RESEARCH ARTICLE

Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer

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Abstract

Seasonal patterns of groundwater and sediment microbial communities were explored in a hydrologically dynamic alpine oligotrophic porous aquifer, characterized by pronounced groundwater table fluctuations. Rising of the groundwater level in consequence of snow melting water recharge was accompanied by a dramatic drop of bacterial Shannon diversity in groundwater from $H' = 3.22 \pm 0.28$ in autumn and winter to $H' = 1.31 \pm 0.35$ in spring and summer, evaluated based on T-RFLP community fingerprinting. Elevated numbers of bacteria in groundwater in autumn followed nutrient inputs via recharge from summer rains and correlated well with highest concentrations of assimilable organic carbon. Sterile sediments incubated to groundwater in monitoring wells were readily colonized reaching maximum cell densities within 2 months, followed by a consecutive but delayed increase and leveling-off of bacterial diversity. After 1 year of incubation, the initially sterile sediments exhibited a similar number of bacteria and Shannon diversity when compared to vital sediment from a nearby river incubated in parallel. The river bed sediment microbial communities hardly changed in composition, diversity, and cell numbers during 1 year of exposure to groundwater. Summing up, the seasonal hydrological dynamics were found to induce considerable dynamics of microbial communities suspended in groundwater, while sediment communities seem unaffected and stable in terms of biomass and diversity.

Introduction

Because impacts on groundwater systems by various sources of pollution are not only a serious risk for human and environmental health but also constitute a substantial economic factor, until recently, investigations of aquifers mainly focused on contaminated sites (Zarda *et al.*, 1998; Cavalca *et al.*, 2004; Hendrickx *et al.*, 2005; Yagi & Madsen, 2009). In pristine, energy-limited groundwater ecosystem microbial communities as well as related biogeochemical processes have received far less attention (Griebler *et al.*, 2002; Detmers *et al.*, 2004; Flynn *et al.*, 2008, 2010).

Physical–chemical conditions and consequently microbial community patterns in surface waters underlie pronounced seasonal dynamics and follow environmental gradients (Van der Gucht *et al.*, 2005; Sapp *et al.*, 2007; Alonso *et al.*, 2010). Shielded by a soil cover and unsaturated sediments, the water-saturated subsurface of aquifers is generally perceived to be environmentally stable. This stability as well as the energy-limited conditions and moderate temperatures is expected to select for low-diversity communities. The hydrological dynamics of aquifers are only recently recognized by microbial ecologists. Studies performed in pristine karst aquifers and associated springs (Farnleitner *et al.*, 2005; Pronk *et al.*, 2009; Wilhartitz *et al.*, 2009) and in contaminated porous aquifers (McGuire *et al.*, 2000, 2005; Haack *et al.*, 2004; Ayuso *et al.*, 2009) hint at serious effects of hydrological seasonal dynamics on bacterial communities. Compared to karst systems, where up to 50% of the porosity is represented by well-developed conduits and short water residence times, porous aquifers are characterized by much smaller voids, reduced water flow velocities, and increased water residence times (Goldscheider *et al.*, 2006). Therefore, the question arises how hydrologically dynamic a

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shallow porous aquifer may be and to what extent its environmental instability affects the microbial communities in biomass, activity, and composition.

Another aspect of ecological interest is the relationship between the energetic constraints in pristine aquifers and the systems' carrying capacity, also evaluated in light of hydrological dynamics. The concept of carrying capacity has been challenged to understand the stability and resilience of ecosystems (May, 1972; Pimm, 1984) and is a pivotal point in the debate of biodiversity (Tilman *et al.*, 1998; Tilman, 1999; McCann, 2000; Aoki & Mizushima, 2001). For groundwater ecosystems, the concept of carrying capacity has not been assessed so far. Similarly, the ecological concept of r/K selection, which has its origin in macroecology and is closely related to the dynamics of carrying capacity, awaits consideration when microbiologically exploring pristine aquifers which underlie periodic hydrological disturbances. The r and K strategy concept has been stressed repeatedly in microbial ecology but hardly with groundwater ecosystems (Hirsch & Rades-Rohkohl, 1990).

Our study focused on a pristine porous aquifer in the alpine region of southern Germany. Hydrologically, the system is influenced by mountain snow melting water in spring and pronounced summer and autumn rain events, as well as by a small river discharging and recharging the valley's aquifer. We hypothesized that the biomass and composition of groundwater and sediment bacterial communities in such an aquifer are driven by the prevailing hydrogeological conditions and consequently will exhibit strong seasonal dynamics. In order to develop a first picture, one hydrological season was followed by repeated sampling of river water and groundwater from four monitoring wells partly located along a gradient between the mountains and the river. Suspended as well as attached bacterial communities were monitored together with the physical–chemical conditions. Finally, results have been analyzed in light of ecological concepts, such as the r/K strategy concept and the concept of carrying capacity.

Materials and methods

Study area

The study site is located in the area of the village of Mittenwald, in the south of Germany. The porous aquifer located on the northern foothills of the Alps is drained by the Isar River. The shallow aquifer consists of quaternary sediments mainly composed of gravel and coarse to medium sands. The four groundwater monitoring wells selected were distributed to two groundwater bodies, one located close to the village of Mittenwald (Hoffeld) and one further upgradient of the Isar River (Riedboden) (Fig. 1). Both sites receive only minor anthropogenic impacts, and thus, the groundwater can directly be supplied to households as drinking water without any further treatment. The only potential impacts to the groundwater originate from irregular fertilization of the grassland with manure (wells MIT039 and MIT038) and grazing of cows on the pasture in spring and autumn (well MIT052). However, both activities underlie strict legal regulations to prevent any possible impact to groundwater quality. Owing to the topological position, the groundwater system receives serious hydraulic disturbances from (1) snow melting water flushing down the valley during spring to early summer, (2) pronounced rain events in summer and autumn, (3) infiltration of Isar River water and exfiltration to the river, and (4) karst water continuously discharging from the mountains.

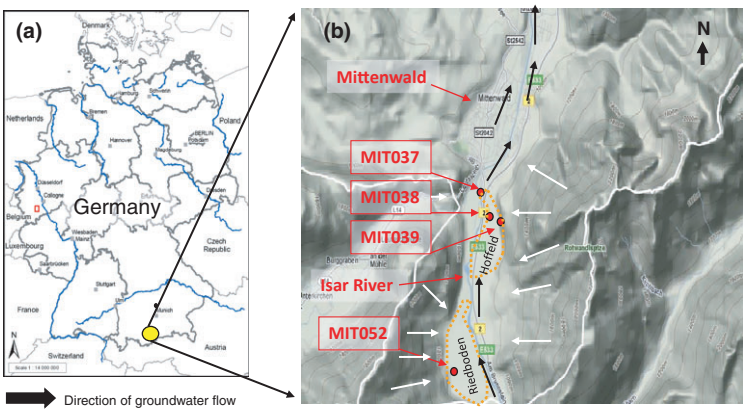

Fig. 1. (a) Geographic position of the study area. (b) Topographic map highlighting the location of the selected groundwater monitoring wells and the Isar River. Arrows indicate the general direction of groundwater flow. Wells MIT037, MIT038, and MIT039 are distributed in the area of 'Hoffeld' close to the village of Mittenwald, while well MIT052 is located in the mountain pasture 'Riedboden'.

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The monitoring wells were distributed within an area of approximately 7 km^2. Well MIT037 is located close (about 10 m) to the Isar River besides a main road. Well MIT038 was at a small street between two pastures in a distance of about 120 m from the river. Well MIT039 was another 120 m away from the river in the middle of grass land near the foot of a mountain (Fig. 1). Well MIT052 was located on a mountain pasture in a recreational area (Fig. 1).

Sampling

Investigations were conducted over 1 year (one hydrological cycle from March 2010 to March 2011). In total, six sampling events were performed (March, May, July, October, and December of 2010, March of 2011). Each time, groundwater, well water (standing water in the pipe), river water, and sediments incubated in the wells were collected. Five liters of groundwater, 2–5 L of river water, and 2 L of well water were filtered through 0.22-μm filter membranes (Millipore, MA) to concentrate suspended bacteria for subsequent DNA extraction. Filters were shock-frozen on dry ice and stored at −20 °C. For physical–chemical analysis, groundwater was collected in 500-mL sterile Schott bottles and stored at 4 °C till further processing. Water samples for DOC measurement were filtered through 0.45-μm pore size cellulose-nitrate filters (Millipore) and acidified with HCl to a pH $\leq$ 2. For total bacterial counts, water and sediment samples were fixed with glutardialdehyde (2.5% final concentration).

To complete the picture of microbial communities in the aquifer, sediments were incubated in groundwater wells. In detail, natural bed sediments from the Isar River were collected and carefully sieved into the medium sand fraction (0.2–0.63 mm) using river water. Subsequently, the sediment was packed into polyethylene columns with a mesh size of 1 × 2 mm. The sediment columns were then soaked in deionized laboratory water and autoclaved five times at 121 °C by repeatedly changing the water. Afterward, the columns were stored at 4 °C in sterile water until incubation. For means of comparison, fresh river sediments sampled on March 2010, carefully sieved in the field, were directly packed into autoclaved PE columns and incubated in the individual wells together with the sterile columns from March 2010 to March 2011. The sediment columns were subsampled along with the groundwater sampling surveys, at a time interval of 49, 106, 186, 253, and 338 days for tracing changes in colonization and bacterial community composition over time. For total cell counts, sediment subsamples (0.5 mL) were fixed with 1 mL 2.5% glutardialdehyde and stored at 4 °C in the dark until further processing. Aliquots for DNA extraction were shock-frozen on dry ice and stored at −20 °C till further analysis.

Physical–chemical conditions

Key physical and chemical parameters such as electric conductivity (EC), temperature, pH, redox potential (E_H), and concentration of dissolved oxygen were directly measured on-site by means of field sensors (WTW, Weilheim, Germany). Dissolved organic carbon (DOC) was determined as nonpurgeable organic carbon (NPOC) in triplicate from acidified samples using high-temperature combustion with infrared detection of CO_2 (Shimadzu, TOC-5050). Phosphate was analyzed colorimetrically as soluble reactive phosphorus (SRP) by the ammonium-molybdate method according to Murphy & Riley (1962). Samples were measured at 880 nm on a spectrophotometer (Varian, Cary 50 Bio). Major anions and cations were analyzed in triplicate by ion chromatography (Dionex Model DX 100, cations: CS 12A 4 mm column, CSRS-Ultra II 4 mm suppressor, anions: AS 4A 4 mm column, ASRS-Ultra II 4 mm suppressor, conductivity detection). Samples were quantified against commercial standards.

Water stable isotope analysis

Stable isotopes of oxygen ($^{18}O/^{16}O$) and hydrogen ($^{2}H/^{1}H$) were determined by isotope ratio mass spectrometry. The $\delta^{18}O$ values in samples were analyzed via equilibration with CO_2 at 18 °C for 5 h under constant shaking and for $\delta^{2}H$ values via reaction with U at 800 °C. Both $\delta^{18}O$ and $\delta^{2}H$ values were determined relative to internal standards that were calibrated using IAEA Vienna V-SMOW, V-GESP, and V-SLAP standards. Data are expressed in parts per thousand 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 $\delta^{2}H$.

Microbiological variables

Assimilable organic carbon (AOC) was determined following a protocol of Escobar & Randall (2001). Forty-five milliliters of water, filtered through a 0.22-μm membrane (Millipore), was inoculated with 5 mL of unfiltered groundwater or river water. After 30 days of incubation at *in situ* temperature in the dark, the amount of AOC was determined by calculating the newly formed biomass from the difference in cell numbers at day 0 and day 30. The applied conversion factor, 1 cell equals 20 μg carbon, was based on the study by Griebler *et al.* (2002).

The total number of bacterial cells in water and sediments were determined by means of flow cytometry. With sediment samples, 0.5-mL sample aliquots were

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fixed with 2.5% glutardialdehyde and kept at 4 °C until further preparations. After centrifugation and replacement of the glutardialdehyde by 1.5 mL PBS, cells were dislodged from sediment using a swing mill (Retsch, MM 200; 3 min, 20 Hz) (Anneser *et al.*, 2010) and separated from abiotic particles via density gradient centrifugation according to the protocol of Lindahl & Bakken (1995). The density fraction containing the bulk (about 90%) of bacterial cells was collected and further treated like water samples. The water samples were transferred to a tube containing 1 mL of PBS and fluorescent beads as internal standard. Bacterial cells in the solution were then stained with SYBR green I (Molecular Probes, Invitrogen, Karlsruhe, Germany) at a ratio of 1 : 10 000 before counting via flow cytometry (BD LSR II).

Extraction of total DNA from frozen filters and sediments was performed as described by Winderl *et al.* (2008) and Brielmann *et al.* (2009), and modified by Anneser *et al.* (2010). Extracted DNA was stored frozen at −20 °C until further processing. Terminal restriction fragment length polymorphism (T-RFLP) analysis of bacterial 16S rRNA gene amplicons was carried out using the primer set Ba27f-FAM/907r for bacteria and MspI digestion as previously described (Winderl *et al.*, 2008). The electropherograms obtained from the fragment analysis were examined using the GeneMapper software (GeneMapper V4.0). Output data were analyzed according to the protocol of Culman *et al.* (2009). T-RFLP fingerprinting was always carried out in duplicate. The T-RFLP data were analyzed by the T-Rex software package (Culman *et al.*, 2009). Shannon diversity and evenness were derived by PAST based on the T-RFLP data.

Clone libraries

For clone libraries, the bacterial 16S rRNA genes from water samples in May and July 2010 were amplified via PCR using the primers 27f (5′-AGAGTTTGATCC TGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGA CTT-3′) (Reysenbach *et al.*, 2000). One hundred and eight clones were constructed (16–20 clones per samples); further details on the clone libraries are given in the Supporting Information, Data S1. Phylogenetic analyses were performed using the ARB software package (Ludwig *et al.*, 2004).

Statistical analysis

Linear relationships among measured variables were explored by a Pearson product correlation (when data set was normally distributed) or by Spearman rank order correlations (when data set failed the normality test). Normality was examined using the Shapiro–Wilk test. A *P*-value of 0.05 was set as significance threshold. All statistical analyses were performed using the statistic package in SigmaPlot 12.0 for Windows. Relationships between hydrological and microbial variables were explored by means of a canonical correlation analysis using R 2.14.1.

Results and discussion

Hydrological dynamics and environmental conditions

The pronounced groundwater table changes observed during the time of investigation pinpointed at striking hydrological dynamics in the aquifer system. With increasing distance to the Isar River, the dynamics increased, as was obvious for the wells in the area of the 'Hoffeld' (Figs 1 and 2a). Wells located in the Hoffeld area exhibited water table changes of 1.05, 1.75, and 5.55 m in wells MIT037, MIT038, and MIT039, respectively. A tremendous change in the water table of 7.48 m was observed at well MIT052, which is located in the mountain pasture, the so-called Riedboden (Figs 1 and 2b). While the river is a fast buffer in terms of water levels, it exhibited pronounced seasonal changes in temperature which arrived at the nearby well MIT037 with a delay of 2–3 months (Fig. 2c). Groundwater temperature ranged from 4 to 9 °C, which was significantly less variable than the temperature dynamics in the river, which peaked at a value of 13.2 °C in July 2010 (Fig. 2c). Only moderate seasonal changes in water chemistry were indicated by several physical–chemical parameters such as the electric conductivity, with an annual mean value of 299 ± 44 μS cm^{-1} for groundwater from all sites investigated (Fig. S1a). Also the river water was found to be stable all over the year with a mean value of 237 ± 13 μS cm^{-1}. The pH of the groundwater ranged between 7 and 8, while it was always slightly above 8 for the Isar River (data not shown). High concentrations of dissolved oxygen all year-round at all sampling sites showed that the shallow aquifer was fully oxygenated with an average DO value of 10.26 ± 1.32 mg L^{-1} (data not shown). More interestingly, individual parameters indicated the different origin of water in spring and autumn. As can be seen from nitrate (Fig. S1b) and potassium (Fig. S1c) concentrations, small peaks in autumn indicate water from summer precipitation percolating through the soil layers before reaching the aquifer. Nitrate concentrations were positively correlated with the water table changes ($r = 0.468$, $P = 0.0211$). Stable water isotope data, that is, $^{2}H/^{1}H$ and $^{18}O/^{16}O$, dropped from March to May before rising again, clearly indicating snow melting water flushing the aquifer in late spring to early summer (Fig. S1d); snow is characterized by significantly lower

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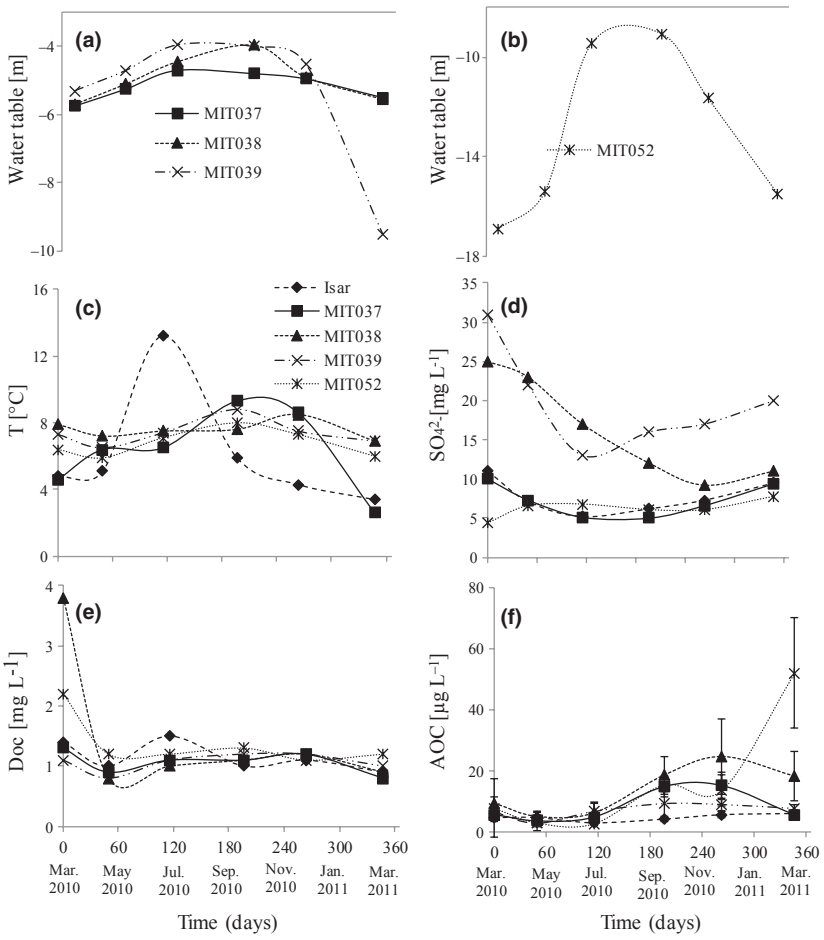

Fig. 2. Seasonal patterns of selected physical–chemical parameters.

stable isotope content of ^{18}O and ^{2}H (lighter isotope signature) as those in surface water and groundwater. An exception is well MIT039. Here, the more enriched isotope values (Fig. S1d) are less conclusive in terms of seasonal patterns but unambiguously point at an origin of the water from a lower recharge area in terms of altitude.

Surface water clearly distinguished from groundwater based on chloride concentrations. Groundwater samples exhibited a mean chloride concentration of 2.96 ± 1.93 mg L^{-1}, whereas the river water contained an average concentration of 0.57 ± 0.37 mg L^{-1}. Extraordinary high chloride concentrations of 19 and 47 mg mL^{-1} were observed in March and May 2010, respectively, at well MIT037 (Fig. S1e) that is located in a 10m distance to a main road. These exceptional high values are explained by the application of salt (NaCl) for deicing the road during winter. Sulfate concentrations separated the groundwater adjacent to the river from groundwater of the more distant wells in the Hoffeld (Fig. 1d). Groundwater pumped from below the Riedboden was found most similar to river water (Fig. 1d). The seasonal sulfate patterns observed at the wells MIT038 and MIT039 reflect the influence of the snow melting in late spring to summer, similar to the isotope data. Measurements of heavy metals and ammonium were always below the detection limit (5–30 µg L^{-1}) in both river water and groundwater. Similarly, concentrations of SRP were below the detection limit (5 µg L^{-1}) in samples from well MIT037 and the river water. SRP concentrations in groundwater samples from the wells MIT038, MIT039, and MIT052 were highest in October 2010 with 53, 65, and 16 µg L^{-1}, respectively, but were close to the detection limit for the rest of the sampling period (data not shown). Highest values in October again point at soil water from summer precipitation recharging the aquifer. Concentrations of DOC ranged from 0.9 to 1.7 mg L^{-1}, with highest values at well MIT038 (3.8 mg L^{-1}) and well MIT052 (2.2 mg L^{-1}) in March 2010 (Fig. 2e). Apart from these extraordinary high concentrations, a slightly elevated DOC level in groundwater is found during summer and winter and

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lowest values occurred in spring (Fig. 1e). The AOC, ranging from 2.5 to 25 μg L^{-1}, accounting for only 0.2–4.3% of the bulk DOC, clearly increased in autumn and winter (Fig. 2f). The higher fraction of assimilable DOC indicates a shorter travel distance and time, which give a hint to local recharge from summer rains. For river water samples, AOC was found negatively correlated with temperature ($r = -0.898$, $P = 0.0152$), indicating that at higher temperatures more DOC is converted into bacterial biomass. Correlation rank analyses revealed a few more correlations between individual abiotic variables (Table S1). However, there is an obvious lack of relationships between individual abiotic and microbial variables ($P > 0.05$), which was further confirmed by a multivariate canonical correlation analysis. This lack in correlations is common with groundwater studies. Possible explanations are as follows: (1) There is a delay between snow melting as well as precipitations and the arrival of these waters in the saturated subsurface; (2) mixtures of organic carbon (DOC) reaching groundwater are because of the travel through soils and sediments often depleted in readily degradable components. Thus, there is no simple relationship between the concentration of DOC and microbial patterns; and (3) groundwater pumped from a fully screened well is a mixture of water from different sediment layers and depths and water of different age with varying proportions. Thus, the microbial communities from pumped groundwater also represent a mixture of communities. Clear relationships between microorganisms and their abiotic conditions frequently get lost when analyzing such integrated water samples.

During the 1 year period of observation, the hydrological dynamics, which is mirrored by pronounced groundwater table changes, can be distinguished into two phases. Phase I starts with the continuous increase in the water table from March 2010 to July 2010 caused by the arrival of snow melting water from the mountains. The water table then stays up till autumn complemented with recharge from the summer rains. The slowly decreasing impact from snow melting water is read from the increasing stable water isotope values (Fig. S1d). Phase II is then characterized by the return of the groundwater table between November 2010 and March 2011 (Fig. 2a and b). From March 2010 to April 2010, only a relative low and constant precipitation (143 mm in March and 140 mm in April) contributed to the groundwater recharge of the area. From May on, the amounts of precipitation increased (248 mm in May, 205 mm in June, 174 mm in July, and 298 mm in August 2010). Clearly documented by our data set, for example groundwater table dynamics, and confirmed by representatives of the local water works, the Riedboden area (Fig. 1) represents an individual groundwater body, less connected with the valley aquifer. Phase II showing the steady decrease in hydraulic head is explained by the dry conditions with only very little precipitation from November 2010 to March 2011.

Suspended bacterial communities

Total bacterial numbers for both groundwater and river water ranged between 1.2×10^4 and 2.7×10^5 cells mL^{-1} (Fig. 3a). Cell numbers in the groundwater were generally lower than in the river water, except for samples from well MIT052 in autumn and winter, which followed the surface water trend overriding surface water numbers slightly (Fig. 3a). The total cell counts peaked together with AOC but with a delay of 2 months with respect to nutrient concentrations, that is, potassium and nitrate. The active microbial biomass estimated from measurements of cellular adenosine triphosphate (ATP) was highest in autumn and lowest in winter (data not shown), pointing at a higher proportion of active cells within the bacterial communities in autumn.

Shannon diversity data derived from T-RFLP fingerprinting revealed strong seasonal dynamics in the composition of the suspended bacterial communities in groundwater, while the dynamics were found to be moderate in river water (Fig. 3b). The number of T-RFs (considered as 'richness') ranged from only 4 to 102 with individual samples, being lowest in groundwater from well MIT039 in July 2010 (Fig. 3b). The average Shannon diversity was 2.57 ± 1.01, varying considerably from 0.47 to 4.00 in groundwater samples. In comparison, the river water exhibited stable Shannon values all over the seasons with a mean of $H' = 3.64 \pm 0.19$ (Fig. 3b). The dramatic drop of the groundwater bacterial diversity went along with the increase in the groundwater table. Until autumn, with the return of the groundwater table to its original low position, the diversity fully recovered even exceeding the spring values (Fig. 3b). Exemplarily, the temporal changes in the relative abundance of bacterial T-RFs are shown for river water samples and groundwater samples from well MIT039 (Fig. 3c and d). The Shannon diversity and the bacterial richness were both found to be positively related to AOC, $r = 0.425$ ($P = 0.0387$) and $r = 0.422$ ($P = 0.0399$), respectively.

Surprisingly, the arrival of the snow melting water in the valley aquifer did not significantly decrease the number of suspended cells; however, it led to a dramatic collapse of bacterial Shannon diversity in groundwater as evaluated by T-RFLP fingerprinting. Clone libraries generated from groundwater samples in May and July 2010 support that picture. Those revealed representatives of seven major bacterial lineages (Fig. S2). *Proteobacteria* were found to be most dominant (73% of a total of 108 clones from samples in May and July 2010), followed by

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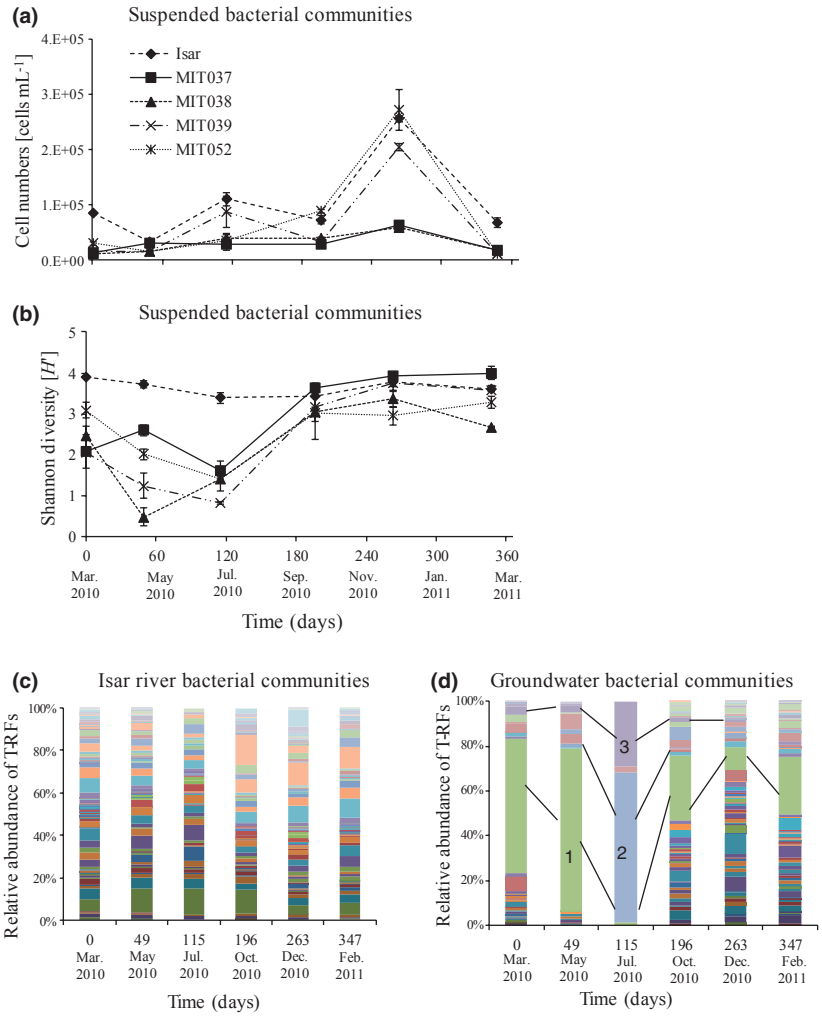

Fig. 3. Seasonal patterns of suspended bacterial communities. (a) bacterial abundance; (b) bacterial Shannon diversity; (c) relative abundance of T-RFs in water samples from the Isar River and (d) in groundwater samples collected at well MIT039. T-RFs are distinguished by color, and its relative abundance corresponds to bar height. Dominant T-RFs are highlighted: (1) bp 267, (2) bp 313, (3) bp 353.

Actinobacteria (4%) and *Firmicutes* (2%). The clones from water samples in July were most similar to sequences of *Comamonadaceae* (54% of 54 clones) and *Rhodocyclaceae* (16%), both *betaproteobacteria*. Compared to July, suspended bacterial communities in May were still more diverse with sequences affiliated to *Rhodocyclaceae* (43% of 63 clones), *Comamonadaceae* (6%), *Alphaproteobacteria* (6%), and *Gammaproteobacteria* (6%). Within the *Rhodocylaceae*, sequences related to *Dechloromonas* and *Ferribacterium* environmental clones, originally isolated from mining-impacted freshwater lake sediments and drinking water (Cummings *et al.*, 1999; Li *et al.*, 2010), were dominating (Fig. S2).

In other studies, the decline in biodiversity along with pulses of recharge caused by storm events has been explained by a dilution of the indigenous bacterial communities (Pronk *et al.*, 2009). However, the clear dominance of single T-RFs in spring 2010, which have been of comparably low relative abundance prior to the impact of the snow melting water, contradicts dilution being the exclusive process responsible but points at either very selective growth conditions or that the dominant bacterial populations detected in spring arrived together with the snow melting water. Both possibilities await further evaluation.

The low evenness of the spring groundwater communities provides further evidence that the system was disturbed at that time, while in autumn and winter the evenness returned to higher values, generally related to a more stable and functionally diverse community (Bell *et al.*, 2005; Wittebolle *et al.*, 2009). It may also be hypothesized that the T-RFs present in samples at times

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of low recharge best represent the autochthonous groundwater communities (Farnleitner *et al.*, 2005; Pronk *et al.*, 2009). In contrast to groundwater, the bacterial diversity in the river water was not affected at all by the hydrological dynamics (Fig. 3b). However, there is substantial indication that the melting water peak in the river, generally lasting no longer than a few weeks in early spring, was missed by our sampling schedule. On the other hand, the much higher diversity in the river water in spring may also be explained by the high turbidity of the water at that time. Consequently, bacterial community analysis from river water unavoidable also included cells attached to fine particles most probably leading to an overestimation of the planktonic bacterial diversity. It is obvious from the T-RFLP analysis that the bacterial community composition in river water differed between phase I (pronounced recharge to the valley) and phase II (decreasing groundwater table) (Fig. 3c). Not surprisingly, the influence of the river to the adjacent groundwater (well MIT037) could be documented by a much higher similarity of the bacterial communities (Bray–Curtis similarity index = 38.2%) when compared to groundwater samples from other wells. From summer to autumn, all samples from groundwater wells showed a high similarity in bacterial community composition (60–75%). In December, this pattern changed to much lower similarities (35–50%). This indicates the impact from snow melting water flushing the valley aquifer kind of disturbed and homogenized individual local bacterial community patterns.

Comparison of groundwater and water in groundwater wells

To obtain an overall picture of the bacterial communities in the investigated aquifer and to determine the frame conditions for the incubation of sediments to the groundwater monitoring wells, bacterial abundance and community composition of well water (= water in the pipes prior to pumping) were investigated and compared with the respective groundwater. On average, bacterial communities in groundwater were characterized by higher cell numbers but lower Shannon diversities than those in well water (Table 1). These differences were smallest in October 2010 and most pronounced in December of the same year. The Bray–Curtis similarity of well water and groundwater derived from T-RFLP data pinpointed at a different composition of communities. The lowest community similarity was found for well MIT052 with only 8.3% ± 3.2% (Table 1). However, similar to groundwater samples, well waters displayed a seasonal dynamic in Shannon diversity (Fig. S3), although less pronounced. Well waters of MIT037 and MIT052 followed the same temporal trend in diversity as found for the adjacent groundwater, while the well water from wells MIT038 and MIT039 displayed a second diversity decline in December 2010 (Fig. S3). The patterns of cell numbers in well water observed are surprising. As reported from several sites, higher cell numbers and/or bacterial biomass were found generally in well water when compared to the surrounding aquifer pore water (= true groundwater) (McNabb & Mallard, 1984; Hirsch & Rades-Rohkohl, 1988, 1990, 1992; Griebler *et al.*, 2002).

Patterns of attached bacterial communities

As groundwater samples alone are not representative of a porous aquifer, we also examined sediment bacterial communities. However, because we could not afford drilling of fresh sediments over time, we decided to incubate two different kinds of sediments, (1) fresh and untreated sediment from the bed of the nearby River Isar and (2) river bed sediment which have been sterilized, in the water of the individual groundwater monitoring wells.

Total cell counts of the fresh river bed sediments dropped slightly from 1.08×10^7 cells mL^{-1} at the early stage of the incubation to $4.2–7.7 \times 10^6$ cells mL^{-1} after 1 year of exposition to well water (Fig. 4a). The sterile sediments were quickly colonized within the first

Table 1. Comparison of suspended bacterial communities of groundwater and well water

	Groundwater		Well water		
Wells	TNC ($\times 10^4$ cells mL^{-1})	Shannon diversity	TNC ($\times 10^4$ cells mL^{-1})	Shannon diversity	Community similarity for groundwater and well water
MIT037	3.5 ± 2.0	3.2 ± 1.0	2.7 ± 2.2	3.9 ± 0.5	22.2 ± 17.8
MIT038	8.5 ± 8.6	2.2 ± 1.2	3.4 ± 3.4	3.4 ± 0.5	12.8 ± 10.7
MIT039	3.9 ± 1.7	2.5 ± 1.4	2.5 ± 2.5	3.4 ± 0.6	13.8 ± 11.2
MIT052	10.3 ± 11.8	2.5 ± 0.8	5.0 ± 3.5	3.3 ± 0.8	8.3 ± 3.2

TNC, total numbers of cells; Shannon diversity was derived from T-RFLP analysis; values are the seasonal averages of means from individual sampling events ± SD. Community composition similarity analysis was performed considering all individual fingerprints and possible combinations of comparison between groundwater and well water samples using the Bray–Curtis similarity index. Values are seasonal averages ± SD. The standard deviation in all cases represents the seasonal variations rather than biological or technical replicates.

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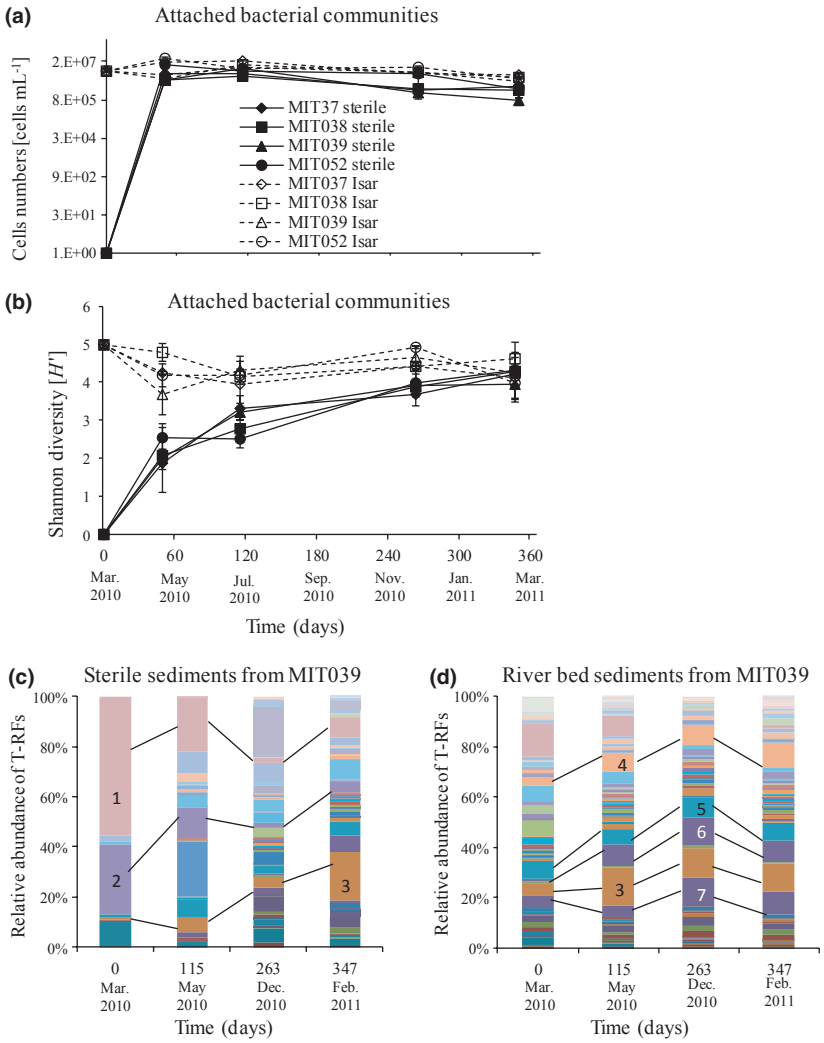

Fig. 4. Sediment bacterial communities. (a) bacterial abundance; (b) bacterial Shannon diversity; and relative abundance of T-RFs of (c) initially sterile sediment and (d) river bed sediments exposed to water in well MIT039. Dominant T-RFs are highlighted: (1) bp 325; (2) bp 233; (3) bp 126; (4) bp 269; (5) bp 132 (6) bp 130; (7) bp 124.

2 months reaching an average cell number of $9.0 \pm 6.5 \times 10^6$ cells mL^{-1}, and then leveling-off at a more or less constant cell density for the rest of the incubation period (Fig. 4a).

The bacterial diversity of the fresh river bed sediments was high ($H' = 5 \pm 0.03$) at the beginning of the incubation period. Toward the end of the exposure, it declined slightly to $H' = 4.24 \pm 0.26$. The Shannon diversity of the sterile sediments constantly increased from zero to $H' = 4.19 \pm 0.18$. Thus, after 1 year, a similar Shannon diversity established for both types of sediments. However, as can be seen in Fig. 4c and 4d, the community composition with the different types of sediments exhibited some differences. Nevertheless, individual dominant T-RFs detected in the communities of the fresh river bed sediments were later found with the initially sterile but then with colonized sediments. The relative abundance of individual T-RFs of the attached bacterial communities is exemplarily shown for river bed sediment and sterile sediment incubated in well MIT039 (Fig. 4c and 4d).

For a better understanding of the dynamics of the bacterial communities from the different kinds of sediments over the time, the consecutive community changes calculated as Bray–Curtis dissimilarity were plotted vs. the Shannon diversity (Marzorati *et al.*, 2008). Samples from March 2010 were considered as starting point. As seen from Fig. 5b, the samples from the initially sterile sediments clustered according to the time of incubation. The fresh river bed sediments revealed only moderate dynamics in bacterial community composition expressed by the

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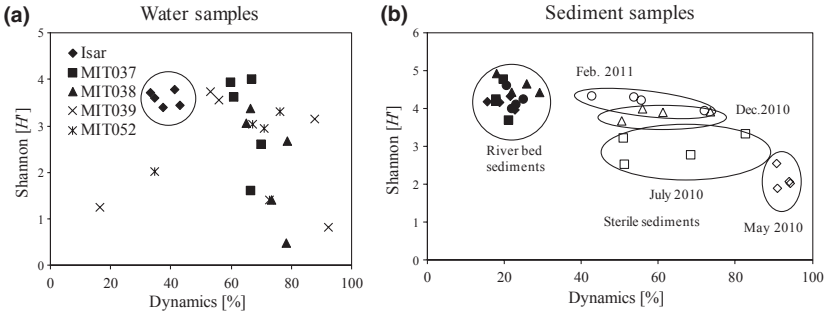

Fig. 5. Scatter plot of bacterial Shannon diversity vs. the degree of change in community composition [Dy] for (a) water and (b) sediment samples. The dynamics of community composition changes based on the Bray–Curtis dissimilarity index calculated for between consecutive sampling events; hence, the data from March 2010 served as starting point.

high and constant Shannon diversities ($H' = 4.31 \pm 0.32$) and small changes in composition (Bray–Curtis dissimilarity = 21.34% ± 3.46%). With water samples, a similar constant clustering was only observed for the river water communities ($H' = 3.59 \pm 0.2$; Bray–Curtis dissimilarity = 37.9% ± 4.2%), while groundwater communities grouped more according to the site and season (Fig. 5a).

It is known that free-living bacteria in aquifers represent only a small fraction of the total bacterial communities in terms of cell numbers, biomass, and diversity (Alfreider *et al.*, 1997; Griebler *et al.*, 2002; Griebler & Lueders, 2009). The ratio of attached to suspended cells is especially high in oligotrophic sediment systems (Griebler *et al.*, 2001; Lehman *et al.*, 2001). In the best case, bacterial communities in groundwater are a subset of the attached communities, but a different presence and/or dominance of individual groups of bacteria have been observed frequently for both the suspended and attached communities resulting in a limited community similarity (Alonso *et al.*, 2010). In the present study, the total number of attached cells was 2–3 orders of magnitude higher than cell counts from groundwater and river water. Impressive was the long-term stability of the river bed bacterial communities when exposed to well water for 12 months. After 1 year, the communities exhibited still a similarity of 80% (79% ± 3.4%) on average compared to the first day of incubation. And although the hydrological dynamics may be expected to be reduced inside the monitoring wells, which was indicated by the reduced dynamics of well water bacterial communities, this is surprising.

r and K strategy and carrying capacity

The different slopes of the increase in cell numbers and bacterial diversity as observed during the colonization of the sterile sediments exposed to water of different groundwater monitoring wells is a nice example for the succession of bacterial communities occupying a new habitat. There are indications for bacteria initiating the colonization, but later being repelled or replaced in numbers by others (Fig. 4c), a pattern that recalls the concept of r and K strategists (Douglas *et al.*, 1990). This succession is also underlined by the changes in community evenness, which was generally poor at the beginning and increased toward the end of incubation (Fig. S4), indicating the development of more functionally diverse and stable communities resilient to environmental disturbances (Wittebolle *et al.*, 2009). There is some preliminary and semi-quantitative evidence from pyrosequencing data that the early colonizers had shorter generation times than some of their dominant successors. However, the concept of r/K selection needs to be systematically investigated with a clear focus on bacterial growth rates. Worth to note is that the initial colonizers have been partly different ones in the different wells, that is, T-RF bp 488 in well MIT037, T-RF bp 401 in well MIT038, and MIT039, and T-RF bp 80 in well MIT052, providing evidence that the different areas in the aquifer investigated may harbor a different repertoire of organisms related to key functions. After 1 year, the attached communities on the formerly sterile sediments incubated in the different wells showed similarities of 31–45% to each other. The continuously increasing community similarity between the two sediment types, initially sterile sediment and colonized river bed sediment, over time implies the effects of an ongoing selection on community composition governed by the environmental conditions and/or interspecific competition.

When examining the total number of attached cells and the bacterial diversity found at the two different types of sediment after 1 year of common incubation, the similarity becomes obvious. The highly divers and densely colonized river bed sediments loss and the sterile sediments gain in numbers and diversity leveling-off at the same range. It looks like there is a certain carrying capacity related to biomass and diversity this environment

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could sustain. The concept of carrying capacity was started to be used in applied ecology and was then later exploited to interpret the relationship of the environmental r/K selection (Greenslade, 1983; Grime, 1988; Douglas *et al.*, 1990). According to the definition of del Monte-Luna *et al.* (2004), the carrying capacity is 'the limit of growth or development of each and all hierarchical levels of biological integration, beginning with the population, and shaped by processes and interdependent relationships between finite resources and the consumers of those resources'. However, the application of the carrying capacity concept at the ecosystem level was frequently questioned and criticized because of its vague interpretations and predictions (Dhondt, 1988). Our results open the door for further detailed research in that direction. Can a microbial carrying capacity be established in suspended communities of hydrologically dynamic systems? For how long does an allochthonous bacterial community persist when exposed to new environmental conditions? Is the stability of diverse bacterial communities regulated by physical–chemical factors, while the establishment of communities is regulated by interspecific competition? These and further questions await further research in the near future.

Conclusions

To our knowledge, this is the first detailed investigation of seasonal patterns of suspended and attached microbial communities in a hydrologically dynamics porous oligoalimonic (energy-limited) shallow aquifer. Our data revealed that the free-living microbial consortia are heavily affected in its composition by the fluctuating hydrological conditions, exhibiting pronounced seasonal changes. On the contrary, the environmentally dynamic aquifer seems to maintain stable and robust attached bacterial communities hardly influenced by the seasonal hydrological cycle. First evidence was collected for the existence of a system-specific microbial carrying capacity, which, however, awaits further exploration.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

Fig. S1. Seasonal patterns of electric conductivity (EC), nitrate, potassium, ^{18}O in water, and chloride content of groundwaters and Isar River water.

Fig. S2. Phylogenetic tree of 16S rRNA gene sequences of groundwater samples collected in May and July 2010.

Fig. S3. Seasonal dynamics of bacterial Shannon diversity in well water from the individual groundwater monitoring wells.

Fig. S4. Development of attached bacterial community evenness over time.

Table S1. Summary of relationships between microbial variables and hydrological characteristics for groundwater and Isar river water provided by a Pearson correlation matrix.

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