Evaluating the performance of water purification in a vegetated groundwater recharge basin maintained by short-term pulsed infiltration events

Birgit Mindl, Julia Hofer, Claudia Kellermann, Willibald Stichler, Günter Teichmann, Roland Psenner, Dan L. Danielopol, Wolfgang Neudorfer and Christian Griebler

ABSTRACT

Infiltration of surface water constitutes an important pillar in artificial groundwater recharge. However, insufficient transformation of organic carbon and nutrients, as well as clogging of sediments often cause major problems. The attenuation efficiency of dissolved organic carbon (DOC), nutrients and pathogens versus the risk of bioclogging for intermittent recharge were studied in an infiltration basin covered with different kinds of macrovegetation. The quality and concentration of organic carbon, major nutrients, as well as bacterial biomass, activity and diversity in the surface water, the porewater, and the sediment matrix were monitored over one recharge period. Additionally, the numbers of viral particles and *Escherichia coli* were assessed. Our study showed a fast establishment of high microbial activity. DOC and nutrients have sustainably been reduced within 1.2 m of sediment passage. Numbers of *E. coli*, which were high in the topmost centimetres of sediment porewater, dropped below the detection limit. Reed cover was found to be advantageous over bushes and trees, since it supported higher microbial activities along with a good infiltration and purification performance. Short-term infiltration periods of several days followed by a break of similar time were found suitable for providing high recharge rates, and good water purification without the risk of bioclogging.

Key words | artificial groundwater recharge, bioclogging, dissolved organic carbon, *E. coli*, nutrient transformation, virus like particles

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INTRODUCTION

Artificial recharge of groundwater is of increasing importance in many areas where groundwater supplies for irrigation, public and industrial use are depleted faster than they are naturally replenished. Common groundwater recharge strategies include the use of surface water via bank filtration (Grünheid *et al.* 2005), well injection (Pyne 1995) and water spreading techniques, especially the use of infiltration ponds (Bouwer 2002). The latter technique has the advantage that the water infiltrating into the shallow aquifer is conditioned by physical (filtration), chemical (e.g. adsorption, precipitation/dissolution) and biological processes (Wotton 2002), where the biological cleaning capacity is the most crucial factor. Recharge ponds are highly dynamic environments, characterized by local spatial and temporal conditions such as the hydrogeology, sediment structure, climate seasonal variations, as well as the raw water quality. The water purification capacity is further determined by the interactions between the different groups of organisms inhabiting the underlying soils and sediments. Here, microbial communities, particularly attached microbial consortia, play a key role in the removal and transformation of organic carbon, nutrients, and allochthonous microbes, including organic and inorganic contaminants as well as pathogens (Stevik *et al.* 1999; van Cuyk *et al.* 2001; Hijnen *et al.* 2004). Increased microbial growth and the accumulation of particles in the sediments, however, may cause a significant reduction of infiltration performance. To prevent sediments from clogging, several strategies have been developed. First, it proved advantageous to maintain recharge ponds in a

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pulsed mode, i.e. several days or weeks of infiltration are followed by periods of sediment 'unsaturating' conditions and reoxidation (Rüetschi 2000). Second, macrovegetation was shown to reduce the development of algal mats at the sediment surface via reduction of incoming radiation and furthermore improved sediment conductivity (Brix 1997; Peters 1998; Rüetschi *et al.* 1998).

At the groundwater recharge plant Stallingerfeld, surface water from the Danube River is infiltrated periodically via infiltration ponds into the local aquifer. Infiltration periods do not last longer than ten days interrupted by pauses of similar length. The recharge ponds are structured by different kinds of macrovegetation to test for optimal recharge management strategies. The purpose of our study was to investigate the water infiltration and purification performance in two differently planted areas within a recharge basin accompanying a typical infiltration event. The main focus was on: (i) the removal efficiency for dissolved organic carbon (DOC), nutrients, and faecal indicators; (ii) the potential risk of bioclogging; and (iii) the effects of different macrovegetation. Furthermore, the study evaluated the qualitative change of DOM with infiltration, spatial and temporal patterns of natural virus and bacterial communities, including abundance, growth activity and diversity, as well as invertebrate fauna.

MATERIAL AND METHODS

Sampling sites

The Marchfeld region resembles an area of about 1,000 km^2, located in Lower Austria east of Vienna. The groundwater recharge plant Stallingerfeld at Deutsch-Wagram consists of several recharge ponds, periodically flooded with water from the Marchfeldkanal, supplied with water from the Danube River (Figure 1).

Our investigations focused on infiltration pond 5 with a surface area of 4,630 m^2 and four sections, where sections A–C were all planted with reed but managed by different harvesting strategies, and section D, planted mainly with willow (*Salix* sp.) and poplar (*Populus* sp.). For the purpose of comparison we selected the areas B and D in pond 5. Section 5B has an area of 1,500 m^2 and reed vegetation, which is cut once a year to avoid the accumulation of massive organic matter. At the time of sampling (May to June 2004), reed stems had an average height of about 1 m. Section 5D with an area of 575 m^2 was covered with 3–5 year-old bushes and small trees. Here, the litter was allowed to accumulate and to decay at the sediment surface.

After a three-weekly alternating infiltration period (1 week infiltration, stop for 1 week, another 1 week infiltration) in April and May 2004, water samples and sediment samples

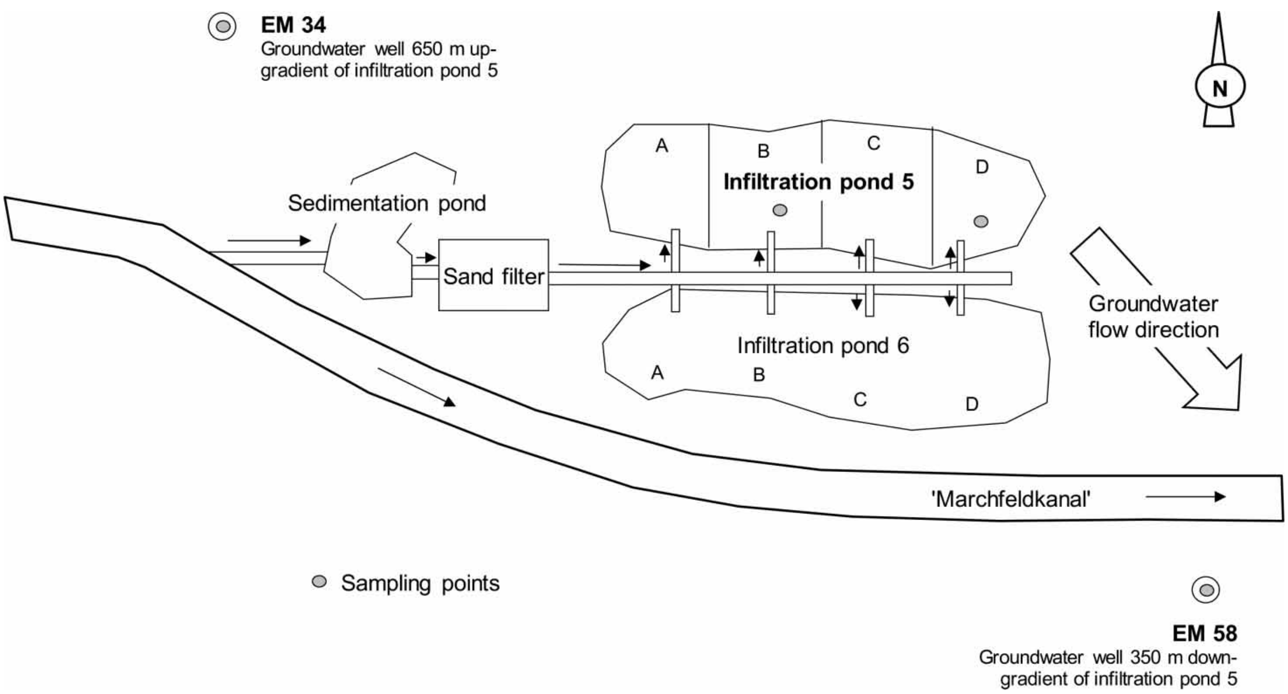

Figure 1 | Schematic illustration of the artificial recharge plant Stallingerfeld at Deutsch-Wagram, Austria.

were collected during a first survey. In detail, water from the pond inflow, the free water column in the pond at the two sections 5B and 5D, and sediment porewater from 0–10 cm, 30–40 cm, 70–80 cm, and 110–120 cm depths of both sites were sampled. Together with the porewater, fine sediments were also collected. Both sediment and porewater samples were withdrawn with a Bou-Rouch suction pump (Pospisil 1992). A second sampling campaign took place in June 2004, following a 9-day infiltration period. Samples were collected on June 4th, 6th, 9th and 11th. Between the surveys in May and June, the infiltration pond was topped up with surface water periodically at weekly intervals. The topping-up rate into the pond was approximately 20 L s^{-1}. Infiltration pond 6 (Figure 1) was not topped up during this study.

Two groundwater monitoring wells, i.e. EM 34 (650 m up-gradient) and EM 58 (370 m down-gradient) (Figure 1), were sampled occasionally. The groundwater flow velocity in the direction to well EM 58 was 2–2.5 m d^{-1}. Groundwater samples were collected with a double-packer-sampler from three selected depths: 0.5, 5 and 10 m below the groundwater table (bgwt). The groundwater table was at 4 to 5 m below ground level at the time of sampling.

Physico-chemical parameters

Groundwater recharge and sediment conductivity (k_f) in the various depths of sites 5B and 5D were estimated using a seepage meter and a minipiezometer following a simple field approach (Lee & Cherry 1978). Temperature, electrical conductivity and pH were measured immediately after sampling using field sensors (WTW, Weilheim, Germany). Dissolved oxygen values were determined either directly in the surface water or inside the piezometers after pumping. Samples for ammonium and total dissolved phosphorus were analysed spectrophotometrically after filtration following the molybdate-blue method of Vogler (1966). Nitrate (NO_3^-) was determined from filtered samples via ion chromatography (DIONEX DX-120). Samples for measurements of DOC were filtered through a pre-rinsed 0.45 μm syringe filter, then acidified to pH 2 with HCl, and later measured as non-purgeable organic carbon (Shimadzu TOC-5000). Qualitative evaluation of DOM was carried out by means of fluorescence spectroscopy (for details see the Supporting Information (SI), available in the online version of this paper). Sediment total organic matter (TOM) was determined as the weight loss after combustion of dried sediment at 450 °C for 5 hours.

Stable isotopes of oxygen ($^{18}O/^{16}O$) and hydrogen ($^2H/^1H$) were determined by isotope ratio mass spectrometry. The $\delta^{18}O$ values in samples were analysed via equilibration with CO_2 at 25 °C for 24 hours (Epstein & Mayeda 1953) and for δ^2H values via reaction with Cr at 850 °C (Coleman et al. 1982). Both $\delta^{18}O$ and δ^2H values were determined relative to internal standards that were calibrated using IAEA SMOW standards. Data were normalised following Coplen (1988) and are expressed relative to V-SMOW. Samples were measured at least in duplicate with a precision of ± 0.1‰ for $\delta^{18}O$ and ± 1.0‰ for δ^2H.

Microbial parameters

The total numbers of bacteria and viruses in water and sediments were determined by means of flow cytometry and epifluorescence microscopy, respectively (for details see the SI). Numbers are expressed in cells or particles per mL water or per cm^3 sediment.

Bacterial community analysis was performed by terminal restriction fragment length polymorphism (T-RFLP) fingerprinting from DNA extracted from triplicate sediment samples (details in SI). The Shannon–Wiener index *H'* was calculated as $H' = -\Sigma\pi \ ln \ \pi$, whereas π is the relative abundance of single terminal restriction fragments (T-RFs) in a given fingerprint (Hill et al. 2003).

Heterotrophic bacterial carbon production (BCP) was estimated by means of incorporation of [^{3}H]leucine into cell biomass (details are provided in the SI).

The amount of extracellular polymeric substances (EPS) was conducted using the phenol-sulfuric acid method described by Dubois et al. (1956).

On the last two days of the second sampling survey, porewater samples from section 5B were analysed for the presence of *Escherichia coli*. Aliquots of 10 and 100 mL were filtered onto 0.45 μm cellulose nitrate filters (Satorius), which were placed on chromocult medium plates for 48 hours at 37 °C.

Statistical analyses

To test for differences between the sampling points 5B and 5D, we used a two-way analysis of variance (ANOVA) with post-hoc comparisons. To control the overall experimental Type I error, we corrected the significance level according to the Bonferroni method as $\{\alpha/[n(n-1)/2]\}$, where α is the probability level and n is the number of observations. Spearman rank correlations analyses were used to evaluate relations between microbial and chemical parameters. All statistical analyses were done using the SigmaStat software package (Version 2.03, SPSS), as well as the statistical package offered in T-REX.

RESULTS

Hydrological situation

Water stable isotope analysis showed that the recharge system can be distinguished into three compartments, i.e. (A) inflow from the supply canal and the standing water in the pond during periods of infiltration, (B) porewater in the upper 120 cm of the sediment, and (C) true groundwater in the surrounding aquifer. Pond surface water showed $\delta^{18}O$ values of -11.86 to $-12.03‰$ and $\delta^{2}H$ values of -86.5 to $-87.1‰$. Almost similar values were obtained for sediment porewaters at section 5B with -11.82 to $-11.97‰$ for $\delta^{18}O$ and -85.9 to $-86.7‰$ for $\delta^{2}H$. In contrast, groundwater from the up-gradient well EM 34 showed significantly different isotope signatures, i.e. $-9.58‰$ and $-70.8‰$ for $\delta^{18}O$ and $\delta^{2}H$, respectively (Figure 2). Water collected at the down-gradient well EM 58 revealed a clear mixture of isotopically heavier groundwater from the up-gradient and the lighter recharge water. Calculations based on isotope values indicated a content of $35 \pm 5\%$ of infiltration water at 5 m bgwt at the time of sampling (Figure 2).

Removal efficiency for nutrients and organic carbon

During the main study period in June 2004, an entire infiltration event was followed by four sequential sampling surveys at two different sections (5B and 5D) in infiltration pond 5 (Figure 1). The surface water donation rate of 20 L s^{-1} and a seepage rate of 1–5 m d^{-1} resulted in a water column of 10 to 30 cm overlying the sediments. Sediment at section 5B was characterised by a compact layer of roots, humus, clay and gravel in the uppermost 2–5 cm. At section 5D this layer was twice as thick (approximately 10 cm) and contained leaves from the bushes, but less gravel. At section 5D the hydraulic conductivity was significantly reduced at sediment depths of 30 to 40 cm and 70 to 80 cm in comparison to section 5B (Table S1, available in the online version of this paper).

Nitrate concentrations in the supply water ranged between 4 and 7.5 mg L^{-1} and decreased with sediment depth at both sections of the recharge pond (ANOVA, $P <$ 0.001, Figure S1, available in the online version of this paper). Much higher nitrate values than in the surface water were found in groundwater from the up-gradient well EM 34 with 81 mg L^{-1} in 0.5 m and 66 mg L^{-1} in 5 m depth below the groundwater table. Groundwater from the down-gradient well EM 58, located on the flow path of the investigated recharge pond, contained 16.5 ± 2.5 mg L^{-1} nitrate at 0.5 m bgwt, successively increasing with depth, i.e. 50 ± 0.03 mg L^{-1} at 5 m and 76 ± 3.7 mg L^{-1} at 10 m bgwt (Figure 2).

A difference between section 5B and 5D was obvious when looking at total dissolved phosphorus (TDP) and ammonium. At section 5B both parameters exhibited an increase with sediment depth, while an opposite trend was obtained at 5D (Figure S1). Concerning groundwater, the highest TDP values were observed in the upper layers of the down-gradient well EM 58 reaching concentrations of almost 100 μg L^{-1} (Table S2, available in the online version of this paper), while TDP values in the groundwater from well EM 34, were similar to concentrations found in the supply water and the sediment porewater. More details on the physical-chemical characteristics of the supply and sediment porewater, as well as the up- and down-gradient groundwater are provided in Table S2.

Key for the evaluation of the purification performance is the DOC. DOC concentration in the supply water was

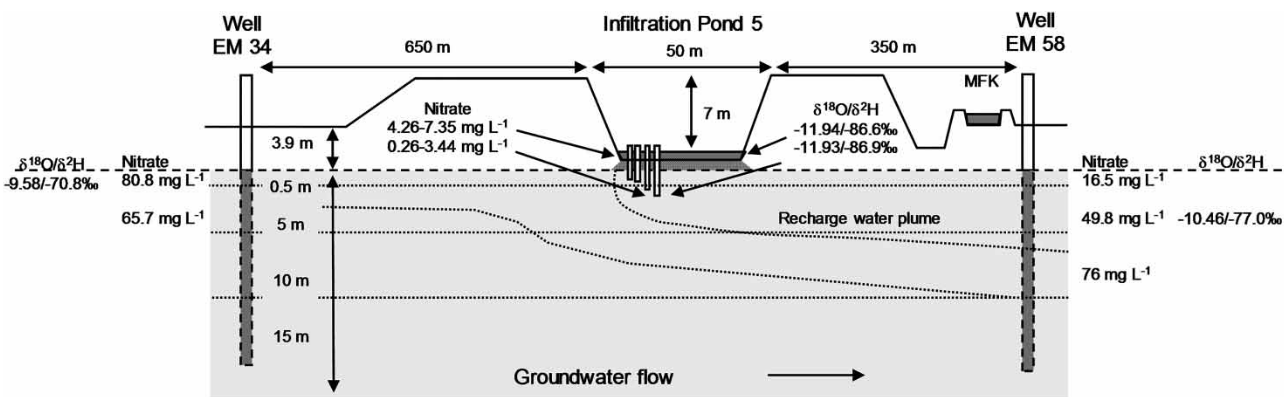

Figure 2 | Transect across the infiltration pond following groundwater flow direction. Via water isotope and nitrate values, surface water recharge and mixing of infiltrated water and groundwater is indicated. Isotope values of sediment porewater are mean values from four different depths (0–10, 30–40, 70–80, 110–120 cm).

2.5 ± 0.07 mg L^{-1}. In the overlying water column, DOC showed slightly increased values with 3.7 mg L^{-1} at section 5B and 2.8 mg L^{-1} at 5D. At section 5B, DOC in sediment porewater decreased on average from 3.56 mg L^{-1} to 2.4 mg L^{-1} with depth during the investigation (Figure 3). At 5D DOC values at 0–10 cm were significantly higher than in the overlying surface water and also higher compared to 5B with a mean of 5.81 mg L^{-1}. Similar to section 5B DOC values decreased with depth to 2.42 mg L^{-1} at 110–120 cm. DOC values in groundwater from the up-gradient well EM 34 showed a concentration of 2.67 mg L^{-1} in 0.5 m and 1.66 mg L^{-1} in water from 5 m below the groundwater table. At the down-gradient well EM 58 DOC showed a similar trend, i.e. 2.3 ± 0.3 mg L^{-1} at 0.5 m, 2.4 ± 0.3 mg L^{-1} at 5 m, and 1.5 ± 0.2 mg L^{-1} at 10 m below the groundwater table (Table S2).

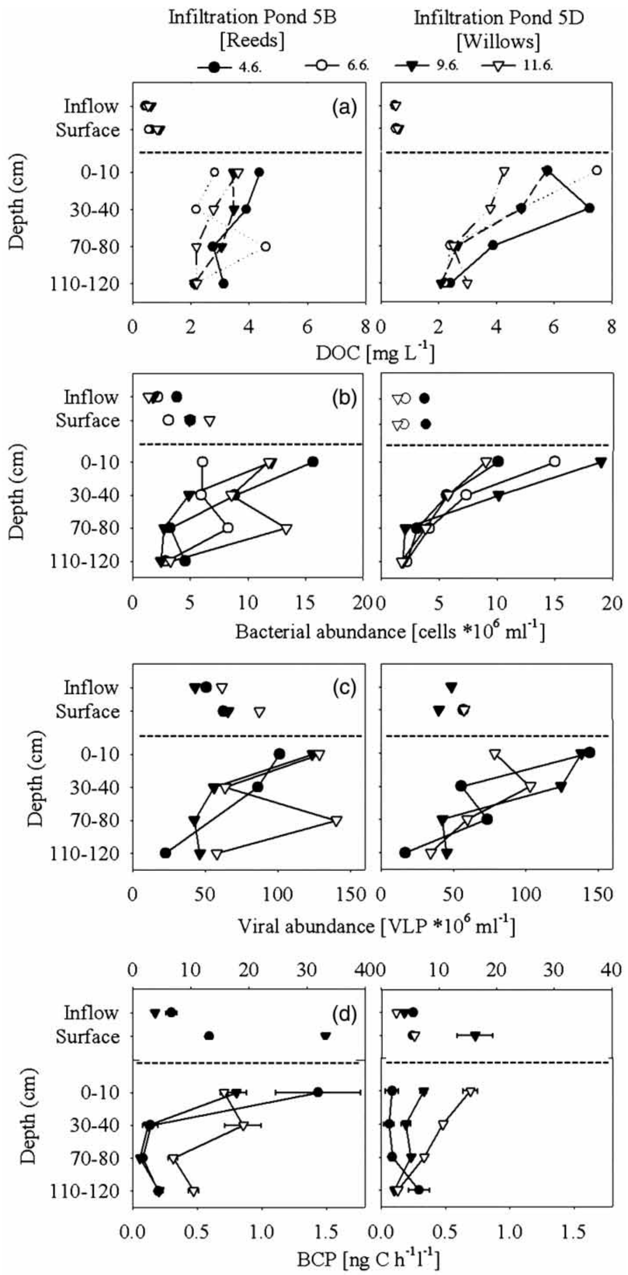

Figure 3 | Depth profiles (connected with lines) of DOC and selected microbial parameters analysed in sediment porewater at two sites from infiltration pond 5. Free symbols on top of each chart refer to values determined in donation water and the free water column in the pond. The different symbols refer to different sampling days during a survey in June 2004 (see particular date on top). Values are means of triplicate measurements ± SD.

The qualitative evaluation of the DOM revealed a higher degree of humification in the surface water (overlying water column) and in porewater of the topmost 10 cm of sediment (mean of emission ≥ 450 nm) than in deeper layers for both sites, section 5B and 5D. With depth, mean values of emission decreased to 440–450 nm. In sediment porewater, fulvic acids (FAs) constituted a more prominent fraction than humic acids (HAs), which accounted for only approximately 10% of the humic substances in the DOM. The lowest HA content was found with the groundwater samples. In general, the high molecular fraction of the DOM decreased with sediment depth at both sampling sites.

Removal of potential pathogens

The efficiency of attenuation of *E. coli* in recharge pond 5 were evaluated twice but only for site 5B. *E. coli* which was continuously imported into the sediment with surface water (53 ± 32 CFUs 100 mL^{-1}) peaked in the porewater of the top sediment layer (715 ± 685 CFUs 100 mL^{-1}) and then constantly decreased with depth to values below the detection limit at 110–120 cm below the soil surface.

Distribution of bacteria, viruses and patterns of microbial activity

The number of suspended bacteria peaked in the porewater of the upmost 10 cm of sediment at 5B and 5D. With increasing depth, a 2–5-fold reduction in cell numbers was observed at 5B and a 5–10-fold reduction at 5D (Figure 3). In comparison, bacterial numbers in the groundwater were 2–3 orders of magnitude lower (Figure S2, available in the online version of this paper).

Bacteria attached to the sediment were 1–2 orders of magnitude higher in number than porewater concentrations. Again, in most cases a decrease with sediment depth was observed (Figure 4). Numbers of attached bacteria were significantly correlated with dissolved oxygen, nitrate,

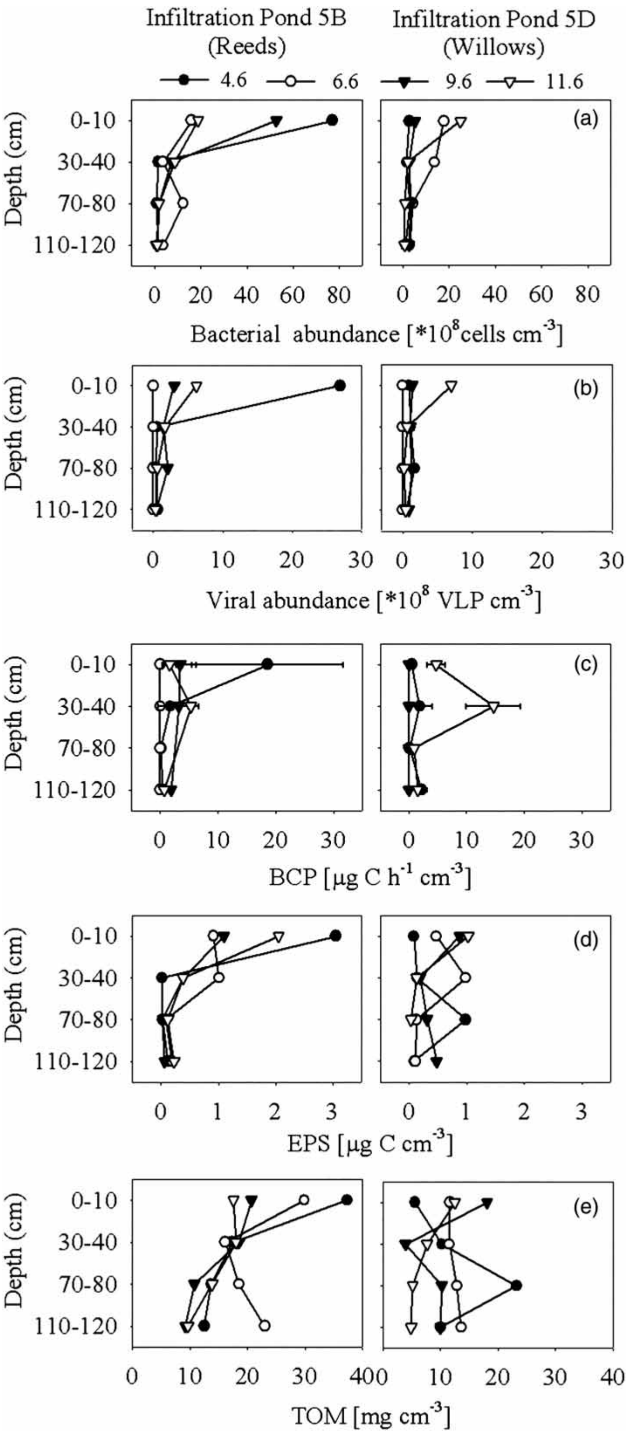

Figure 4 | Depth gradients of sediment parameters analysed in sediment from infiltration pond. The different symbols refer to different sampling days during a survey in June 2004 (see particular date on top). Values are means of triplicate measurements ± SD.

TOM, and the concentration of EPS at both sampling sites (Spearman rank, $P < 0.03$).

The spatial and temporal patterns of viruses were similar to those of bacteria and significantly correlated to each other in both porewater and sediment samples (Spearman rank, $P < 0.001$, $r = 0.9$). In porewater, the highest numbers of viruses always occurred at 0–10 cm and decreased with depth at both sampling sites (Figure 3). The number of viruses attached to particles was 2–3-fold higher compared to the numbers in porewater, except for sampling day 1 (Figure 4). The virus to bacteria ratio (VBR) was in the range of 10–20 (pond 5B) and 5–10 (pond 5D) in the top 10 cm sediment porewater. At 1 m sediment depth the ratio was between 10–20, independent from the site. With the sediment samples, the total number of attached viruses was less than the number of bacteria in the top sediment and almost similar at 110–120 cm depth. For comparison, groundwater numbers of viruses were 2–3 orders of magnitude lower (Figure S2). The VBR in groundwater was around 5.

Although the number of bacteria in the standing pond water was always lower than in sediment porewater (Figure 3), the bacterial carbon production (BCP) showed an inverse picture with 15–20-fold higher values in the surface water. Within the sediment, the BCP of suspended bacteria tended to decrease with depth (Figure 3). Attached bacteria showed 1000-fold higher BCP rates when compared to those in porewater. Again, at both sites the highest BCP rates were found in the upmost sediment layers and decreased 10-fold with depth (Figure 4).

The number of EPS from attached bacteria (biofilms) was low. The highest value was detected at section B5 in 0–10 cm sediment with 3.06 µg C cm^{-3}. At 5B a decreasing trend of EPS was observed with depth for all sampling days. EPS values at 5D did not show any clear trend with depth (Figure 4). EPS data from 5D were significantly different from 5B data (ANOVA, $P < 0.01$, Figure 4). This picture was similar for TOM, which also showed a significant difference between sampling sites 5B and 5D (ANOVA, $P < 0.01$, Figure 4).

Bacterial community fingerprints

Bacterial community analyses, carried out once with sediments collected from 0–10 cm and 110–120 cm depth at both sampling sites, showed no significant differences between the surface sediments at 5B and 5D as well as at 110–120 cm depth at 5B with regard to the Shannon-Wiener diversity, community richness and evenness. Sediments from 110–120 cm at 5D showed lower values (Table S3, available in the online version of this paper). A principal component analysis revealed a very close clustering of the surface sediment samples of 5B and 5D, whereas sediments of the deeper areas at 5B and 5D separated from each other as well as from the surface sediments according to their community composition (Figure S3, available in the online version of this paper). More details on the community analysis are provided in the SI.

DISCUSSION

The combined action of abiotic and biotic processes, such as filtration and degradation, are responsible for the high purification potential of aquatic sediment systems. A number of factors related to the structure of the sediment body (i.e. grain size distribution, mineralogy, hydraulic conductivity) and to the activity state of the microbial community clearly influence the purification potential in recharge ponds (Wotton 2002; Li *et al.* 2013; Alidina *et al.* 2014). An important factor that may limit the purification capacity is the quality of the supply water or, in other words, the total load of particles, organic carbon and nutrients being introduced into the system. However, the infiltration and purification performance may be optimized by choosing an appropriate duration of infiltration. At the investigated groundwater recharge plant (Figure 1) Danube River water supplied by the Marchfeldkanal is infiltrated periodically. Areas within individual ponds are structured by different vegetation to test for optimal recharge management.

Water purification efficiency

Monitoring of physico-chemical and microbial parameters revealed that the time required for the initialization of high water purification efficiency in the periodically infiltrated pond areas is negligible. Dissolved oxygen measurements indicated that the aerobic microbial respiration was already present to a high extent in the unsaturated sediment during the infiltration pauses. Already at sampling day one (which equals day two of infiltration) about 75% of the oxygen was consumed within the first 120 cm of the newly saturated sediment (Figure S1(b)). In other words, stable microbial activity is achieved from re-activation of well-conditioned microbial communities outlasting infiltration pauses. Experience from other sites and from laboratory experiments supports the idea of a fast initialization even after days of a break in infiltration. It was further shown that bacterial populations attached to the sediment matrix were taxonomically stable for several months despite pronounced dynamics in environmental conditions (Zhou *et al.* 2012) and may regain full activity within hours to a few days (Mellage *et al.* 2015).

The DOC, which, from its qualitative characteristics, suggests that it originates mainly from algal exudates and dead plant leachates rather than from source water (Figure 3(a)), was efficiently reduced by 56% and 75% at section 5B and 5D, respectively, to about 2.0 mg L^{-1} at 110–120 cm sediment depth. Porewater DOC concentrations at this sediment depth already represented natural groundwater conditions, when compared to groundwater samples from the up-gradient well EM 34 (Table S1). No further decline in DOC concentration could be observed after the aquifer passage (a distance of about 350 m) from the pond to the down-gradient well EM 58. A similar picture was obtained by Kivimäki *et al.* (1998) who found efficient DOC degradation in the upper soil and sediment zones but concluded that the reduction of DOC in the aquifer was mainly driven by sorption and dilution. When compared with other recharge plants, the reduction of DOC we observed seems rather efficient. Comparably high DOC reduction was reported for a forest soil and fluvial sediment system in Switzerland (Rüetschi 2000) and an artificial groundwater recharge site in Sweden (Jacks 2001). However, in both cases the distance of the sediment passage was much longer.

Qualitative evaluation of the DOM revealed a higher degree of humification in the surface water (overlying water column) and in porewater of the topmost 10 cm of sediment for both sampling sites. This seems in contradiction with the overall concept of DOM transformation in aquatic systems, where it is assumed that low molecular weight compounds are biotransformed first and the remainder is composed mainly of high molecular weight molecules such as humic substances. However, in sediment systems, sorption needs to be considered as an important process. High molecular weight fractions of DOM are significantly retained (Juhna *et al.* 2003; Lindroos *et al.* 2002). In our system, the content of HAs continuously decreased with depth and was lowest in the true groundwater samples. The efficient removal of large molecular fractions from porewater was documented by Lindroos *et al.* (2002); Drewes *et al.* (2006); Kolehmainen *et al.* (2007). FAs, however, constituted a more prominent fraction in the water. However, the share between microbial transformation and sorption to surfaces remains poorly understood and awaits further evaluation. In recent experiments we could show that sandy aquifer sediments contained high amounts of DOM sorbed to the particle surfaces. While the porewater exhibited DOC concentrations of only 1–3 mg L^{-1}, alkalization or heating (90 °C) of sediments increased DOC concentrations in the porewater 10–30-fold (Griebler *et al.* in prep.).

Besides the effective removal of DOC, the sediment passage of 1.2 m at our test site was also sufficient to reduce the amount of nitrate present in the supply water by 50 to 65% (Figure S1(d)). However, nitrate reduction was of no importance since nitrate concentrations in groundwater exceeded those in the source water (Table S1). Conversely, the low nitrate values could be, together with water isotopic values, used to interpret the effects of surface infiltration and mixing of different water masses in the aquifer (Figure 2). Although the water collected at the down-gradient groundwater well did not, due to the comparably long travelling time in the aquifer, represent water infiltrated during the investigation period, but rather water infiltrated about 3 weeks earlier. Nitrate values clearly showed that shallow groundwater at well EM 58 was a mixture of recharge water and true groundwater, exhibiting a gradual increase in concentration with depth (Figure 2). Water δ^2H and $\delta^{18}O$ values further proved that no mixing of infiltration water and groundwater took place in the first 120 cm of the sediment passage in the recharge pond. Here, physical-chemical changes in sediment porewater observed with depth can therefore be attributed exclusively to biotic transformation and abiotic filtration and sorption processes, rather than dilution.

In contrast to other studies where total counts of prokaryotic cells significantly decreased during the sediment passage (e.g. Kolehmainen *et al.* 2007), cell numbers in porewater of one metre sediment depth were fairly similar to those in the infiltrating surface water. However, when compared to the abundance of suspended cells in the top sediment porewater, a significant reduction by 3–10-fold took place (Figure 3(b)). The reason for this decrease in cell numbers is mainly related to the successive depletion of available energy in the form of favourable organic matter and nutrients, rather than a sole physical filtration effect. This is in accordance with the results from bacterial carbon production measurements that clearly underlined a ready decline in bacterial growth rates with sediment depth. Active microbial food web interactions, such as protozoan grazing and viral lysis, have been shown to regulate the bacterial standing stock (Weinbauer & Höfle 1998; Hahn & Höfle 2001). The microbially active natural sediments of the infiltration pond proved to be efficient in removing 'extrinsic' microorganisms (pathogens) from the surface water as exemplarily shown for the faecal indicator *E. coli*. The number of colony forming units (CFUs) was below the detection limit at a sediment depth of 110–120 cm at section 5B, although the supply water, and especially the porewater from the top 10 cm of sediment, contained high numbers of *E. coli*. Besides a die-off of pathogens, microbial antagonistic processes are believed to substantially contribute to their elimination (Krauss & Griebler 2011). Since numbers of prokaryotes in groundwater were found at 1–2 orders of magnitude lower than in one metre sediment porewater below the recharge basin, this decreasing trend must have continued with distance to the infiltration site. While a few studies have tackled the fate of (surrogate) pathogenic viruses during artificial recharge of surface and waste water (Anders & Chrysikopoulos 2005), there are no data available with respect to the abundance and fate of natural viruses (bacteriophages) from comparable systems. Viral numbers observed in our study are in accordance with findings from freshwater and marine sediments (Fischer *et al.* 2003; Danovaro *et al.* 2008). This is also true for the VBR. While in the supply water and porewaters VBR accounted for 5:1 to 20:1, an almost 1:1 ratio was found with the sediment matrix. The number of viruses showed a significant positive correlation with bacterial abundance. This suggests that there is an intimate link between both. Similar to the bacteria, viruses decreased in number from the recharge basin to the down-gradient groundwater monitoring well. A similar reduction of bacterial and viral numbers by 1–5 orders of magnitude from raw water to groundwater is reported for bank filtration at three different American rivers (Weiss *et al.* 2005). The decrease in numbers of viruses can be explained by sorption, decay and microbial antagonist activities (Krauss & Griebler 2011).

With regard to the composition of the sediment *eubacterial* communities, a high similarity in composition of the two surface sediment samples from 5B and 5D was found, whereas sediments from the deeper areas at 5B and 5D separated from these and from each other (principal component analysis; Figure S3). The clear dominance of one operational taxonomic unit (T-RF 487) with almost 40% relative abundance at 5D 110–120 cm, may be linked to the sediment anomalies found in 5D (see SI). Moreover, the sediment anomalies as well as the composition of the different microbial community at 5D may be a result of the different rooting of the bushes and trees when compared to the reed in section 5B. For further evidence on communities' functional differences, sequencing and proteomic studies are required.

Risk of bioclogging

Clogging is considered the limiting factor affecting the artificial recharge of aquifers. It is usually a result from deposition of suspended material, precipitation and dissolution processes, gas formation and, importantly, microbial growth. As shown in many studies, 90–99% of microbial biomass is found in the upper 0–10 cm layers of the sediment systems (Stevik *et al.* 1999; van Cuyk *et al.* 2001). A similar situation was found in our recharge pond. It is important to mention that bacteria suspended in sediment porewater generally contribute only 0.01 to 10% of the total bacterial biomass in aquatic sediment system, (Griebler *et al.* 2002), while the major part is attached to the sediment matrix. Consequently, data based on suspended bacteria provide little information on the microbial biomass present in the system. The ratio of attached to suspended cells provides a good estimate of the nutrient conditions; the more limited a system, the higher the ratio (Griebler *et al.* 2001, 2002). The mean ratios of sessile to free-living bacteria in the sediments of recharge pond 5 ranged from 340 (5B) to 110 (5D) and showed an increasing trend with depth at both sites. Besides the number of microorganisms, the amount of EPS produced by bacteria is a crucial criteria for bioclogging. The amount of EPS as well as the TOM concentrations in the sediments of the pond provided no indication for the risk of bioclogging during the short-term infiltration periods as conducted at the investigation site (Figure 4). Thus, although bioclogging has been shown for a large number of recharge sites where the top layers of sediments have to be removed repeatedly or injection wells have to be flushed and regenerated (Baveye *et al.* 1998), the pulsed short-term maintenance of the recharge ponds at Stallingerfeld is key to the sustainable maintenance.

Macrovegetation and infiltration performance

The comparison of the two differently planted sections in recharge pond 5, i.e. with reed at section 5B and poplar and willow bushes and small trees at section 5D, exhibited a number of different patterns. At 5D, the topmost sediment was characterized by a higher organic content and higher silt and clay content. This did not result in a pronounced reduction of the hydraulic conductivity (Table S2). The roots may have created preferential flow paths. The different vegetation also caused deviations in other parameters. The higher DOC concentrations in the porewater of the uppermost sediment layer at 5D (Figure 3(a)) can be attributed to the leaching of dead leaves. However, these elevated DOM concentrations did not break through into the aquifer. As a further difference between the two sections, 5D porewater from 0–10 cm exhibited higher concentrations of total dissolved phosphorus (Figure S1(c)), possibly caused also by leaf litter leachate. An opposite trend was observed for ammonia (Figure S1(e)).

Occasionally, microbial abundance and productivity indicated that 5B sediments were microbially more active (Figure 3 and 4). At the reed-covered site 5B, the sediment surface was less shadowed and higher prokaryotic cell counts in the water column can be attributed to the contribution from unicellular cyanobacteria. On small open spots in the reed vegetation green algal mats regularly formed from *Cladophora* sp. (Neudorfer pers. Comm.). On one hand, such algal blooms bear the risk of bioclogging of the sediment (Verheijden *et al.* 1997). On the other hand, algal oxygen production during the day may fuel some additional oxygen into the upper sediment layer, which may result in higher respiration rates at night. This effect is also obtained by regular drying of the sediments, which leads to unsaturation and reoxidation of reduced zones (e.g. Greskowiak *et al.* 2005). Problems with algal blooms are less pronounced in areas planted with bushes and trees and can also be avoided, by using an intermittent operation technique. The advantage of plant cover is supported by earlier studies (Brix 1997; Platzer & Mauch 1997). Rüetschi *et al.* (1998) demonstrated that reed vegetation offers good conditions for long-term artificial recharge. Other authors propose to use combined plant-covers by mixing reed-fields with trees to create extensive shadow at the water surface and subsequently maintain moderate growth rates of algae during sunny summer days (Peters 1998; Rüetschi *et al.* 1998).

Benthic communities

When comparing the two differently planted sites it is also interesting not only to consider microbial communities but meiofauna, which was studied by Danielopol *et al.* (2006). They showed that the abundance of invertebrates followed the patterns of the microbes. A higher taxonomic diversity was observed at 5B (Table S4, available in the online version of this paper) with highest numbers of specimen, in the upper 10 cm and a strong decline with depth (Danielopol *et al.* 2006). The total number of animals and individual taxa such as oligochaetes positively correlated with the total abundance of bacteria. It has been shown in other studies that a local high density of invertebrates in sand filters may stimulate microbial communities, but at the same time keeps the interstitial spaces open, resulting in a sustainable infiltration performance (Westphal & Rumm 1997; Mermillod-Blondin *et al.* 2003).

CONCLUSIONS

Investigations of an artificial groundwater recharge (AGR) pond revealed an efficient transformation and attenuation of organic carbon, nutrients and faecal indicator bacteria. Full water purification performance is re-established within one day of infiltration after several days of infiltration pause. No indication of bioclogging could be found in the studied sediments over the course of our investigations. Reed cover was found to be advantageous over bushes and trees, since it supported higher microbial activities, meiofaunal diversity, and allowed for easier vegetation management. Short-term infiltration periods of several days followed by a break of similar time, were found suitable to guarantee high recharge rates and good water purification. Nevertheless, future research will have to address a better mechanistic understanding of carbon and nutrient turnover in the complex food webs of AGR systems.

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