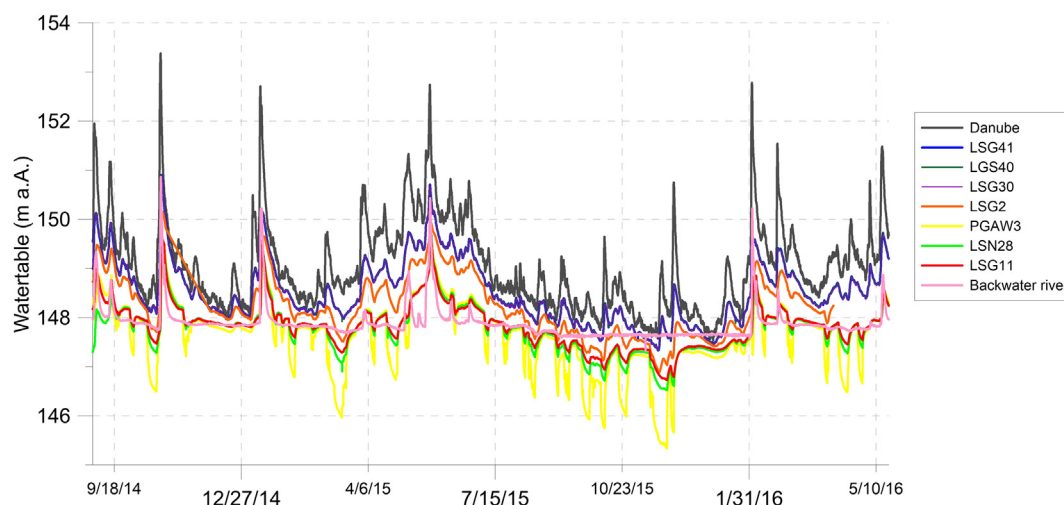
Water table, gradient, temperature and conductivity range of the surface and groundwater bodies during the studied period.

	Water table difference (in m a.A.)	Gradient (%) <sup>a</sup>	Temperature range (in °C)	Electrical conductivity (µS/cm)
Danube	147.54–153.38	n.a.	2.8–23.2	329–882 <sup>b</sup>
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 <sup>b</sup>
LSN28	146.52–150.22	−0.75–0.05	9.8–14.6	533–729 <sup>b</sup>
LSG11	146.73–150.35	−0.26–0.06	9.8–14.5	438–548
Backwater	147.32–150.86	n.a.	0 <sup>c</sup> –31.3	414–639 <sup>b</sup>

<sup>a</sup> 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.

<sup>b</sup> Logger values for this parameter were not available. Instead, hand held measurements taken during the sampling campaigns were used.

<sup>c</sup> 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  $4.64 \times 10^4$  cells/mL up to  $4.04 \times 10^5$  cells/mL (Table 2 and Fig. 3).

They were in a similar range as reported by Alfreyder 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 Flat-head 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  $7.87 \times 10^{-3}$  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 ( $\text{HCO}_3^-$  and  $\text{Cl}^-$ ) 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  $4.18 \times 10^{-6}$  in PGAW3 up to  $1.64 \times 10^{-3}$  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 (Alfreyder 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.

Parameter	Danube	LSG41	LSG40	LSG30	LSG2	PGAW3	LSN28	LSG11	Backwater
	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)
NH <sub>4</sub> -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 <sub>3</sub> <sup>-</sup> (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)	$3.78 \times 10^6$ ( $1.77 \times 10^6$ – $6.14 \times 10^6$ )	$1.99 \times 10^5$ ( $1.35 \times 10^5$ – $3.08 \times 10^5$ )	$1.89 \times 10^5$ ( $9.77 \times 10^4$ – $4.04 \times 10^5$ )	$1.55 \times 10^5$ ( $5.83 \times 10^4$ – $2.94 \times 10^5$ )	$9.14 \times 10^4$ ( $4.64 \times 10^4$ – $1.45 \times 10^5$ )	$7.31 \times 10^4$ ( $4.80 \times 10^4$ – $9.55 \times 10^4$ )	$1.85 \times 10^5$ ( $1.37 \times 10^5$ – $2.41 \times 10^5$ )	$1.69 \times 10^5$ ( $8.21 \times 10^4$ – $2.81 \times 10^5$ )	$8.75 \times 10^6$ ( $2.35 \times 10^6$ – $2.45 \times 10^7$ )
Large cells (cells/mL)	$1.83 \times 10^6$ ( $9.5 \times 10^5$ – $2.68 \times 10^6$ )	$6.09 \times 10^4$ ( $2.65 \times 10^4$ – $1.64 \times 10^5$ )	$5.92 \times 10^4$ ( $2.54 \times 10^4$ – $1.62 \times 10^5$ )	$4.09 \times 10^4$ ( $1.73 \times 10^4$ – $1.02 \times 10^5$ )	$2.30 \times 10^4$ ( $1.51 \times 10^4$ – $4.10 \times 10^4$ )	$1.34 \times 10^4$ ( $9.94 \times 10^3$ – $2.03 \times 10^4$ )	$3.86 \times 10^4$ ( $2.54 \times 10^4$ – $5.18 \times 10^4$ )	$4.47 \times 10^4$ ( $2.16 \times 10^4$ – $1.64 \times 10^5$ )	$2.81 \times 10^6$ ( $1.53 \times 10^6$ – $5.76 \times 10^6$ )
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)	$1.35 \times 10^{-1}$ ( $7.87 \times 10^{-3}$ – $5.93 \times 10^{-1}$ )	$4.27 \times 10^{-4}$ ( $4.35 \times 10^{-5}$ – $1.54 \times 10^{-3}$ )	$3.85 \times 10^{-4}$ ( $3.90 \times 10^{-5}$ – $1.53 \times 10^{-3}$ )	$1.06 \times 10^{-4}$ ( $2.03 \times 10^{-5}$ – $6.39 \times 10^{-4}$ )	$2.26 \times 10^{-5}$ ( $1.13 \times 10^{-5}$ – $5.16 \times 10^{-5}$ )	$1.26 \times 10^{-5}$ ( $4.18 \times 10^{-6}$ – $3.49 \times 10^{-5}$ )	$5.72 \times 10^{-5}$ ( $1.65 \times 10^{-5}$ – $3.26 \times 10^{-4}$ )	$1.16 \times 10^{-4}$ ( $9.73 \times 10^{-6}$ – $1.64 \times 10^{-3}$ )	$1.40 \times 10^{-1}$ ( $1.08 \times 10^{-2}$ – $3.83 \times 10^{-1}$ )

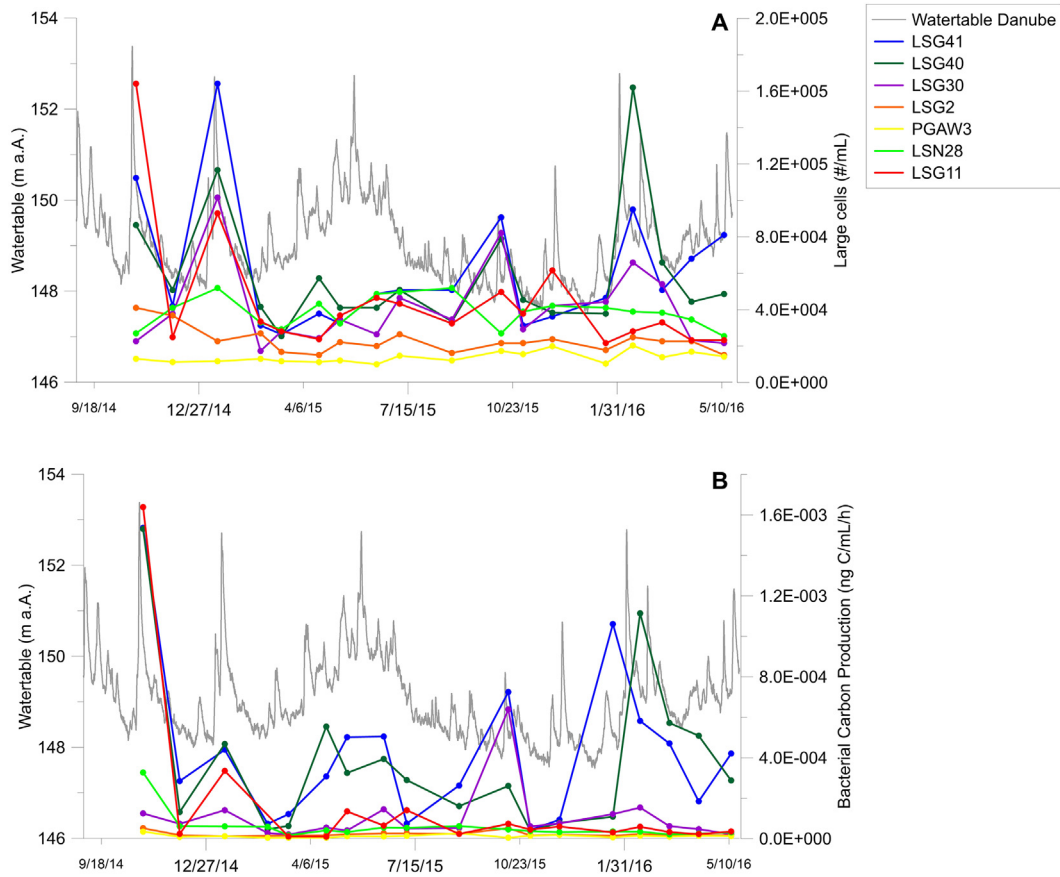


Fig. 3. a) Abundance of large bacterial cells and b) the bacterial carbon production in all groundwater monitoring wells versus the water table of the Danube.

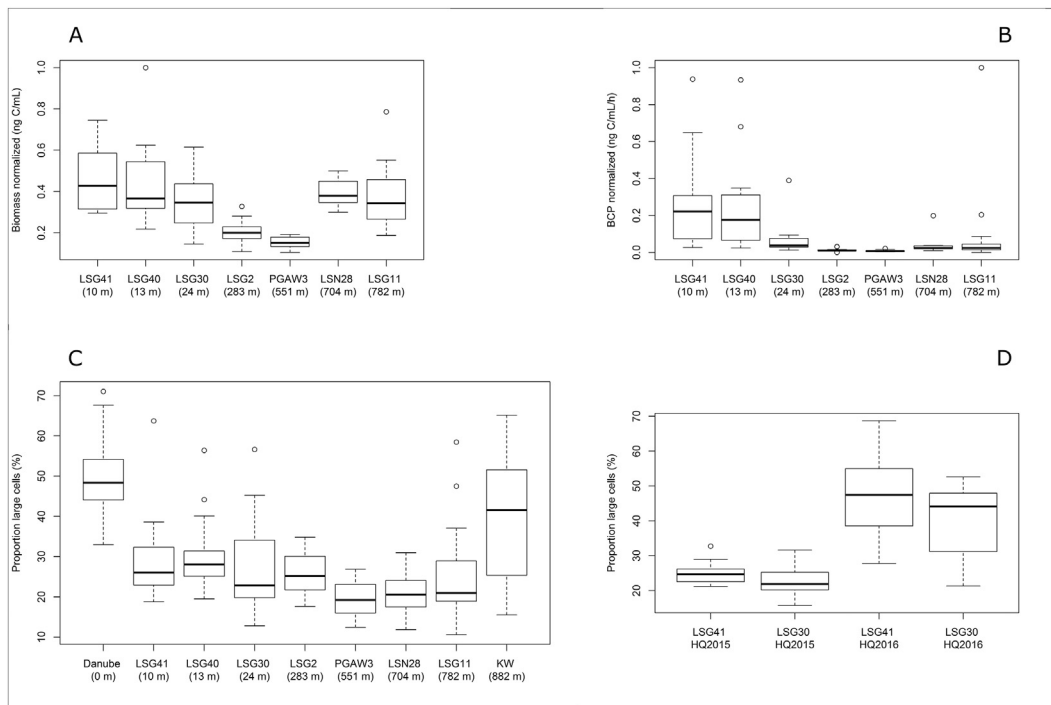


Fig. 4. Boxplots of a) normalized biomass in the groundwater monitoring wells during the monthly sampling campaign, b) normalized BCP in the groundwater monitoring wells during the monthly sampling campaign, c) the proportion of large cells during the monthly sampling campaign and d) the proportion of 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	11.6	12.7
	(11.7–15.2)	(11.0–13.9)	(12.0–13.9)
EC (µS/cm)	345	505	397
	(329–365)	(425–560)	(391–401)
pH	7.94	7.28	7.40
	(7.52–8.05)	(7.05–7.45)	(7.27–7.52)
TOC (mg/L)	2.73	0.90	1.00
	(1.60–3.60)	(0.80–0.90)	(1.00–1.00)
NO <sub>3</sub> <sup>-</sup> (mg/L)	7.44	5.40	6.60
	(6.00–8.10)	(4.30–6.60)	(6.30–6.80)
Bacterial abundance (cells/mL)	n.a.	$2.12 \times 10^5$	$1.06 \times 10^5$
		$(1.46 \times 10^5 - 2.58 \times 10^5)$	$(9.56 \times 10^4 - 1.20 \times 10^5)$
Large cells (cells/mL)	n.a.	$5.29 \times 10^4$	$2.44 \times 10^4$
		$(3.40 \times 10^4 - 6.37 \times 10^4)$	$(1.51 \times 10^4 - 3.38 \times 10^4)$
Proportion large cells (%)	n.a.	25.1	23.1
		(21.2–32.7)	(15.7–31.6)
Biomass (ng C/mL)	n.a.	2.65	1.30
		(1.80–3.22)	(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 <sub>3</sub> <sup>-</sup> (mg/L)	11.0 (9.80–12.0)	9.97 (8.80–11.0)	10.9 (10.0–12.0)
Bacterial abundance (cells/mL)	$3.80 \times 10^6$ ( $1.87 \times 10^6$ – $4.97 \times 10^6$ )	$4.48 \times 10^5$ ( $2.46 \times 10^5$ – $7.32 \times 10^5$ )	$2.63 \times 10^5$ ( $1.88 \times 10^5$ – $3.46 \times 10^5$ )
Large cells (cells/mL)	$3.30 \times 10^6$ ( $1.26 \times 10^6$ – $4.48 \times 10^6$ )	$2.28 \times 10^5$ ( $9.50 \times 10^4$ – $4.32 \times 10^5$ )	$1.08 \times 10^5$ ( $6.26 \times 10^4$ – $1.66 \times 10^5$ )
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

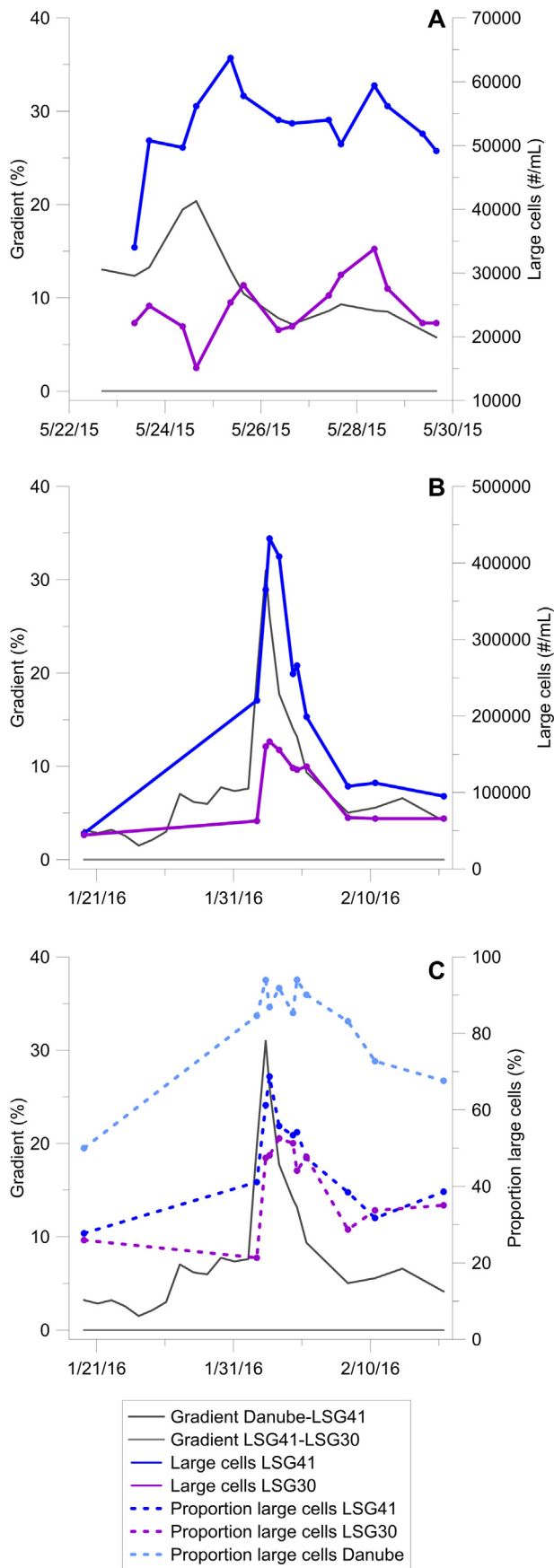
Lin et al. (2012) showed the influence of the temporal dynamics in water level on the community composition of the Hanford aquifer. During higher water levels two groups of Actinobacteria were found which were not present under lower water levels. A distinction between inflow of riverine bacteria, elution 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  $1.67 \times 10^5$  to a maximum of  $7.32 \times 10^5$  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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