RESEARCH ARTICLE

Effects of thermal energy discharge on shallow groundwater ecosystems

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Abstract

The use of groundwater as a carrier of thermal energy is an important source of sustainable heating and cooling. However, the effects of thermal use on geochemical and biological aquifer characteristics are poorly understood. Here, we have assessed the impacts of heat discharge on an uncontaminated, shallow aquifer by monitoring the hydrogeochemical, bacterial and faunal parameters at an active thermal discharge facility. The observed variability between wells was considerable. Yet, no significant temperature impacts on bacterial or faunal abundance and on bacterial productivity were observed. Also, we did not observe an improved survival or growth of coliforms with temperature. In contrast, the diversity of bacterial terminal restriction fragment (T-RF) length polymorphism fingerprints and faunal populations was either positively or negatively affected by temperature, respectively, and the abundance of selected T-RFs was clearly temperature dependent. Canonical correspondence analysis indicated that both the impact of temperature and of surface water from a nearby river, were important drivers of aquifer biotic variability. These results demonstrate that aquifer thermal energy discharge can affect aquifer bacteria and fauna, while at the same time controlling only a minor part of the total seasonal and spatial variability and therefore posing no likely threat to ecosystem functioning and drinking water protection in uncontaminated, shallow aquifers.

Introduction

Climate forcing by anthropogenic greenhouse gas emissions is of increasing concern to humanity. Therefore, the development and exploitation of low- or zero-emission energy technologies is becoming more and more relevant. Groundwater and the use of the thermal energy stored therein for heating and cooling represents one option for the sustainable climatization of buildings. Throughout the last decade, ground source heat pumps (GSHPs) connected to below-ground heat exchangers (closed loop) or directly fed by groundwater from a well (open loop) have been increasingly implemented. In 2004, *c.* 600 000 GSHPs were operational worldwide, most of them in the United States and Europe, with annual growth rates of *c.* 10% (Lund *et al.*, 2004). In Germany, *c.* 44 000 shallow geothermal facilities are currently operational (Boehme *et al.*, 2007). There, plant operation is usually authorized for maximum temperature changes of $\pm 6\,^{\circ}C$ compared with unaffected local groundwater temperatures. The recommended maximum reinjection temperature in open-loop systems is 20 °C (VDI, 2000).

In shallow aquifers (down to depths of *c.* 10 m), groundwater temperature is usually 1–2 °C higher than the mean annual surface temperature (Parsons, 1970) and, depending on the recharge and geology of the aquifer, only minimally influenced by diurnal or annual temperature fluctuations (Silliman & Booth, 1993). Because temperature is a key driver of hydrogeochemical and biological processes, anthropogenically induced temperature changes can be hypothesized to influence groundwater systems, which are normally more or less isothermal. Up to now, aquifer thermal energy discharge has been shown to influence a variety of geochemical parameters depending on the sediment characteristics, temperature divergence and organic matter content. Particularly, the reinjection of heated groundwater can lead to carbonate precipitation (Griffioen & Appelo, 1993), increased dissolution of silicate minerals (Arning *et al.*, 2006), the mobilization of organic

compounds from sediments (Brons *et al.*, 1991) and to decreasing groundwater oxygen saturation (Stumm & Morgan, 1995).

In contrast to these reports on hydrogeochemical aspects of aquifer thermal energy usage, studies on the impact on groundwater biota (especially microorganisms) and biotic ecosystem functions are scarce. A few available studies did not detect significant effects of temperature changes on total bacterial or viable counts (Adinolfi *et al.*, 1994; York *et al.*, 1998; Schippers & Reichling, 2006). However, while these authors have hypothesized the possibility of temperature-related microbial population shifts, no respective reports are available. While natural groundwater habitats are considered to harbor mainly psychrophilic and psychrotolerant microorganisms, increased temperatures may foster mesophilic populations instead. Besides community shifts, increased groundwater temperatures are expected to enhance general microbial activities, which can be argued to be a beneficial side effect of GSHP systems improving the self-purification of groundwater especially in polluted urban areas (Ruck *et al.*, 1993). On the other hand, elevated temperatures may also cause hygienic problems wherever groundwater is used as a reservoir of drinking water, due to a potentially improved survival, transport or even growth of infiltrating faecal and pathogenic microorganisms. Still, temperature is certainly not the only important factor controlling groundwater microorganisms, and it can be expected to closely interact with others, such as salinity, pH and the presence of electron donors or acceptors (Pomeroy & Wiebe, 2001).

Although much less abundant than microorganisms, groundwater fauna can also be hypothesized to be affected by temperature. Aquifer metazoans usually thrive in very defined ecological niches and therefore may even be more susceptible to minor temperature changes than their prokaryotic counterparts. However, the relationship between faunal assemblages and temperature in groundwater was only studied in terms of temperature as an indicator for surface water inflow to date (Datry *et al.*, 2005) or as a secondary effect of altitude (Ward & Voelz, 1994; Reeves *et al.*, 2007). To our knowledge, temperature itself has not been regarded as a shaping factor for groundwater faunal assemblages so far.

Here, we present a comprehensive field investigation of an aquifer downstream of an industrial facility using considerable amounts of groundwater for cooling purposes. An integrated methodological approach comprising hydrogeochemical analyses, monitoring of microbial activities, bacterial community profiling and the investigation of groundwater faunal assemblages was applied. Thus, we aim to identify temperature-induced changes in aquifer ecosystem characteristics and functioning, as a basis for the development of a knowledge-driven authorization practice and sustainable operation schemes for aquifer thermal energy use.

Materials and methods

Site description

Groundwater samples were taken from the existing monitoring well network of an active temperature discharge facility at an oligotrophic shallow quaternary aquifer. The aquifer has a mean depth of 8–15 m composed of gravels that overlay a secondary aquifer characterized by tertiary gravel and sands. The facility is located *c.* 2 km east of Freising, Germany, adjacent to the floodplains of the Isar River, and the main groundwater flow is towards the river (Fig. 1). Therefore, the developing temperature plume is naturally confined in its distribution. Eight sampling wells were chosen with respect to their position to the temperature plume (unaffected, temporary or continuous impact). The aquifer is characterized by a high hydraulic conductivity ($k_f = 0.023\ m\ s^{-1}$) and relatively high average groundwater flow velocities (18 to 29 $m\ day^{-1}$). The mean natural groundwater temperature in the study area is usually $11 \pm 1\ ^{\circ}C$, depending on the seasonal and also Isar River influence. Groundwater discharge temperature by the facility should not surpass 21 °C due to system authorization.

Sample collection

Groundwater sampling was conducted in April (May for first fauna sampling, respectively), June, August and November 2007. This fully covers the main cooling period of the facility. Before sample collection from the observation wells, the water table was measured using a portable depth gauge, and a temperature profile was recorded. Afterwards, groundwater fauna in the well was collected using a planktonic net as described in Fuchs (2007). The net was drawn three times covering the entire depth of the well screen. Subsequently, groundwater samples were taken by pumping from a depth of between 3 and 5-m below the water surface. A minimum of three well volumes was pumped before sampling. Then, the first few liters were used to determine the physicochemical parameters [temperature, electrical conductivity, pH, dissolved oxygen (DO) and redox potential] with field sensors (WTW). Subsequently, triplicate 50-mL samples were filtered through 0.45-μm membrane filters into sterile polyethylene tubes and glass flasks for measurement of dissolved organic carbon (DOC), soluble reactive phosphorus (SRP) and major anions and cations. For bacterial cell counts, triplicate 50-mL samples were immediately fixed with glutaraldehyde (1% final concentration). Filtered, fixed and fauna samples were stored at 4 °C in the dark during transport and until analysis in the laboratory. DOC, SRP and major ions were analyzed within 2 days.

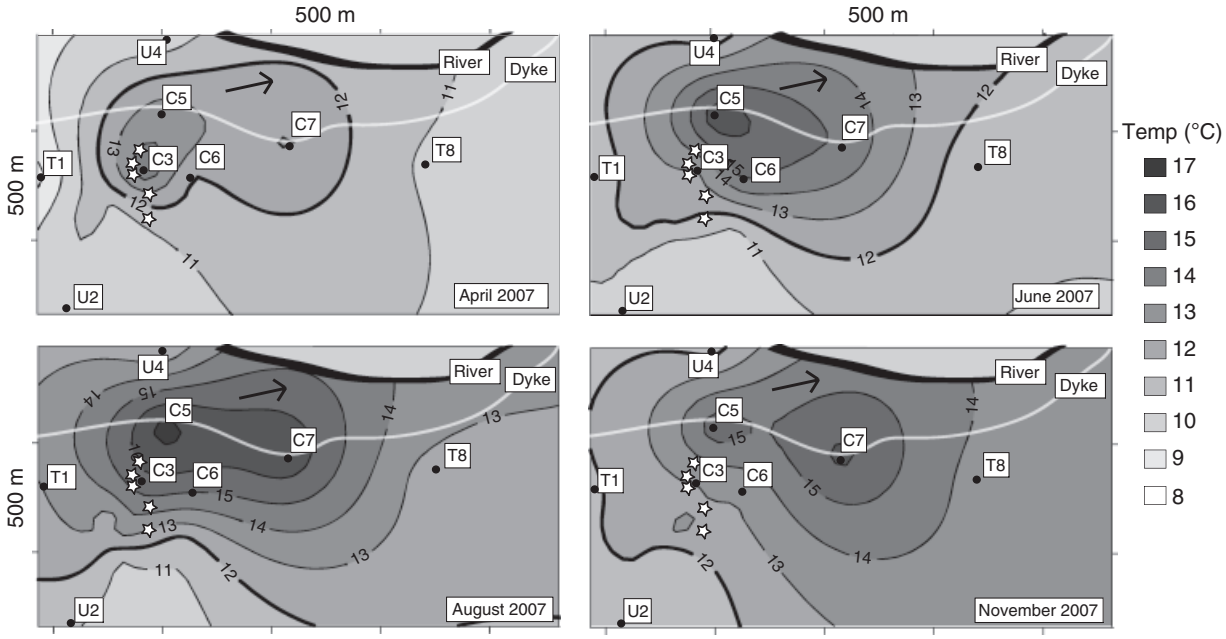

Fig. 1. Spatial dynamics of the temperature plume and location of sampling wells at the investigated field site. The extent of the plume is indicated in +1 K isotherms between 10 and 17 °C as interpolated by Kriging from the mean groundwater temperatures. Prefixes in the well names indicate unaffected (U), temporary (T) or continuous (C) temperature impacts. Stars show the location of the reinjection wells of the discharge facility; the arrow indicates the main groundwater flow direction towards the confining river.

Water chemistry

DOC was analyzed as nonpurgeable organic carbon in acidified samples using high-temperature combustion with infrared detection of CO_2 as described previously (McIntyre *et al.*, 2005) on a Shimadzu TOC-5050. SRP was determined colorimetrically according to Murphy & Riley (1962) and Pote & Daniel (2000) modified for 10-mL samples. Samples were read at 880 nm on a spectrophotometer (Varian, Cary 50 Bio). Ions (chloride, nitrate, sulphate, sodium, potassium, magnesium and calcium) were analyzed by ion chromatography (Dionex Model DX 100, cations: CS 12A 4 mm column, CSRS-Ultra II 4 mm suppressor, eluent 20 mM CH_4O_3S; anions: AS 4A 4 mm column, ASRS-Ultra II 4 mm suppressor, eluent 3.5 mM Na_2CO_3+1 mM $NaHCO_3$ and conductivity detection). Samples were quantified using commercial standards; data acquisition was performed with the PEAKNET software (Dionex). Bicarbonate ions were analyzed using a titration unit (Schott). Samples were analyzed in triplicate for all parameters.

Bacterial and faunal abundances

The total bacterial abundances (BAs) were determined by epifluorescence microscopy of stained cells following a modified protocol of Martens-Habbena & Sass (2006). Glutaraldehyde-fixed samples were stained with a final concentration of 1 × SYBR Green I (Molecular Probes) and incubated for 20 min at 4 °C in the dark. Subsequently, the sample was filtered onto a black polycarbonate filter (0.2 μm, Millipore), followed by a washing step with cell-free sterile water. The filter was embedded in antifading low-fluorescence immersion oil (Type A, Cargille Labs) on a microscope slide and either analyzed immediately or frozen at −20 °C in the dark (Lunau *et al.*, 2005). Samples were counted with a Zeiss Axiolab microscope at × 1000 magnification (filter set: Zeiss, Ex 450–490 nm, FT 505 nm, LP 520 nm) using a counting grid. At least 300 cells were counted per slide. Freshwater invertebrates were counted and sorted to class or order level at × 16 magnification under a dissecting stereomicroscope (Leica MZ 16).

In addition to our regular bimonthly sampling routine, standard microbiological drinking water analyses were performed for the different wells in accordance to the European Union Council Directive 98/83/EC for the assessment of the quality of water intended for human consumption during a sampling in May 2007. This included the enumeration of total CFU at 22, 37 and additionally at 27 °C on R2A-agar, as well as the quantification of coliform bacteria and of *Escherichia coli* (Oehmichen *et al.*, 2003).

Bacterial activities

Extracellular phosphatase activity (EPA) was estimated using modified protocols of Hoppe (1993) and Hendel *et al.* (2001). Methylumbelliferyl phosphate (MUF-P, Sigma)

was used as a substrate. For each sampling procedure, a stock solution of 10 mM MUF-P was prepared freshly in autoclaved deionized water. One hundred and twenty-five microliters of stock solution was added to triplicate 4.875-mL groundwater subsamples directly in the field, resulting in a final concentration of 250 μM fluorogenic substrate. In a preceding experiment, phosphatase activity did not reach enzyme saturation at concentrations of up to 500 μM, and uptake of MUF-P was linear for at least 26 h. Nevertheless, we used a final substrate concentration of 250 μM for better comparability with previous studies (Hendel *et al.*, 2001). Samples were incubated for 24 h at *in situ* temperatures (± 1 °C) in adjustable thermoboxes (Waeco). After incubation, 300 μL of ammonium glycine buffer (0.1 M, pH 10.5) was added to the samples and fluorescence emission was immediately recorded at 446 nm (excitation at 363 nm) using a Bowman Series 2 spectrofluorometer (SLM Aminco). Blank measurements allowed correcting for nonenzymatic hydrolysis of the substrate and/or fluorescent contaminants. The fluorescent product was quantified using a standard solution (MUF, 100 μM) added in increasing final concentrations (0–2.9 μM) to blanks.

The bacterial carbon productivity (BCP) of heterotrophic groundwater microorganisms was estimated via the incorporation of [*methyl*-^{3}H]-thymidine by adopting the protocols of Fuhrman & Azam (1982) and Kirschner & Velimirov (1997). [*methyl*-^{3}H]-thymidine (85 Ci mmol^{-1}, 1 mCi mL^{-1}, GE Healthcare) was added to triplicates of 30 mL groundwater directly in the field, to a final concentration of 10 nM. This saturation concentration was determined in preceding experiments. Samples were incubated at *in situ* temperatures (± 1 °C) in thermoboxes for 6 h. Label incorporation was determined to be linear for at least 8 h. Groundwater samples fixed with formaldehyde (3.7% final concentration) before substrate addition were run as blanks. Incubation of samples was stopped after 6 h with formaldehyde and stored at 4 °C in the dark until further analysis. Later, samples were treated with 1.5 mL of 100% ice-cold trichloroacetic acid (TCA) and placed on ice for 15 min. Before sample filtration, cellulose nitrate filters (0.2 μm, Whatman) were rinsed with ice-cold deionized water and an ice-cold 5% TCA solution. After sample filtration, the filters were repeatedly rinsed with ice-cold 5% TCA solution and deionized water. Filters were then placed in scintillation vials, and dried for 1 h at room temperature before dissolving them in 1 mL ethylacetate and shaking for 1 h. After the addition of scintillation cocktail (Ultima Gold XR, Perkin Elmer), samples were stored at 4 °C in the dark overnight and subsequently subjected to liquid scintillation counting on a Tri-carb 1600 TR (Perkin Elmer). Counts were manually corrected for quenching using a predetermined quench curve and a machine-counting efficiency program. The actual incorporation of label into DNA was determined to be 10% of the total [*methyl*-^{3}H]-thymidine uptake. Incorporation rates were converted to carbon production rates using conversion factors of 1×10^{18} cells mol^{-1} radiolabelled thymidine (Bell, 1990) and 20 fg carbon per cell (Griebler *et al.*, 2002).

Groundwater DNA extraction and Terminal restriction fragment length polymorphism (T-RFLP) analysis

Approximately 6 L of groundwater was sampled into autoclaved Duran bottles that were allowed to overflow. Samples were kept cool and dark during the time of sampling and transport, and were immediately filtered through 0.2-μm sterile membrane filters (Neolab) upon return to the lab. Filters were subsequently frozen and maintained at -20 °C. DNA was extracted from filters using a modification of a previously described protocol. Freshly thawed filters were aseptically cut into small pieces (*c.* 2 mm^2), which were then transferred into bead-beating cups and extracted as described in Winderl *et al.* (2008). After extraction and precipitation, DNA pellets were resuspended in 25 μL of EB buffer [10 mM Tris-HCl (pH 8.5), Qiagen] and stored frozen (-20 °C) until further analyses.

T-RFLP analysis of bacterial 16S rRNA gene amplicons was performed as described previously (Winderl *et al.*, 2008) with the primers Ba27f-FAM/907r and MspI digestion. Primary electropherogram analysis was performed using the GENEMAPPER 5.1 software (Applied Biosystems). Relative T-RF abundances were inferred from peak heights, and T-RFs with a peak height below 100 relative fluorescence units or with a peak area contribution $< 1\%$ were considered as background noise and excluded from further analysis (Lueders & Friedrich, 2003). The Shannon–Wiener index H' was calculated as $H' = -\Sigma pi \ln pi$, whereas pi is the relative abundance of single T-RF in a given fingerprint (Hill *et al.*, 2003).

The reproducibility of our workflow and of T-RFLP analysis was exemplarily verified via replicate fingerprinting analyses of duplicate DNA extracts from six of the wells sampled in April 2007 (U2, T1, T8, C3, C5 and C7). Community fingerprints and inferred T-RF abundances were shown to be highly reproducible. The total T-RF reappearance in replicate fingerprints was 64% (150 of 234 in total), whereas most nonreproducible T-RFs were just above the detection limit of 1% (1.3% abundance, in average) and thus likely to represent rare species or fingerprinting artifacts. The total average SD of the reproducible T-RFs was only 0.6% relative T-RF abundance (between 0% and maximally 5.4% peak abundance variation). The Mann–Whitney rank sum test was applied to test whether fingerprints from the duplicate DNA extracts were statistically different ($P < 0.05$). No statistically significant

difference for the replicated fingerprints from the six wells was detected.

Statistical analyses

Field data resulted in a dataset of 15 quantitative physicochemical variables and three quantitative biological variables: groundwater temperature, pH, electrical conductivity, redox potential, DO, DOC, SRP, major anions (HCO_3^-, Cl^-, NO_3^- and SO_4^{2-}), major cations (Na^+, K^+, Mg^{2+} and Ca^{2+}), and BA, BCP and bacterial EPA. For statistical analyses, variables failing the normality test (EC, DOC, Ca^{2+}, and BA) were log-normalized by adding a translation constant when necessary. All variables underwent a *z*-score standardization, resulting in dimensionless quantitative variables (Legendre & Legendre, 1998). In addition, two sets of biotic population data were generated: bacterial community data were expressed as relative abundance data of detected T-RFs, while the counts of recovered faunal populations in wells were square root-transformed as described by Clarke & Warwick (1994). Detected bacterial T-RFs, as well as invertebrate classes and orders, were treated as operational taxonomic units (OTUs) in subsequent analyses.

A variety of statistical analyses were applied to detect the potential impacts of temperature on the quantitative physicochemical and biological variables, as well as on bacterial community structure and faunal assemblages. In a first step, Spearman's rank correlation was used to test for the direct influence of temperature. Subsequently, forward stepwise linear regressions were deployed to predict the dependent variables BA, BCP and EPA as a function of the 15 physicochemical and population variables. One-way ANOVA was used to test for significant differences among the means of physicochemical parameters, BA and activities, as well as bacterial and faunal Shannon–Wiener diversity among wells. Common Mann–Whitney rank sum tests were applied to check for the reproducibility of T-RFs from independent DNA extracts. For both tests, samples were regarded as significantly different if P was < 0.05. To assess the similarity between bacterial and faunal communities obtained from different samples, distance matrices based on Bray–Curtis coefficients were calculated (Legendre & Legendre, 1998). The matrices served as an input for the MANOVA analysis to test whether bacterial and faunal communities were significantly different among wells. R indicates the separation in MANOVA (complete separation: $R = 1$; no separation: $R = 0$).

Canonical correspondence analysis (CCA), a direct constrained ordination method that uses linear combinations of environmental (explanatory) variables to maximally separate patterns of OTU distribution, was applied to optimally explain the observed variance in OTUs (Legendre & Legendre, 1998) and to investigate a hypothetical temperature dependence of bacterial and faunal assemblages. CCA was chosen because of its robustness towards the absence of OTUs in certain samples of a data set (Ramette, 2007), and towards relatively high numbers of OTUs compared with sampling locations (Legendre & Legendre, 1998). In addition, CCA is based on unimodal species–environment relationships. The combination of explanatory variables describing the most influential gradients was determined by applying the forward selection method outlined by ter Braak & Verdonschot (1995). The influence of rare bacterial OTUs on pattern discrimination was investigated by repeating CCA with different T-RF cut-offs defined by the number of times a specific T-RF appeared in the entire data set. Thus, CCA was repeated for data sets including T-RFs appearing ≥ 1, ≥ 5, ≥ 10 and ≥ 15 times in all samples.

Variables with a significant influence on the population structure were combined into variable categories for variance partitioning (Borcard *et al.*, 1992). This procedure allows to partition variations in bacterial and faunal assemblages into separate effects of categories and into their interactions using partial CCA (pCCA) and unbiased estimators of the variation fractions (Peres-Neto *et al.*, 2006). The significance of the variables and fractions was tested by ANOVA-like permutation tests. The congruence between the variability of bacterial communities and faunal assemblages was assessed by Procrustean superimposition (PROTEST) as outlined by Peres-Neto & Jackson (2001). The strength of the correlation between the two multidimensional scalings of faunal and bacterial communities is expressed in the Procrustes correlation statistic r. This correlation can then be assessed by permutation tests to indicate significant differences between the two projections.

All statistical analyses were performed with the R software, version 2.6.2, using the packages VEGAN 1.11-0, following the recommendations of the authors (Oksanen, 2008). T-RFLP (C3/11 and U4/8) and fauna results (C7/8, C6/5) from two samples each showed almost no consistency with the comprehensive data set and were therefore defined as outliers and excluded from further statistical analysis.

Results

Groundwater temperature and physicochemistry

Over the period of sampling, the reinjection of heated groundwater resulted in the development of a pronounced temperature plume at the field site. The spatial development of the temperature plume (Fig. 1), as well as the temperature amplitudes monitored within sampling wells (Fig. 2) were dependent on the seasonal fluctuation of cooling demand met by the discharge plant. During the study period, measured groundwater temperatures ranged from 8.5 to 17.8 °C (Fig. 2), the latter significantly exceeding the natural

mean annual groundwater temperature of *c.* 11 °C at the site. All groundwater temperatures above 12 °C were considered to be anthropogenically impacted. The extent of the heat plume was the smallest in April and the largest in November (Fig. 1). Generally, two of the eight monitoring wells were always unaffected by anthropogenic temperature influence (U2 and U4), two wells were temporarily impacted (T1 and T8) and four wells were continuously affected (C3, C5, C6 and C7). While the unaffected wells showed temperature fluctuations within ±1 °C of the mean natural groundwater temperature, the affected wells underwent temperature dynamics of up to ±5 °C (Fig. 2). For the upstream well T1, however, direct impacts by the investigated discharge facility cannot be assumed. Here, annual groundwater temperature dynamics indicated anthropogenic temperature influence at least in August, possibly connected to housing directly surrounding this well or other upstream anthropogenic temperature impacts.

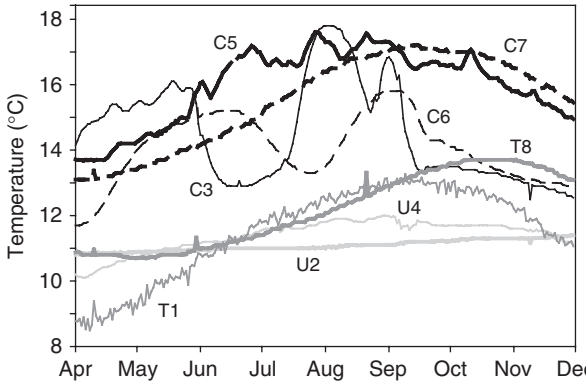

Fig. 2. Groundwater temperature dynamics within sampling wells at the thermal discharge site during the investigation period. Naming of wells is as in Fig. 1.

The physicochemical data of the site are indicative of an oxygenated, oligotrophic, oligoalimonic quaternary carbonate aquifer with low mean annual concentrations of DOC ($1.3 \pm 0.4\ mg\ L^{-1}$), SRP ($46 \pm 23\ \mu g\ L^{-1}$) and nitrate ($15.0 \pm 3.2\ mg\ L^{-1}$; Fig. 3, nitrate data not shown). DOC clearly peaked in August, while pH and DO were minimal in

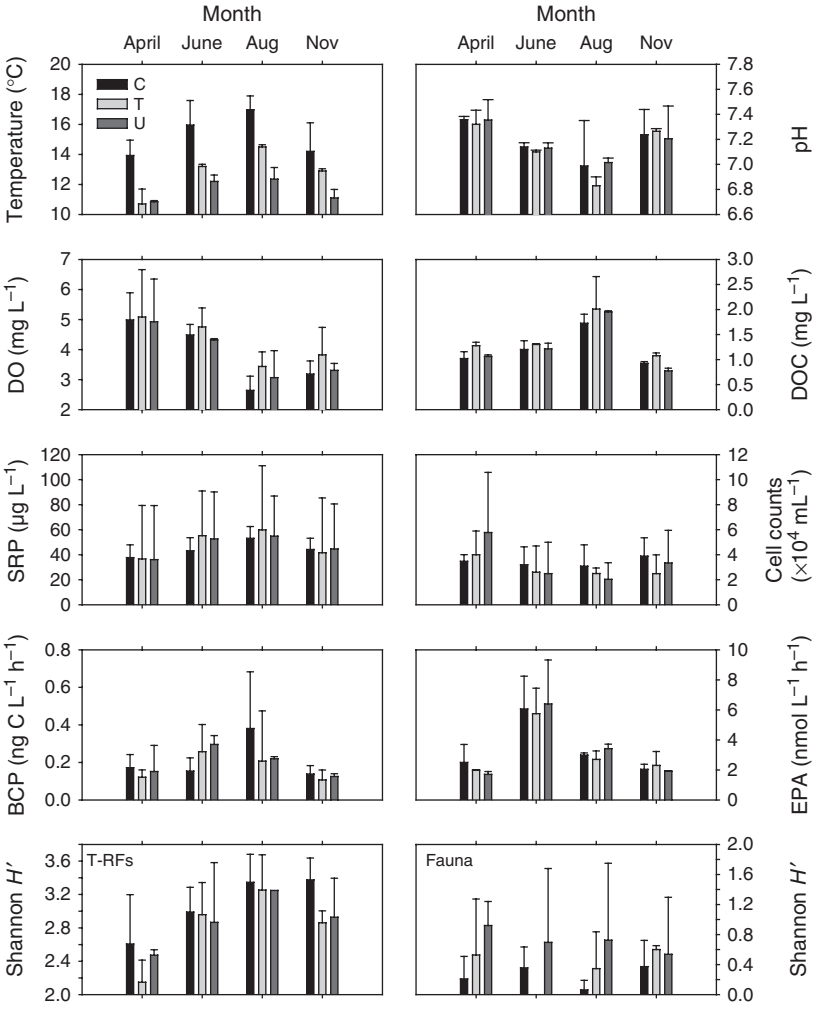

Fig. 3. Seasonal dynamics of selected physicochemical and biotic parameters in groundwater from sampling wells at the investigated field site. Monthly mean values for wells either unaffected (U), temporarily affected (T) or continuously affected (C) by heat discharge for the four sampling dates ±SD (three independent measurements per well) are shown. First sampling of fauna was in May 2007.

Table 1. Groundwater temperature, microbial activities and bacterial and faunal community indicators in the investigated wells

	Wells	U2	U4	T1	T8	C3	C6	C7	C5
	Temperature (°C)*	11.3 (0.6)	11.9 (1.0)	12.7 (1.9)	13.0 (1.2)	14.4 (1.6)	14.5 (2.2)	15.9 (1.5)	16.3 (1.8)
Bacteria*	Total counts (10^4 cells mL^{-1})	1.4 (0.7)	5.4 (2.7)	3.9 (1.1)	1.8 (0.7)	3.6 (0.8)	2.2 (0.5)	2.8 (0.5)	5.0 (1.0)
	BCP (ng C L^{-1} h^{-1})	0.21 (0.07)	0.19 (0.12)	0.17 (0.14)	0.18 (0.15)	0.34 (0.31)	0.21 (0.07)	0.13 (0.07)	0.16 (0.11)
	EPA (nmol L^{-1} h^{-1})	2.7 (1.3)	4.0 (3.1)	3.1 (1.1)	3.2 (2.5)	2.8 (1.0)	2.8 (1.5)	3.3 (2.0)	4.7 (3.0)
	Shannon H'	2.9 (0.4)	2.7 (0.5)	2.8 (0.6)	2.8 (0.5)	2.6 (0.3)	3.1 (0.6)	3.4 (0.2)	3.0 (0.6)
Drinking water	CFU (22 °C) (mL^{-1})	26	103	195	38	80	29	28	359
indicators†	CFU (27 °C) (mL^{-1})	47	131	304	51	138	57	43	779
	CFU (37 °C) (mL^{-1})	5	6	7	9	9	5	2	11
	Coliforms (in 250 mL)	–	–	4	–	–	–	–	–
	E. coli	–	1	–	–	–	–	1	–
Fauna*,†	Total counts (per well)	1.8 (1.0)	70.5 (78.9)	2.5 (1.3)	3.0 (1.8)	4.3 (2.1)	1.0 (0.8)	12.2 (20.5)	88.5 (83.2)
	Taxa	2.2 (0.5)	5.8 (0.5)	2.5 (0.6)	2.8 (1.0)	2.7 (0.6)	1.8 (0.5)	2.5 (0.6)	4.5 (0.6)
	Shannon H'	0.17 (0.3)	1.26 (0.2)	0.33 (0.4)	0.40 (0.5)	0.38 (0.3)	0.00 (0.0)	0.33 (0.4)	0.29 (0.1)

*Values represent the mean values out of four sampling dates (April, June, August and November 2007), SDs are given in parentheses.
†Microbiological drinking water indicators were monitored in May 2007. First sampling of fauna was also in May 2007.

summer. However, none of the monitored physicochemical parameters were found to be significantly (at $P < 0.05$) affected by or correlated to heat discharge, as revealed by Spearman rank correlations of the respective data.

Bacterial and faunal abundances and activities

BAs differed significantly between the wells (one-way ANOVA, $P < 0.001$). The mean BA over four sampling dates ranged from 1.4×10^4 cells mL^{-1} in well U2 to 5.4×10^4 in well U4 (Table 1). Both were unaffected by temperature, but U4 was located in the floodplains of the Isar River (Fig. 1). Again, groundwater temperature seemed to exert no significant influence on the total bacterial cell counts (Spearman's $\rho = 0.03$, $P = 0.86$). Instead, based on forward stepwise regression, BA was shown to be positively related to SRP concentrations ($P < 0.001$) and negatively related to DOC concentrations ($P = 0.016$).

Groundwater temperature alone was also not a key driver of bacterial carbon production (Spearman's $\rho = 0.06$, $P = 0.74$), which exhibited maximum and minimum seasonal values in August (0.81 ng C L^{-1} h^{-1} in well C3, and 0.02 ng C L^{-1} h^{-1} in well T1, respectively). BCP was generally higher in summer, although due to the high SDs, these differences were not significant (Fig. 3). In contrast, temperature appeared to be positively correlated to EPA (Spearman's $\rho = 0.45$, $P = 0.01$). In addition, stepwise linear regressions revealed that phosphatase activity was predictable by a combination of groundwater temperature, Mg^{2+} and HCO_3^-. With season, phosphatase activity varied significantly (one-way ANOVA, $P < 0.001$) and peaked in June (Fig. 3).

In addition to our regular samplings, a survey of standard microbiological drinking water indicators was performed for the different wells in May 2007 (Table 1). The legal thresholds for total CFUs at 22 and 27 °C of the drinking water directive (100 CFU mL^{-1}) were exceeded in three (22 °C) and four (27 °C) wells, respectively, and *E. coli* or coliforms were found in three wells. Very likely, these detections could be linked to local impacts of housing, agriculture or the Isar River. However, neither total CFUs nor coliforms and *E. coli* counts were significantly related to groundwater temperature.

Faunal abundances were generally low (Table 1), with often only one or two taxa present. One sample was devoid of fauna (C6 in May 2007). Among the detected groundwater fauna, especially niphargids (*Amphipoda*) were present in substantial numbers in some wells, for example in wells C5 and U4, where between 50 and 212 individuals were counted on some samplings. Other frequently detected taxa were affiliated to isopods, ostracods and cyclopoids.

Bacterial and faunal diversity

T-RFLP fingerprinting was used to monitor the diversity and structure of groundwater bacterial communities. The mean annual Shannon–Wiener diversity H' inferred from the fingerprints did not differ significantly between the investigated wells (one-way ANOVA, $P = 0.38$) and varied from 2.6 in well C3 to 3.4 in well C7 (Table 1). However, groundwater temperature appeared to weakly but significantly (Spearman's $\rho = 0.46$, $P = 0.01$) affect bacterial diversity, resulting in generally increased diversity at elevated temperatures (Fig. 4). Consequently, the average H' for all wells was the highest during August, when the extent and temperature of the heat plume were maximal (Fig. 3). However, the diversity in the unaffected wells was also clearly increased in August. The apparently higher bacterial diversity in impacted wells was, to a large extent, based on the presence of rare T-RFs. Of the total of 109 distinct T-RFs

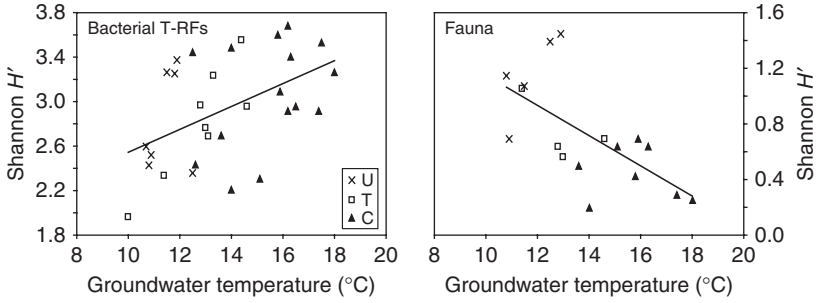

Fig. 4. Shannon–Wiener diversity H' of bacterial T-RFLP fingerprints and fauna samplings in relation to groundwater temperature. The ordination discriminates wells unaffected (U), temporarily affected (T) or continuously affected (C) by heat discharge.

detected within this study, 50% appeared in only five or less fingerprints, and 17% were found only once. However, these rare T-RFs were normally of low relative abundance (*c.* 1.7% in average). In contrast, 25% of the T-RFs were conserved in more than half of the fingerprints and identified to contribute more abundant constituents of the groundwater bacterial community.

For groundwater fauna, sometimes only one taxon was found in some wells, as a consequence of which H' became 0. This already indicates that the distribution of specific groundwater fauna was much patchier than that of dominant bacterial OTUs. Nevertheless, faunal H' indicated highly significant differences among wells (one-way ANOVA, $P = 0.001$). A pairwise comparison showed that the diversity in U4, the well closest to the Isar River, was significantly different from all others (Table 1). This well generally yielded high numbers of faunal taxa. Overall Shannon diversity ranged from 0.2 (C5 in August) to 1.45 (U4 in August). As for bacteria, groundwater temperature appeared to significantly affect faunal diversity (Spearman's $\rho = -0.67$, $P = 0.004$), but conversely resulted in decreased diversity at elevated temperatures (Fig. 4). Also in contrast to bacteria, no significant general seasonal dynamics were observed for faunal diversity (Fig. 3). To test whether there was any significant correlation between faunal and bacterial communities, a Procrustes rotation with a subsequent permutation test was performed. The correlation between bacterial and faunal diversity was low and not significant ($r = 0.085$; $P = 0.97$).

Multivariate analyses of temperature effects on groundwater bacteria and fauna

Significant differences in the structure of bacterial and faunal communities were observed between wells (MANOVA, $R = 0.64$ and $R = 0.71$, $P < 0.01$, respectively). CCA was applied to explain the observed variance in OTU distribution and to unravel the hypothetical temperature dependencies of bacterial and faunal assemblages. For bacteria, CCA revealed concentrations of calcium, magnesium, potassium and groundwater temperature to be the strongest determinants of community composition as monitored via T-RF distribution (Table 2). The concentrations of SRP and temperature were key determinants for faunal populations. This indicates that both bacterial and faunal community variation was mainly influenced by two environmental gradients. The first, most dominant gradient was caused by infiltrating surface water of the nearby river. In CCA ordination (Fig. 5), this gradient is indicated by the dilution of calcium, magnesium and potassium ions with increasing influence of surface water. Consequently, samples from well U4, located adjacent to the Isar River, are ordinated towards lower concentrations of Ca^{2+}, Mg^{2+} and K^{+}, thus substantiating the computed model. For fauna, as shown by CCA (Fig. 6), the influence of surface water is indicated by higher phosphorus concentrations. Here, U4 tended to ordinate towards the upper concentrations of SRP.

Table 2. Key determinant parameters for groundwater bacterial and faunal community variance as identified by forward selection in CCA

	Variable	λ	*P*	N_P
Bacteria				
1	Ca^{2+}	0.18	0.005	200
2	Mg^{2+}	0.17	0.005	200
3	K^{+}	0.15	0.020	400
4	T	0.12	0.040	2400
Fauna				
1	SRP	0.19	0.033	1300
2	T	0.17	0.053	10 000

λ, Eigenvalues inferred for controlling ion concentrations and temperature; *P*, significance of correlations inferred from CCA; *N*, number of permutations.

For both bacterial and faunal populations, temperature was identified as the second dominant driver of the observed community variation, besides surface water influence (Table 2). For bacteria, samples from wells in the continuously temperature-impacted zone (especially C5 and C7) were mostly ordinated in the high-temperature range (Fig. 5), again supporting the validity of the deduced CCA model. The influence of the two controlling gradients on the variation of total bacterial community composition was quantified by variance partitioning. Thus, groundwater temperature accounted for 5% of T-RF variability, and 'surface water-related' variables explained 15% of the

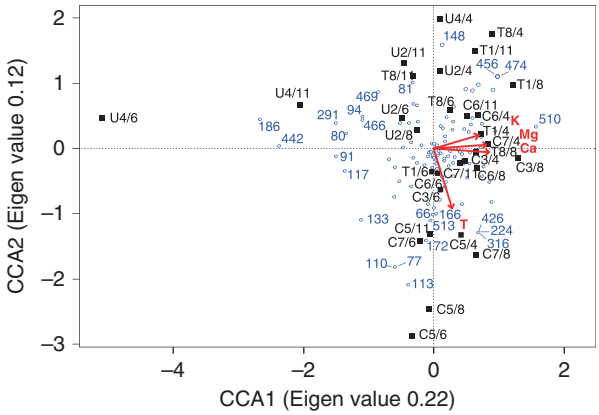

Fig. 5. Species-conditional triplot based on CCA of bacterial community data comprising 14% of the inertia (weighted variance) in T-RF abundances and 69% of variance in the weighted averages and class totals of T-RFs with respect to environmental variables. Quantitative environmental variables are indicated by arrows and show the direction of the increase of each variable. The length of the arrow indicates the degree of correlation with the ordination axes. Fingerprints from well samplings are indicated by black squares and are labelled in accordance to Fig. 1 and the sampling month. Only selected specific T-RFs (blue circles) with OTU scores in CCA > |1| are displayed; some are labelled with the respective T-RF length (bp), ordination space allowing.

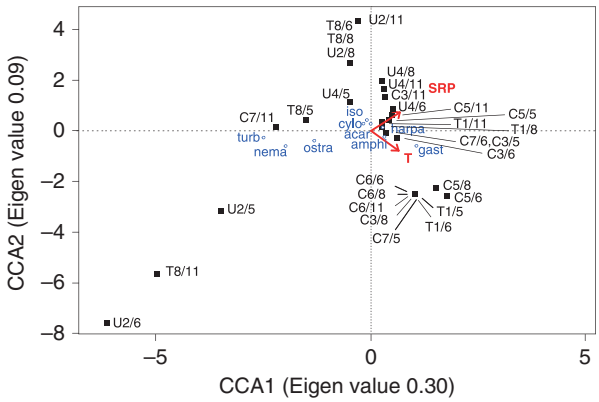

Fig. 6. Species-conditional triplot based on CCA of faunal community data comprising 15% of the inertia (weighted variance) in faunal abundances and 100% of variance in the weighted averages and class totals of OTUs with respect to environmental variables. Quantitative environmental variables and well samplings are indicated as in Fig. 5. Faunal OTUs are indicated by blue circles and abbreviated as follows: acari, *Acari*; amphi, *Amphipoda*; cyclo, *Cyclopoida*; gast, *Gastropoda*; harpa, *Harpacticoida*; iso, *Isoptera*; nema, *Nematoda*; ostra, *Ostracoda*; and turb, *Turbellaria*.

observed T-RF variation. While there was no covariation of groundwater temperature and 'surface water-related' variables, 80% of the observed bacterial community variation remained unexplained. The influence of rare T-RFs on the results of the CCA was tested by using different cut-offs for T-RF data to be included in CCA. Consecutively, T-RFs appearing in < 1, 5, 10 or 15 samples were omitted from CCA, and the respective results were compared with CCA including all T-RFs (Table 3). Except for the $n \leq 10$ cut-off, all models identified groundwater temperature as a significant determinant of bacterial community variation (Table 3). This clearly illustrates the robustness of our CCA approach, irrespective of whether rare T-RFs are included or not.

Table 3. Key determinant parameters for groundwater bacterial community variance identified in repetitive CCA with increasing cut-offs for T-RF conservation

T-RF appearance cut-off (n)*	Significant determinant variables identified in CCA ($P \leq 0.05$)	$\Sigma\lambda^{\dagger}$
1	Ca^{2+}, Mg^{2+}, T, K^{+}	0.63
5	Mg^{2+}, K^{+}, Ca^{2+}, T, Na	0.50
10	Ca^{2+}, Mg^{2+}, K^{+}, SRP, Na^{+}	0.25
15	T	0.04

*Numbers indicate the minimal reappearance criterion for given T-RFs in the entire data set to be included into CCA.

†$\Sigma\lambda$, summarized Eigenvalues inferred for controlling ion concentrations and temperature.

For the fauna, SRP concentrations and groundwater temperature each accounted for 8% of the observed variability. The covariation of temperature-related and surface water-related variables was negative (-1%) and was therefore set to 0 (Legendre, 2007). Accordingly, 84% of the faunal variation remained unexplained. In the ordination of faunal distribution, none of the faunal OTUs tended heavily towards the extremes of any variable, indicating that although faunal assemblages were well described by the two variable sets, the data contained considerable noise. This noise may partly stem from the fact that faunal abundance and diversity was generally low and patchy, and that fauna was not resolved to a more detailed genus or species level.

Specific T-RF abundance in relation to temperature

The screening of bacterial T-RF frequencies based on the results of CCA (Fig. 5) identified three major types of correlations to groundwater temperature: (1) Some T-RFs were detected preferentially at natural groundwater temperatures (e.g. the 81-, 88-, 94-, 148-, 466- and 469-bp T-RFs, Fig. 5). (2) Furthermore, a variety of T-RFs were preferentially detected at elevated groundwater temperatures (e.g. the 66-, 77-, 110-, 113-, 166-, 172-, 224-, 316-, 426- and 513-bp T-RFs). (3) A third and actually the most common T-RF type appeared to be more or less unaffected by temperature and was detected in similar frequencies over the entire temperature range, such as the 74-, 84-, 91-, 123-, 153- and 160-bp T-RFs. The ordination of the different

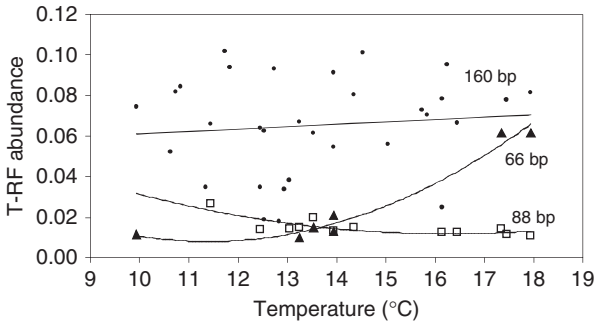

Fig. 7. Temperature-dependent abundance distribution of selected T-RFs detected in different wells and at different time points of sampling.

T-RFs towards higher, lower or median temperature ranges in CCA is indicative of this grouping (Fig. 5).

Although most of these candidate 'signature' T-RFs were rarely occurring (≤ 5 recoveries), some of the more-conserved T-RFs showed significant changes in relative abundance depending on the temperature. As examples, the 66-bp (positive correlation, Spearman's $\rho = 0.90$, $P < 0.001$) or 88-bp T-RFs (negative correlation, Spearman's $\rho = -0.75$, $P = 0.004$), as well as the temperature-independent distribution of the ubiquitous and generally more abundant 160-bp T-RF are plotted (Fig. 7).

Discussion

Temperature changes are known to affect a variety of aquifer geochemical reactions, depending on the water and sediment characteristics (Brons *et al.*, 1991; Griffioen & Appelo, 1993; Stumm & Morgan, 1995; Arning *et al.*, 2006). However, most of these reactions are of little or no importance within the temperature variation encountered in this study (8.5–17.8 °C). Thus, it is not surprising that we did not detect any significant effects of heat discharge on physicochemical aquifer characteristics. Rather, the dynamics observed for some of these parameters were caused by the influence of seasonal groundwater recharge and surface water infiltration, than by temperature changes.

Also, BA and productivity showed no direct correlation with groundwater temperature. Both parameters were at the lower range of values reported for uncontaminated aquifers (Griebler & Mösslacher, 2003; Johnson *et al.*, 2004; Goldscheider *et al.*, 2006). Instead, BA was positively related to concentrations of SRP and negatively correlated to concentrations of DOC, which may indicate enhanced microbial growth and the exhaustion of DOC at higher phosphorus concentrations, and thus nutrient limitation of the groundwater microorganisms. Also, the total CFU counts and the detection of coliforms appeared to be unconnected to the heat plume. Here, the impacts of local housing, agriculture or the Isar River can be hypothesized to exert a much stronger influence on groundwater hygienic parameters.

This lack of a clear relation between groundwater temperatures and bacterial counts and activities is likely to be related to the very oligotrophic and oligoalimonic nature of the investigated aquifer. Thus, energy limitation can be hypothesized to prevent the influence of temperature on bacteria, which may well be expected for more productive systems. However, we have to clearly state that some uncertainty in this interpretation arises from the extremely low bacterial activities encountered, which were just above the sensitivity limits of the applied assay. Although thymidine incorporation is a very sensitive assay, activities in pristine groundwater can be close to detection limits (Alfreider *et al.*, 1997). For future studies, considerable effort should thus be focused on improving such generic activity indicators.

Different from the abundance and activity of groundwater microorganisms, bacterial diversity clearly increased with temperature, which was accompanied by the appearance of specific OTUs and the disappearance of others. Hypothetically, some of these OTUs may represent community components that are more competitive for available resources under elevated temperatures (Hall *et al.*, 2008), and that will not be equally thriving in natural aquifers. Unfortunately, we have not been able to identify any of these OTUs by cloning and sequencing, or by cultivation approaches in the present study. Certainly, the identification of such putative indicator bacteria will be the focus of our future research.

Although temperature was shown to be an important driver of bacterial diversity, a more relevant impact on bacterial populations resulted from infiltrating surface water as shown by CCA. The exchange of surface and groundwater through the hyporheic zone is known to undergo seasonal dynamics and to introduce gradients of physicochemical conditions, nutrients and organic matter (Storey *et al.*, 2003; Hunt *et al.*, 2006). Thus, surface water influence can be expected to induce strong seasonal shifts in groundwater organismic populations, which are otherwise assumed to be rather stable over time (Gsell *et al.*, 1997; Dumas, 2002).

Nevertheless, beyond temperature and surface water influence, by far the most important fraction of bacterial community variation observed remained unexplained. This indicates that probably, the most important drivers of aquifer bacterial community dynamics at the site were not covered by our analyses, or that temporal variability and spatial heterogeneity in uncontaminated aquifers is much higher than expected (Griebler & Lueders, 2008). In our study, only groundwater sampling was conducted, mainly due to limited possibilities for sediment sampling at the site. However, attached microorganisms are generally more abundant in aquifers than planktonic ones (Alfreider *et al.*,

1997; Griebler *et al.*, 2002). Thus, temperature effects can be hypothesized to be more pronounced for sedimentary aquifer microbial communities.

We used relativized T-RF peak abundances to identify the effects of temperature on groundwater bacterial communities. This approach was chosen to de-emphasize the influence of 'rare' or low-abundance T-RFs on statistical results, and to focus on the more dominant community constituents in interpretation. As shown by repetitive CCA, with increasing T-RF reappearance cut-off (Table 3), the key determinants of groundwater bacterial community composition were readily identified even with increasing numbers of less widely distributed T-RFs excluded from the models. As reported here and elsewhere (Fahy *et al.*, 2005; Euringer & Lueders, 2008), relative T-RF abundances can be effectively reproduced for aquifer microbial communities. However, it has been shown in other studies that the use of binary T-RFLP data sets can provide even more robust results in the interpretation of T-RFLP data sets (Culman *et al.*, 2008; Enwall & Hallin, 2009). Therefore, the use of relativized T-RF abundance in statistical models should always be carefully considered and used with caution (Thies, 2007).

A further cautioning statement concerning the interpretation of our results with respect to on-site variability and heterogeneity is that true negative controls for the impacted wells, as would have been possible only by investigating all wells in the absence of actual temperature impacts, were unfeasible. Nevertheless, we are confident that our sampling scheme of temperature-impacted and nonimpacted wells as well as the elaborate applied statistics warrant an ecological interpretation of our results within acceptable confidence limits.

No congruence was detected between bacterial and faunal community descriptors, even though groundwater microorganisms are a food source for groundwater invertebrates (Griebler & Mösslacher, 2003; Datry *et al.*, 2005). For fauna, the identification of potential temperature impacts may be more difficult to address, especially because populations were very patchy and fauna was not resolved to ultimate taxonomic detail. It is likely that a number of species with potentially opposing temperature preferences were present, for example, among the cyclopoids. In contrast, the amphipod populations recovered in high numbers in some wells did not appear to sustain temperature preferences.

Faunal abundance and diversity at the site were comparable to that in similar habitats (Datry *et al.*, 2005; Fuchs, 2007). While faunal abundance showed no relation to impacted groundwater temperatures, faunal diversity decreased with temperature, possibly emphasizing the sensitivity of individual groundwater invertebrates towards heat discharge. This correlation, however, must be interpreted with caution as most of the high-diversity samples were from the temperature-unaffected well close to the Isar River. Nevertheless, in contrast to bacterial communities, which are characterized by more or less ubiquitous dispersal and a high functional redundancy, groundwater fauna is not readily replaced by temperature-tolerant counterparts. True groundwater invertebrates (stygobites) are assumed to be cold stenotherm and can hardly persist at water temperatures exceeding 16 °C for extended periods of time (T. Weber & S.I. Schmidt, unpublished data). Because groundwater invertebrates were previously found to react sensitively to stressors such as oxygen depletion (Mösslacher, 1998; Malard & Hervant, 1999) or organic pollution (Sinton, 1984; Culver *et al.*, 1992), specific representatives of groundwater fauna may also serve as very sensitive indicators for temperature impacts. However, as for bacteria, by far the most important fraction of faunal variation encountered remained unexplained. Groundwater fauna in pristine aquifers is of generally low abundance and diversity, and distributed in very patchy patterns dependent on the local resources' availability (Gibert, 2001; Datry *et al.*, 2005) and hydrodynamics (Dumas *et al.*, 2001; Hahn, 2006; Schmidt *et al.*, 2007). Obviously, this complicates the unambiguous establishment of faunal indicators for groundwater ecosystem stress.

In summary, we show that elevated groundwater temperatures clearly impacted groundwater bacterial and faunal community composition and diversity at the investigated site. At the same time, at least for bacteria, we provide no evidence for the effects of these population shifts on aquifer integrity and ecosystem functions. Generally, it is assumed that a low level of biodiversity may allow for stable ecosystem functions (i.e. in an unaffected groundwater ecosystem), but that a greater biodiversity may be called for in fluctuating environments (Humbert & Dorigo, 2005). In our case, bacterial diversity was considerable *ex ante* in the unaffected aquifer, and further increased under temperature impact. This is consistent with the intermediate disturbance hypothesis, which predicts that microbial diversity in natural systems may peak at intermediate intensities or frequencies of small-scale disturbances (Lake, 2000; Ibekwe *et al.*, 2002). Profound shifts in community composition at similar levels of diversity and richness or at increasing biodiversity have also been detected upon anthropogenic perturbations in other groundwater-related studies (Cho & Kim, 2000; Röling *et al.*, 2001; Johnson *et al.*, 2004).

Because of the lack of scientific studies investigating the impact of heat discharge on groundwater ecosystems, consistent guidelines for the authorization of such facilities exist at present. In Switzerland, temperature deviations of not more than 3 °C from the mean natural temperatures are normally approved. In Germany, temperature changes of up to ± 6 °C are usually considered acceptable (VDI, 2004). Based on our findings, this *modus operandi* of ± 6 °C temperature deviation seems definitely approvable for

bacterial indicators of groundwater ecosystem functioning. For the more elusive and patchy groundwater fauna, more dedicated research is certainly called for.

Evidently, these interpretations can be expected to hold true only for the temperature ranges operative at the investigated site (reinjection at a maximum of 21 °C) and for systems comparable to the examined oligotrophic aquifer. This concept may not apply to groundwater systems with an elevated concentration of organic substrates and nutrients, and it also has to be verified towards more elevated temperature limits of groundwater thermal energy use. For planktonic microorganisms in a pond ecosystem, it has been shown that the direct effects of warming were far less important than the nutrient effects, and that a combination of warming and nutrients triggered very complex dynamics (Christoffersen *et al.*, 2006). Hence, temperature impacts for contaminated groundwater systems remain to be further established.

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References

Adinolfi M, Koch M & Ruck W (1994) *Ökologische und mikrobielle Folgen der Wärmespeicherung im Aquifer. Stuttgarter Berichte zur Siedlungswasserwirtschaft*, *124*. Oldenbourg Industrieverlag GmbH, Münich, pp. 89–106.

Alfreider A, Krössbacher M & Psenner R (1997) Groundwater samples do not reflect bacterial densities and activity in subsurface systems. *Water Res* **31**: 832–840.

Arning E, Kölling M, Schulz HD, Panteleit B & Reichling J (2006) Einfluss oberflächennaher Wärmegewinnung auf geochemische Prozesse im Grundwasserleiter. *Grundwasser* **11**: 27–39.

Bell RT (1990) An explanation for the variability in the conversion factor deriving bacterial cell production from incorporation of [^{3}H]thymidine. *Limnol Oceanogr* **35**: 910–915.

Boehme D, Duerrschmidt W, Staiß F, Linkohr C & Kratzat M (2007) *Entwicklung der erneuerbaren Energien im Jahr 2006 in Deutschland*. Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Referat KI III 1, Germany.

Borcard D, Legendre P & Drapeau P (1992) Partialling out the spatial component of ecological variation. *Ecology* **73**: 1045–1055.

Brons HE, Griffioen J, Appelo CAJ & Zehnder AJB (1991) (Bio)geochemical reactions in aquifer material from a thermal energy storage site. *Water Res* **25**: 729–736.

Cho JC & Kim SJ (2000) Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input. *Appl Environ Microb* **66**: 956–965.

Christoffersen K, Andersen N, Sondergaard M, Liboriussen L & Jeppesen E (2006) Implications of climate-enforced temperature increases on freshwater pico- and nanoplankton populations studied in artificial ponds during 16 months. *Hydrobiologia* **560**: 259–266.

Clarke KR & Warwick RM (1994) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*. Natural Environment Research Council, UK.

Culman SW, Gauch HG, Blackwood CB & Thies JE (2008) Analysis of T-RFLP data using analysis of variance and ordination methods: a comparative study. *J Microbiol Meth* **75**: 55–63.

Culver DC, Jones WK & Holsinger JR (1992) Biological and hydrological investigation of the Cedars, Lee County, Virginia, an ecologically significant and threatened karst area. *Ground Water Ecology* (Stanford JA & Simons JJ, eds), pp. 281–290. American Water Resources Association, Bethesda, MD.

Datry T, Malard F & Gibert J (2005) Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer. *J N Am Benthol Soc* **24**: 461–477.

Dumas P (2002) Stability of interstitial crustacean communities in an isolated alluvial aquifer. *Hydrobiologia* **468**: 63–76.

Dumas P, Bou C & Gibert J (2001) Groundwater macrocrustaceans as natural indicators of the Ariege alluvial aquifer. *Int Rev Hydrobiol* **86**: 619–633.

Enwall K & Hallin S (2009) Comparison of T-RFLP and DGGE techniques to assess denitrifier community composition in soil. *Lett Appl Microbiol* **48**: 145–148.

Euringer K & Lueders T (2008) An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. *J Microbiol Meth* **75**: 262–268.

Fahy A, Lethbridge G, Earle R, Ball AS, Timmis KN & McGenity TJ (2005) Effects of long-term benzene pollution on bacterial diversity and community structure in groundwater. *Environ Microbiol* **7**: 1192–1199.

Fuchs A (2007) Erhebung und Beschreibung der Grundwasserfauna in Baden-Württemberg. PhD Thesis, University Koblenz-Landau, Landau, Germany.

Fuhrman J & Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar Biol* **66**: 109–120.

Gibert J (2001) Basic attributes of groundwater ecosystems. *Groundwater Ecology – A Tool for Management of Water Resources* (Griebler C, Danielopol DL, Gibert J, Nachtnebel HP & Notenboom J, eds), pp. 39–54. Office for Official Publications of the European Communities, Luxembourg.

Goldscheider N, Hunkeler D & Rossi P (2006) Microbial biocenoses in pristine aquifers and an assessment of investigative methods. *Hydrogeol J* **14**: 926–941.

Griebler C & Lueders T (2008) Microbial biodiversity in groundwater ecosystems. *Freshwater Biol* **54**: 649–677.

Griebler C & Mösslacher F (2003) *Grundwasser-Ökologie*. Facultas Universitätsverlage, Vienna, Austria.

Griebler C, Mindl B, Slezak D & Geiger-Kaiser M (2002) Distribution patterns of attached and suspended bacteria in pristine and contaminated shallow aquifers studied with an *in situ* sediment exposure microcosm. *Aquat Microb Ecol* **28**: 117–129.

Griffioen J & Appelo CAJ (1993) Nature and extent of carbonate precipitation during aquifer thermal energy storage. *Appl Geochem* **8**: 161–176.

Gsell TC, Holben WE & Ventullo RM (1997) Characterization of the sediment bacterial community in groundwater discharge zones of an alkaline fen: a seasonal study. *Appl Environ Microb* **63**: 3111–3118.

Hahn HJ (2006) A first approach to a quantitative ecological assessment of groundwater habitats: the GW-Fauna-Index. *Limnologica* **36**: 119–137.

Hall EK, Neuhauser C & Cotner JB (2008) Toward a mechanistic understanding of how natural bacterial communities respond to changes in temperature in aquatic ecosystems. *ISME J* **2**: 471–481.

Hendel B, Marxsen J, Fiebig D & Preuss G (2001) Extracellular enzyme activities during slow sand filtration in a water recharge plant. *Water Res* **35**: 2484–2488.

Hill TCJ, Walsh KA, Harris JA & Moffett BF (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol Ecol* **43**: 1–11.

Hoppe H-G (1993) Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. *Handbook of Methods in Aquatic Microbial Ecology* (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds), pp. 423–431. Lewis Publishers, Boca Raton, FL.

Humbert JF & Dorigo U (2005) Biodiversity and aquatic ecosystem functioning: a mini-review. *Aquat Ecosyst Health Manag* **8**: 367–374.

Hunt RJ, Strand M & Walker JF (2006) Measuring groundwater–surface water interaction and its effect on wetland stream benthic productivity, Trout Lake watershed, northern Wisconsin, USA. *J Hydrol* **320**: 370–384.

Ibekwe AM, Kennedy AC, Frohne PS, Papiernik SK, Yang CH & Crowley DE (2002) Microbial diversity along a transect of agronomic zones. *FEMS Microbiol Ecol* **39**: 183–191.

Johnson A, Llewellyn N, Smith J, van der Gast C, Lilley A, Singer A & Thompson I (2004) The role of microbial community composition and groundwater chemistry in determining isoproturon degradation potential in UK aquifers. *FEMS Microbiol Ecol* **49**: 71–82.

Kirschner AKT & Velimirov B (1997) A seasonal study of bacterial community succession in a temperate backwater system, indicated by variation in morphotype numbers, biomass and secondary production. *Microb Ecol* **34**: 27–38.

Lake PS (2000) Disturbance, patchiness, and diversity in streams. *J N Am Benthol Soc* **19**: 573–592.

Legendre P (2007) Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *J Plant Ecol* **1**: 3–8.

Legendre P & Legendre L (1998) *Numerical Ecology*. Elsevier Science BV, Amsterdam, the Netherlands.

Lueders T & Friedrich MW (2003) Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and *mcrA* genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. *Appl Environ Microb* **69**: 320–326.

Lunau M, Lemke A, Walther K, Martens-Habbena W & Simon M (2005) An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environ Microbiol* **7**: 961–968.

Lund JW, Sanner B, Rybach L, Curtis R & Hellström G (2004) Geothermal (ground-source) heat pumps – a world overview. *Geo-Heat Center Quart Bull* **25**: 1–10.

Malard F & Hervant F (1999) Oxygen supply and the adaptation of animals in groundwater. *Freshwater Biol* **41**: 1–30.

Martens-Habbena W & Sass H (2006) Sensitive determination of microbial growth by nucleic acid staining in aqueous suspension. *Appl Environ Microb* **72**: 87–95.

McIntyre C, McRae C, Batts BD & Piccolo A (2005) Structural characterisation of groundwater hydrophobic acids isolated from the Tomago Sand Beds, Australia. *Org Geochem* **36**: 385–397.

Mösslacher F (1998) Subsurface dwelling crustaceans as indicators of hydrological conditions, oxygen concentration and sediment structure in an alluvial aquifer. *Int Rev Hydrobiol* **83**: 349–364.

Murphy J & Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* **27**: 31–36.

Oehmichen U, Schmitz M & Seeliger P (2003) *Die neue Trinkwasserverordnung: Der Kommentar aus rechtlicher und technisch-wirtschaftlicher Sicht*. Wirtschafts- und Verlagsgesellschaft Gas und Wasser mbH, Bonn, Germany.

Oksanen J (2008) Multivariate analysis of ecological communities in R: vegan tutorial. Available at http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf, Last accessed 24 March 2009.

Parsons ML (1970) Groundwater thermal regime in a glacial complex. *Water Resour Res* **6**: 1701–1719.

Peres-Neto PR & Jackson DA (2001) How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* **129**: 169–178.

Peres-Neto PR, Legendre P, Dray S & Borcard D (2006) Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* **87**: 2614–2625.

Pomeroy LR & Wiebe WJ (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquat Microb Ecol* **28**: 187–204.

Pote DH & Daniel TC (2000) Analyzing for dissolved reactive phosphorus in water samples. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Southern Coop. Series Bulletin No. 396* (Pierzynski GM, ed), pp. 91–93. Southern Extension/Research Activity-Information Exchange Group (SERA-IEG-17), Kansas State University, Manhattan, KS.

Ramette A (2007) Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol* **62**: 142–160.

Reeves JM, Deckker PD & Halse SA (2007) Groundwater ostracods from the arid Pilbara region of northwestern Australia: distribution and water chemistry. *Hydrobiologia* **585**: 99–118.

Röling WFM, van Breukelen BM, Braster M, Lin B & van Verseveld HW (2001) Relationships between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer. *Appl Environ Microb* **67**: 4619–4629.

Ruck W, Koch M & Adinolfi M (1993) *Die Kombination von Wärmespeicherung und Altlastensanierung. Stuttgarter Berichte zur Siedlungswasserwirtschaft*, *124*. Oldenbourg Industrieverlag GmbH, Munich, pp. 117–126.

Schippers A & Reichling J (2006) Laboruntersuchungen zum Einfluss von Temperaturveränderungen auf die Mikrobiologie des Untergrundes. *Grundwasser* **11**: 40–45.

Schmidt SI, Hahn HJ, Hatton TJ & Humphreys WF (2007) Do faunal assemblages reflect the exchange intensity in groundwater zones? *Hydrobiologia* **583**: 1–17.

Silliman SE & Booth DF (1993) Analysis of time-series measurements of sediment temperature for identification of gaining vs. losing portions of Juday Creek, Indiana. *J Hydrol* **146**: 131–148.

Sinton LW (1984) The macroinvertebrates in a sewage-polluted aquifer. *Hydrobiologia* **119**: 161–169.

Storey RG, Howard KWF & Williams DD (2003) Factors controlling riffle-scale hyporheic exchange flows and their seasonal changes in gaining stream: a three-dimensional groundwater flow model. *Water Resour Res* **39**: 1034.

Stumm W & Morgan JJ (1995) *Aquatic Chemistry*. John Wiley & Sons, New York, NY.

ter Braak CJF & Verdonschot PFM (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquat Sci* **57**: 255–289.

Thies JE (2007) Soil microbial community analysis using terminal restriction fragment length polymorphisms. *Soil Sci Soc Am J* **71**: 579–591.

VDI (2000) Thermal use of the underground – fundamentals, approvals, environmental aspects, VDI-Richtlinien 4640, Part I. *VDI/DIN Handbuch Energietechnik*. Beuth Verlag, Berlin.

VDI (2004) Thermal use of the underground – direct uses, VDI-Richtlinien 4640, Part 4 *VDI/DIN Handbuck Energietechnik*. Beuth Verlag, Berlin.

Ward JV & Voelz NJ (1994) Groundwater fauna of the South Platte River System, Colorado. *Groundwater Ecology* (Gibert J, Danielopol DL & Stanford JA, eds), pp. 391–423. Academic Press, San Diego, CA.

Winderl C, Anneser B, Griebler C, Meckenstock RU & Lueders T (2008) Depth-resolved quantification of anaerobic toluene degraders and aquifer microbial community patterns in distinct redox zones of a tar oil contaminant plume. *Appl Environ Microb* **74**: 792–801.

York KP, Jahangir ZMGS, Solomon T & Stafford L (1998) Presented at the 2nd Stockton International Geothermal Conference, The Richard Stockton College of New Jersey, Pomona, NJ.