TECHNISCHE UNIVERSITÄT MÜNCHEN

Lehrstuhl für Grundwasserökologie

Spatio-temporal patterns of suspended and attached bacterial communities in a hydrologically dynamic aquifer (Mittenwald, Germany)

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigten Dissertation.

Vorsitzender: Univ. - Prof. Dr. R.F. Vogel

Prüfer der Dissertation:

1. Univ. - Prof. Dr. R.U. Meckenstock
2. Univ. - Prof. Dr. J. Geist

Die Dissertation wurde am 03.04.2013 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 02.07.2013 angenommen.

天行健，君子以自强不息
地势坤，君子以厚德载物

——《周易.乾》

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II. ABSTRACT

Groundwater ecosystems are perceived energy-poor systems very stable in environmental conditions and consequently harboring low numbers of microbes with stable community composition. To date, there is a paradigm change taking place pointing at so far ignored abiotic and biotic dynamics of shallow aquifers. The microbial communities are considered to follow seasonal changes, triggered mainly by hydrological dynamics. These findings inspired us to take a closer look at the spatio-temporal patterns of bacterial communities in a hydrologically highly transient porous aquifer in southern Germany, Mittenwald.

The studied aquifer is characterized by the strong hydraulic dynamics, i.e. large amounts of snow melting water from the mountains draining the aquifer in spring, followed by heavy rain events in summer and autumn. The significant impact of melting water caused a dramatic increase of the water table up to 8 meters starting in late spring with a return of the water table in late autumn and winter. The aquifer is located in a water protection area and receives only minor anthropogenic impacts. The aquifer in Mittenwald thus provides an excellent site to study the seasonal patterns of bacterial communities under most natural conditions.

Water samples from the aquifer and the Isar River were collected every two to three months over the period of one year. Fresh river bed sediments and sterile sediments were exposed to groundwater in selected wells and subsampled regularly. Beside a physical-chemical characterization of the water samples, suspended and attached bacterial communities were evaluated by DNA fingerprint in T-RFLP and 454 pyrosequencing. Moreover, the microeukaryotic community composition was determined by traditional cloning and sequencing in combination with a PCR/T-RFLP approach. The abundance of total bacterial cells and virus particles were measured via Flow cytometer. Additionally, the abundance of protozoa was determined with fluorescence *in situ* hybridization (FISH). Further microbial variables such as the concentration of ATP, assimilable organic carbon (AOC) and bacterial carbon production (BCP) were determined in river water and groundwater.

This study revealed pronounced seasonal changes in the composition and abundance of the bacterial communities suspended in groundwater, particularly exhibiting a dramatic drop in Shannon diversity in spring and lately summer at time of snow melting. In contrast, bacterial communities established at the sediment surface were found comparably stable year-round. It is suggested that the prevailing hydrological dynamics are the drivers for the

seasonal dynamics of suspended bacteria. The succession of dominant taxa within the bacteria during colonization of sterile sediments exposed to groundwater in situ and differences in growth rates provided evidence for the presence of individual bacterial r/K- strategists. Similarities of bacterial communities in groundwater and at the sediment surfaces as well as the extraordinary low activity of microbes in the groundwater indicate that the groundwater acts as a ‘seed-bank’. Based on the first measurements of protozoa and viruses, and complemented with values from the literatures, a first estimation of the carbon flux through the suspended microbial food web was made. It hints at a considerable control of bacterial production by protozoan grazing and viral lysis.

1 INTRODUCTION

1.1 Groundwater ecosystems

1.1.1 Basic features

Knowledge on groundwater ecology and aquifer microbial biocenoses seems to be most advanced for two types of groundwater environments: the hyporheic zone and contaminated aquifers. In contrast, the knowledge of microbial communities in pristine aquifers is still very insufficient. The term 'pristine aquifers' in this thesis is used to describe aquifers that are obviously not contaminated. I am aware that by definition there is no 'pristine' aquifer existing any more. Uncontaminated aquifers represent extreme environments for life. These aquifers are most often oligotrophic, i.e. characterised by a limited amount of organic carbon, energy and nutrient. This shortage is responsible for low microbial activities, small cell sizes and a reduced cell number. As soon as there is a change in the chemical water composition due to human activities, this picture may immediately change. Oligotrophic characterizes anthropogenically unaffected groundwater habitats and plays an important role in shaping the peculiar biotic communities and general function of groundwater ecosystems (Gibert, *et al.*, 1994). Groundwater ecosystems due to the lack of light depend on organic carbon coming from the surface and chemical energy. Living conditions (e.g. temperature, pH, water chemistry, flow velocity) are comparably constant in many aquifers, and the biocenoses are somehow shielded against from the seasonal dynamics in environmental conditions present at the surface (Figure 1.1).

Microbial biocenoses in pristine aquifers are composed of Bacteria, Archaea, Viruses and Protozoa. Besides, there is an invertebrate fauna consisting of crustaceans, nematodes, oligochaetes, mites and others (Griebler & Mösslacher, 2003). Eukaryota, namely protozoans (flagellates, amoebae and ciliates) and fungi, seem restricted in their distribution to shallow groundwater habitats (Hirsch, *et al.*, 1992, Madsen & Ghiorse, 1993). However, the knowledge on the distribution of Protozoa may be biased by the lack of investigations and appropriate sampling and analysis methods.

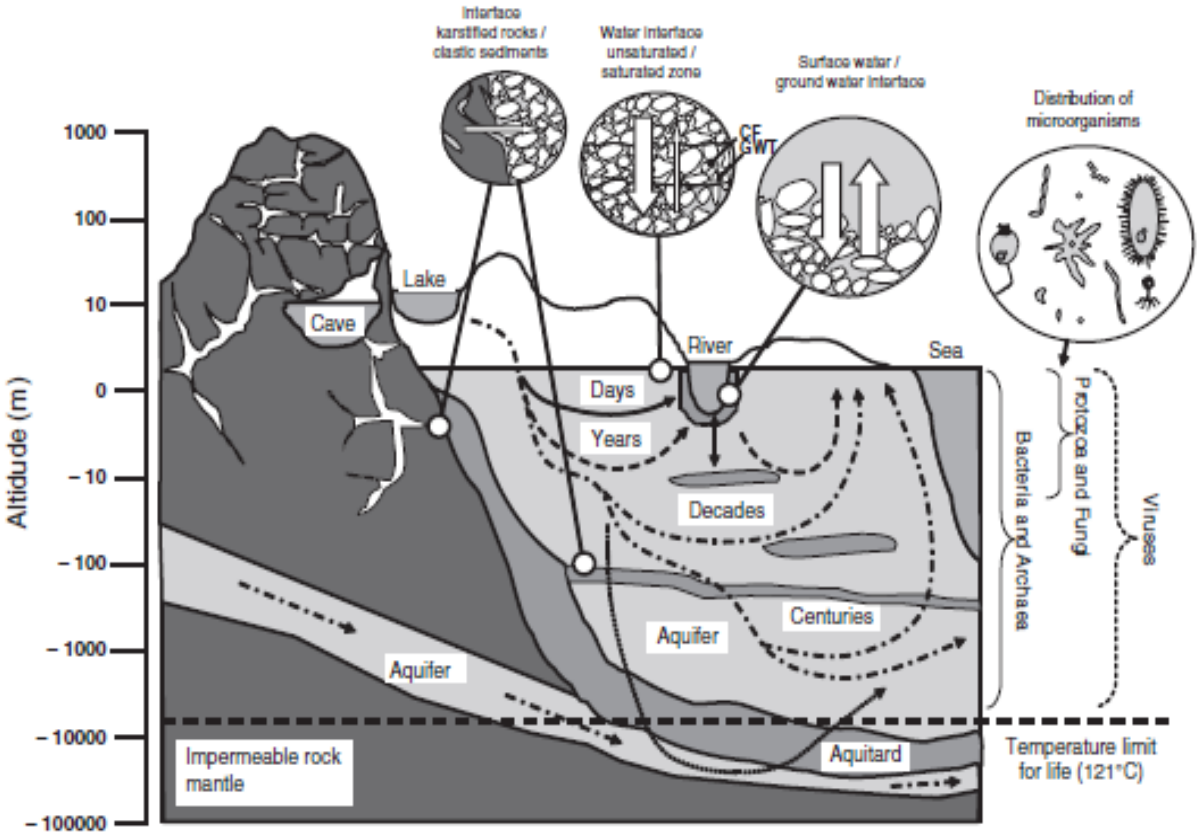


Figure 1.1 Schematic view of aquatic surface and subsurface habitats (Griebler & Lueders, 2009).

1.1.2 Environmental factors controlling the spatio-temporal distribution of microbes

It is well known that geologic, hydrologic and geochemical properties display some heterogeneity in the subsurface. Microbiological patterns in aquifers are affected by lithological-mineralogical parameters (grain-size distribution, rock type and mineral spectrum), geometric parameters (fissure aperture, pore size and karst conduit diameter), hydraulic parameters (porosity and hydraulic conductivity), physical-chemical parameters (pH, T, DOC, oxygen, etc.).

The composition of groundwater may determine microbial activities and the other way round. As pristine aquifers are carbon-limited environments, an input of biodegradable organic contaminants is likely to stimulate aerobic microbial activities and consequently consumption of dissolved oxygen. The stress/toxicity from the organic pollutants can affect the structure and composition of microbial communities. Organically contaminated aquifers generally turn anoxic which is accompanied by a significant change in community structures.

Thus the presence or absence of oxygen is, also for microbial communities, a key factor shaping community composition. It could be shown that the change in redox conditions, driven by the biodegradation of some organic pollutants, was the principal parameter responsible for microbial community shifts in groundwater (Fahy, *et al.*, 2005, Aburto, *et al.*, 2009).

Besides oxygen, temperature is an important parameter which influences the distribution and dynamics of microbial communities in groundwater. Temperature correlated with microbiological variables and seemed to be the most important physical factor controlling microbial communities throughout the year in a coastal Spanish aquifer (Ayuso, *et al.*, 2009). The temperature of groundwater in central Europe is typically at around 10-12 ℃. Seasonal temperature effects on microbial processes may be evident in very shallow groundwater, but an obviously absent at greater depths (Pedersen, 2000). Groundwater systems are similar to deep oceans environments in many features, such as lack of light, low cell numbers and substrate-limited. Aquifers have been considered to be oligotrophic environments similar to open marine systems and with bacterial densities close to that observed for the deep sea (Pedersen & Ekendahl, 1990). In a review, Pomeroy and Wiebe (2001) discussed the combined effects of temperature and substrate limitation for the activity of deep marine bacteria. They conclude that most bacteria in the permanently cold oceans are always living at temperatures well below their growth optima, which implies a higher concentration threshold for substrate utilization in these systems. It was suggested that bacteria growing at low temperature (~0 ℃) require higher substrate concentrations to obtain optimal growth rates compared to bacteria growing at high temperature (Pomeroy & Wiebe, 2001).

The effect of different pH values on microbial processes will be indirect, as pH influences many geochemical parameters, such as mineral dissolution and precipitation, carbon dioxide solubility and various solid-aqueous phase equilibria. The other way round, high or low pH values are processes releasing H^+ or OH^-. It was repeatedly suggested that pH is not a key factor controlling the spatio-temporal distribution of microbial communities present in aquifers, e.g. (Gounot, 1996, Ayuso, *et al.*, 2009); however, pH may be a central factor controlling the diversity and the taxonomic composition of microbial communities as shown for the Donana's groundwater system (Lopez-Archilla, *et al.*, 2007). In the very acidic and alkaline range, pH of course significantly shapes the composition of communities. The presence and growth of a variety of *alkaliphilic δ -Proteobacteria, Bacillus*, and *Clostridium* species at pH up to 13.2 (Roadcap, *et al.*, 2006), extended upward the known range of pH

tolerance for a microbial community by as much as 2 pH units.

Sediment heterogeneity, mineralogy and sediments grain size, have often been considered to be most important factors controlling microbial abundance and activity in aquifers (Musslewhite, *et al.*, 2003) also. It will affect the attached as well as the planktonic microbial communities because there is a permanent exchange between suspended and attached consortia taking place (Hirsch & Rades-Rohkohl, 1990). The apparent control of grain size over microbial abundance is determined by the relationship of grain size to surface area available for colonization. In consequence, smaller grain size fractions contain more cells per volume unit (Griebler & Slezak, 2001). Another control is given due to some hydrogeological characteristics, such as permeability, porosity or transmissivity (Brockman & Murray, 1997, Musslewhite, *et al.*, 2003). Areas that show higher hydraulic conductivities tend to display higher bacterial biomasses (Fredrickson, *et al.*, 2004) and activities (Chapelle & Lovley, 1990). Hydraulic conductivity determines the carbon and nutrient supply to microbes (Lehman, *et al.*, 2001) as well as the movement (transport) of cells through the aquifers system (Balkwill, *et al.*, 1989).

Due to the high heterogeneity and complexity in natural environments, spatially non-homogeneous distributions of numerous physical, and chemical properties yield patches of optimal, suboptimal or constrained and excluding conditions for microorganisms (Parkin, 1993). Therefore, different sampling sites and the distance between sites could result in different conclusions about the existence and scale of spatial continuity or spatial cross-correlation. Differences in microbiological properties between geologic strata typically is caused by the availability and solubility of nutrients or electron donors/acceptors, and whether the transport of these constituents is controlled primarily by diffusion or advection, and finally the distance to other local sources of nutrient(s) or electron donors/acceptors (Brockman & Murray, 1997).

1.2 Biomass, activity and composition of microbial communities

1.2.1 Microbial biomass and activity

The total number of bacteria found in groundwater ecosystems may vary by several orders of magnitude between 10^3 and 10^6 cells per cm^3 of groundwater and between 10^5 and 10^8 cells per cm^3 of sediment (Griebler & Lueders, 2009). Between 0.00084 and 14.8% of the total

numbers could be cultivated and detected using most probable number (MPN) methods (Haveman & Pedersen, 2002). Although subject to a great uncertainty, these estimates therefore suggest that the total prokaryotic biomass of intraterrestrial life is immense.

As microbial mass itself is difficult to measure directly, various proxies are commonly used, including the analysis of cell numbers: e.g. analysis of total cell numbers using epifluorescence microscopy or various types of viable number counts obtained with solid or liquid media. Viable counts depend strongly on the ability of the investigator to develop growth media that suits most bacteria present (Pedersen, 1993). Most studies confirmed that less than 10% of the cells in pristine groundwater are culturable under laboratory conditions. In some cases, however, the number of culturable counts was nearly equal to the number of direct microscopic counts, indicating that most cells were in an active state (Sinclair, *et al.*, 1990). Alternatively, analysis of specific cell components is possible when biomass and its particular constituents occur in stable ratios. Biomass can be estimated relative to the amounts of DNA (Tranvik, 1997), membrane lipids (White, *et al.*, 1979), proteins (Bradford, 1976), and adenosine triphosphate (ATP) (Lundin, *et al.*, 1986). Biomass estimation from ATP data has the advantage of estimating only the viable, living biomass.

As already mentioned above, transport of nutrients and organic matter with groundwater flow is an important factor for microbial activity. The presence of high microbial activity often goes along with high cell numbers. As seen from Table 1.1, the microbial abundance in pristine aquifers is one to two orders of magnitude less than that in oligotrophic lake and deep sea.

Microbial communities can mediate the degradation of organic pollutants in groundwater. In most cases, pollutants introduced into aquifers shift the local microbial diversity (Baker, *et al.*, 2001, Johnson, *et al.*, 2004). Contamination of an aquifer may cause a pronounced decrease in activity and biodiversity due to toxic compounds (Griebler & Lueders, 2009). However, as pristine aquifers are carbon-limited environments, a moderate input of biodegradable organic contaminants is likely to stimulate microbial activity and biodiversity. Many microorganisms can rapidly adapt to changing environmental conditions due to their metabolic flexibility and over a long-term due to the ability to exchange genetic information (Dröge, *et al.*, 1999, Chapelle, 2001).

Table 1.1 Bacterial abundance and production in different subsurface environments.

System	Site		BA (cells L^{-1})	BP (thymidine) (μg C L^{-1} h^{-1})	BP (leucine) (μg C L^{-1} h^{-1})	Reference
Pristine aquifer	Garzweiler,Germany		7.3×10^{8}			(Detmers, *et al.*, 2004)
	Rainier Mesa, Nevada	Ashfall tuff	10^{5}			(Amy, *et al.*, 1992)
	Äspö study area Stripa research mine Sweden	Crystalline granitic bed-rock	10^{6}-10^{9}			(Pedersen & Ekendahl, 1990)
	Madison Formation Great Plain,USA	Dolomitic limestone	10^{6}			(Olsonac, *et al.*, 1981)
	The coastal plain of South Carolina, USA	Gravel	10^{6}-10^{7}			(Hazen, *et al.*, 1991)
	Paris Basin, France	Thermal groundwater	10^{5}-10^{8}			(Daumasa, *et al.*, 1986)
	Northern Calcareous Alps, Austria	Dolomite karst aquifer	1.5×10^{7}			(Farnleitner, *et al.*, 2005)
		Limestone karst aquifer	6.3×10^{7}			
	Donana, SW Spain	Meso- or eutrophic	$1.35\pm1.16\times10^{10}$			(Ayuso, *et al.*, 2009)
Lake / River	Mackenzie River and coastal Beaufort Sea	arctic	6.7×10^{8}	3.17×10^{-3}-17.3×10^{-3}		(Vallieres, *et al.*, 2008)
	Ria de Aveiro, NW Portugal	shallow estuarine system		0.1125–31		(Almeida, *et al.*, 2005)
	Elbe, Germany	Estuaries system	2.0×10^{9}-7.5×10^{9}	2.3×10^{-3}-11.125×10^{-3}		(Goosen, *et al.*, 1999)
	Westerschelde, The Netherlands		2.0×10^{9}-6.5×10^{9}	0.0127-0.125		
	Gironde, France		1.5×10^{9}-7×10^{9}	9.17×10^{-4}-97.9×10^{-4}		
	Antarctica lake	ultra-oligotrophic epishelf	7.60×10^{7}		0.82-1	(Laybourn-Parry, *et al.*, 2001)
Marine	Northern Barents Sea		$3.6\pm3.0\times10^{8}$	$6.5\pm4.17\times10^{-2}$	6.9 ±4.8	(Sturluson, *et al.*, 2008)
	Central Atlantic Ocean		1.0×10^{8}-1.0×10^{9}	4.17×10^{-2}		(Vazquez-Dominguez, *et al.*, 2008)
	Black Sea		1.4×10^{9}-2.9×10^{9}	0.1-0.3		(Morgan, *et al.*, 2006)
	North Water Arctic pelagic system	surface	0.4×10^{9}-1.6×10^{9}	$1\text{-}22\times10^{-1}$		(Middelboe, *et al.*, 2002)
		Below 20 m	2×10^{8} -5×10^{8}	$<1\times10^{-1}$		

1.2.2 Microbial composition and diversity

1.2.2.1 Microbial community composition

Aerobic and anaerobic microorganisms represent two very distinct physiological groups within microbial communities. Groundwater ecosystems, especially in deeper zones and in areas organically contaminated are dominant by anaerobic microorganisms (Capuano, *et al.*, 1995, Ghiorse, *et al.*, 1996, Santegoeds, *et al.*, 1999). Aerobes play an important role in shallow and oligotrophic systems.

It is currently not possible by any method, on the other hand, available to measure precisely the complete composition of a microbial community. Although the molecular approach used here which are T-RFLP may have its flaws (Farrelly, *et al.*, 1995, von Wintzingerode, *et al.*, 1997, Simon, *et al.*, 2001, Kisand & Wikner, 2003, Lopez, *et al.*, 2003), it enables to evaluate the spatial and temporal differences between bacterial communities, and allows to estimate the bacterial diversity. The classical way to study the diversity of bacterial communities is to inoculate different solid and liquid media with environmental samples and subsequently incubate at different temperatures and gas compositions. This approach indicated only a little number of microorganisms to be presented in aquifers. Groups of microorganisms detected in subterranean systems included Bacteria, Protozoa and representatives of yeasts and other Fungi (Hirsch & Rades-Rohkohl, 1992). As in other freshwater systems, a large proportion of the abundant microorganisms belonged to the *δ-Proteobacteria* (Pedersen, *et al.*, 1996, Crump, *et al.*, 1999, Glöckner, *et al.*, 1999). It is reported for a pristine aquifer that Bacteria are dominant, representing 51.9% of the total cell number, while 25.7% of total cell were affiliated with the domain Archaea (Detmers, *et al.*, 2004). The *Comamonas–Variovorax* group (δ-1 subgroup) seems to be of particular importance. Microorganisms belonging to these taxa were frequently isolated from subsurface environments (Balkwill, *et al.*, 1997, Chandler, *et al.*, 1997, Crump, *et al.*, 1999).

1.2.2.2 Diversity of microbial communities

The analysis of microbial diversity in pristine shallow groundwater is based on the classical approach (isolation and cultivation) and the molecular approach. In 1956, the first systematic studies of Bacteria from a shallow aquifer in Germany suggested a distinct indigenous

microbial community are revealed 260 isolates (Wolters & Schwartz, 1956). During 1983-1992, Hirsch and Rades-Rohkohl cultivated several hundred isolates, more than 100 different morphotypes. The main groups were *Proteobacteria*, *Actinobacteria*, *Bacteriodestes* and *Firmicutes*.

The subsurface microbial culture collection (SMCC) in the USA was established to take care and characterize the nearly 10,000 strains of microorganisms isolated from terrestrial subsurface environments (Balkwill, *et al.*, 1997). Among these isolates members of six major phylogenetic groups of bacteria: the high-G+C and low-G+C Gram-positive bacteria; the *alpha-*, *beta-*, and gamma-subdivisions of the *Proteobacteria*; and the *Flexibacter-Cytophaga-Bacteroides* group dominated. Most of the isolates assigned to genera such as *Arthrobacter* and *Sphingomonas* appeared to be new species, thereby indicating that a reasonable amount of novelty is present.

Lehman *et al.* (2004) compared the character of microorganisms associated with the basalt core matrix were compared to those suspended in groundwater pumped from the same well in the eastern Snake River Plain Aquifer. The majority of the bands in a DGGE analysis were most closely related to members of the *Proteobacteria, Firmicutes,* or *Cytophaga-Flexibacter-Bacteroides* groups. Haveman *et al.* (2005) cultured bacteria from Saint John River and from water which were recharged the Fredericton aquifer. They found that microorganisms significantly contribute to water quality improvements during bank filtration by removing organic carbon. Bacteria isolated from groundwater samples, identified by 16S ribosomal RNA gene sequencing, were *alpha-*, *beta-*, *gamma-*, and *delta-Proteobacteria, Actinobacteria*, and *Firmicutes*. Farnleitner *et al.* (2005) characterized spring water from dolomite karst aquifer and limestone karst aquifer respectively by microscopic direct examination of bacterial cells, molecular bacterial 16S-rDNA profiling as well as conventional microbiological cultivation. The distribution of morphotypes and mean cell volumes was different between the two systems, indicating the influence of the hydrogeology on microbial communities. The sequence types aligned mainly to the division and subdivisions of *Proteobacteria* and *Flexibacter-Cytophaga-Bacteroides* as well as *Nitro*spirae. It is reported that in the beach aquifer at Huntington Beach, California, the taxonomically rich and novel denitrifying communities were found, with all nirK clones exhibiting <85% identity and nirS clones exhibiting < 92% identity at the amino acid level to those of cultivated denitrifiers and other environmental clones in the database (Santoro, *et al.*, 2006). The gathered literatures indicate the existence of microbial diversity in shallow groundwater systems, however, to date there is no indication for an endemic groundwater microbiota

(Griebler & Lueders, 2009).

1.2.2.3 Protozoa in groundwater

Protists are eukaryotic, single-celled microorganisms comprising groups commonly referred to as algae, protozoans and lower fungi. The most common protists in aquifers are protozoa, and here flagellates and amoebae. Protozoa are known to be effective predators, feeding on bacteria or other microorganisms (including other protists), and therefore playing a key role in the microbial food web. Protozoa in pristine aquifers are present in very low numbers, with concentrations ranging from $<10^0$ to 10^2 cells per gramme of aquifer material.

Several studies demonstrated that a higher diversity and abundance of flagellates and amoebae was observed in organically contaminated sites compared to pristine, uncontaminated sites (Novarino, *et al.,* 1994). The relationship between protozoan diversity and organic contamination are poorly understood. It was hypothesized that the greater abundance and variety of food sources in the contaminant plume (bacteria, colloidal and dissolved organic matter) is likely to support a larger number of protozoan species (Novarino, *et al.*, 1997).

Taxonomy and systematics of protozoa still mainly based on morphological and behavioural features. Molecular analysis slowly gain in importance but the sequence data banks are still small. Morphological characters used to identify flagellates include the following: cell size, cell shape, ability to form pseudopodia, flagella ornamentation and relative flagella length, presence and position of contractile vacuoles, presence or absence of organelles, and presence or absence of scales. The behavioural features such as swimming modes were also found to be useful for taxonomic purposes.

The flagellates and all of the amoebae are bacterivorous. It was reported that size-selective grazing was the major force controlling both the morphological and the taxonomic structures of the microbial community (Hahn & Höfle, 1999). Grazing on bacteria community capable of degrading organic compounds may have an indirect effect on the overall rates of biodegradation. However, the possibility that the number of heterotrophic flagellates may not always correlate strongly with bacterial number (Gasol & Vaqué, 1993) support the hypothesis that flagellates may depend on alternative or additional food sources e.g. colloidal and dissolved organic matter (Novarino, *et al.*, 1994).

1.3 Viruses in groundwater

1.3.1 Characterization, distribution and diversity of viruses

Viruses are a group of biological entities with a genome consisting either of DNA or RNA and encapsulated in a protein coat (capsid). Virioplankton is typically 10 times more abundant than bacterioplankton. Bacterial viruses (bacteriophages) are bacterial parasites, and as such are genetically and structurally simple with life cycles as short 20-60 min.

The abundance of viruses varies strongly in different environments and is related to bacterial abundance or activity suggesting that the majority of the viruses found in the environment is typically phage. The variability of viral abundance is larger than the typical range of corresponding bacterial numbers indicating that the viral abundance is less tightly controlled than the bacterial abundance. So far reports about virus abundances in groundwater are sparse. Total viral abundance in marine and freshwater systems typically ranges lower than 10^4 and higher than 10^8 mL^{-1} (Wommack & Colwell, 2000, Weinbauer, 2004). Kyle *et al.* (2008) have reported fluorescence microscopic counts of virus-like particles in the range of 10^5-10^7 mL^{-1} for groundwater at the Äspö HRL from depths between 69-455m. A view under a transmission electron microscope (TEM) showed morphologically diverse viral communities.

Reconsidering viral taxonomy, it has been suggested that dsDNA, ssDNA, and ssRNA represent domains, which probably evolved independently. A typical phage has a head and a tail hold together by a connector, however, cubic, spindle, lemon-shaped, filamentous or pleomorphic viruses are also known.

Studies on the morphology of viral communities were often restricted to grouping viruses into size classes. The majority of viruses are usually found in the size range of 30-60 nm, exceptions are a backwater system of the Danube where 84% of the viruses had capsids larger than 60 nm (Mathias, *et al.*, 1995). The largest virus-like particles with a head diameter of up to 750 nm were found in the food vacuole of *Phaeodarian radiolarians* (Gowing, 1993). Recently, giant mimivirus was isolated from the cooling tower. The giant virus has been found by La Scola (2003) already more than a decade earlier in the UK, but mistaken for a bacterium because of its size. In 2008, La Scola *et al.* isolated a new strain of *Acanthamoeba polyphaga* mimivirus (APMV), with a genome harbouring more than 900 protein-coding genes, and denoted this as the mamavirus because it seemed to be even larger than mimivirus

when observed by transmission electron microscopy (La Scola, *et al.*, 2008). The main features of mamavirus closely resembled those for mimivirus, including the formation of a giant viral factory and the typical particle morphology with a multi-layered membrane covered with fibrils (Suzan-Monti, *et al.*, 2007). Associated with the new strain of APMV, they found an icosahedral small virus, Sputnik, which is a 'virophage', much like the bacteriophage viruses that infect and sicken bacteria.

Today, diversity of viruses is studied by isolation and culture-independent molecular analysing. No data are available so far on the genome size distribution of viruses from groundwater systems. The genomes distribution of viruses from marine systems was multimodal with major peaks between 31-36 and 58-63 kb and an average of ca. 50 kb (Steward, *et al.*, 2000). Mesocosm studied simulating phytoplankton blooms have revealed that the viral community composition is dynamic and closely linked to changes of the algal and bacterioplankton community (Larsen, *et al.*, 2001). Others have shown that the variability of viral community composition during a phytoplankton bloom was low (Riemann & Middelboe, 2002). Cyanophages are among the most common studied phages, whose diversity can be studied with cyanophage specific primers targeted against a region of the capsid assembly protein gene. A divers and variable cyanophage community composition was found along depth profiles. Changes in genotype richness were associated with water stratification, nutrient concentration and cyanobacterial abundance. Diversity of viruses was also studied using phages infecting a single bacterial host species. In a recent study, Eydal and his colleagues (2009) reported the isolation of a bacteriophage lytic to *Desulfovibrio aespoeensis,* an indigenous bacteria species from deep groundwater at the Äspö Hard Rock Laboratory (HRL). It is also the first time viable viruses have been isolated from deep groundwater. The viruses were found fall into in the *Podoviridae* morphology group.

1.3.2 Role of viruses in groundwater

The composition, sources and degradation of DOC has been a long standing focus for research in biogeochemistry and microbial ecology. Prokaryotes take up DOM, which mainly originates from primary production. Viral lysis of cell at all trophic levels converts a significant amount of biomass into DOM (viral shunt) and thus elements such as C, N, P, S and Fe became available again for other bacteria (Thingstad, *et al.*, 1993, Fuhrman, 1999, Wilhelm & Suttle, 1999, Wilhelm & Suttle, 2000, Wommack & Colwell, 2000).

There is a close relationship between viruses and bacteria. Manipulating viral abundance caused a change of the bacterial abundance and viral infection of bacteria (Weinbauer & Peduzzi, 1995, Noble & Fuhrman, 1999), enzymatic activity (Noble, *et al.*, 1999), bacterial production and respiration (Noble & Fuhrman, 1999, Middelboe & Lyck, 2002), concentration and composition of DOM and POM (Peduzzi & Weinbauer, 1993), and the dynamics of flagellates (Peduzzi & Weinbauer, 1993, Pesan, *et al.*, 1994). A short-term stimulation of bacterial abundance was also reported (Lammers, 1992). All these data suggest that viral lysis induces changes in the relative importance of individual functional groups in the microbial food web.

Phages and heterotrophic nanoflagellates are the two main predators of prokaryotes in the aquatic systems. Viral infection of bacteria is on average as significant for bacterial mortality as grazing by protozoa (Fuhrman & Nobel, 1995). However, the affect may vary with space and time and environmental conditions (Weinbauer & Peduzzi, 1995, Pedros-Alio, *et al.*, 2000, Guixa-Boixereu, *et al.*, 2002). Guixa-Boixerez *et al.* (2002) suggested that low water temperatures such as those prevailing in Antarctic marine waters might reduce heteronanoflagellates (HNF) activity and thus favour viral infection of bacterioplankton. This agrees with the hypothesis that viral mortality of bacteria increases with host abundance and system productivity. Since bacteria need additional carbon for respiration, uptake of viral lysis products of cells (particularly the cell content) by bacteria should result in a net release of inorganic nitrogen and phosphorus. Lysis products are available to bacteria at the expense of a reduce growth efficiency (Middelboe, *et al.*, 1996, Middelboe & Lyck, 2002) and could at certain times be the dominant source for meeting bacterial carbon demand (Wilhelm & Suttle, 2000). The DOM released during lysis of bacterial cells may stimulate the non-infected bacterial populations (Middelboe, *et al.*, 1996, Gobler, *et al.*, 1997).

Many studies have speculated that viruses have the potential to influence the diversity of bacterial communities. Models suggest that bacterial abundance and diversity should be controlled by predators such as phages in high-productivity systems and by competition in low-productivity systems (Bohannan & Lenski, 2000). One of the underlying concepts is that phages control competitive and dominant species once they get abundance (Fuhrman & Suttle, 1993) and they may even sustain species diversity by ‘killing the winner’ and allowing the losing competitors to co-exist (Thingstad, 2000), however, the experimental data are still poor. It has been reported that bacterial morphotypes show different infection frequencies (Weinbauer & Peduzzi, 1994) and only rod shaped bacterial were significantly affected (Weinbauer & Peduzzi, 1995). Suttle reported that addition of virus concentrates changed the

bacterial community composition (Suttle, 1992). A mass lysis event of cyanobacteria caused a change in the composition of bacteria (van Hannen, *et al.*, 1999). These data support the hypothesis that viral lysis indeed influences diversity.

Prokaryotic viruses can mediate gene transfer between prokaryotes. The three types of gene transfer known for prokaryotes are conjugation, transformation and transduction. Transduction is the phage-mediated gene transfer between a donor and a recipient host cell followed by phenotypic expression of transferred genetic traits in the progeny of the recipient. Most studies on viral gene transduction were performed for lake, soil and sea (Ogunseitan, *et al.*, 1990, Nielsen, *et al.*, 1998, Paul, 1999). Its importance for groundwater systems waits to be studied.

1.4 Concept and objectives of the thesis

1.4.1 Spatio-temporal patterns of bacterial communities in a hydrologically dynamic pristine porous aquifer

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, Flynn, *et al.*, 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 are 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, Haack, *et al.*, 2004, McGuire, *et al.*, 2005, 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 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 that 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. In order to understand the seasonal patterns of microbial communities in porous aquifers, two specific questions were raised: (1) Do the spatio-temporal hydrological variations cause dynamics in microbial and physic-chemical patterns? (2) Is the different spatio-temporal patterns of suspended from attached bacterial communities? 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. We further speculated that, groundwater microbial community patterns underlie strong seasonal dynamics, while sediment communities are more stable (Farnleitner, *et al.*, 2005). 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, T-RFLP results have been analyzed in light of ecological concepts, such as the r/K strategy concept and the concept of carrying capacity.

1.4.2 Bacterial colonization in groundwater explored by 454 pyrosequencing

Colonization (i.e. dispersal, immigration and attachment) is a key process for the assembly of microbial communities in all kinds of habitats. In groundwater ecosystems, colonization generally refers to the attachment of cells to sediment surfaces. An intensive exchange between the mobile (pore water) and the immobile (sediment) matrix is expected. Along with changes in environmental conditions (e.g. flow velocity, substrate availability, etc.), cells may attach but also detach. The process of colonization is thus likely accompanied by changes in the community composition. Especially newly introduced or sterile surfaces are readily colonized when exposed to water as shown for surface water (Findlay, *et al.*, 1992, Augspurger, *et al.*, 2010), the deep sea (Kanzog & Ramette, 2009) and groundwater (Hirsch & Rades-Rohkohl, 1990, Griebler, *et al.*, 2002, Flynn, *et al.*, 2008). in marine systems (Hewson, et al., 2007, Galand, et al., 2010, Gomez-Pereira, et al., 2010), in stream (Hewson, et al., 2007, Östman, et al., 2010), rock pools (Langenheder & Ragnarsson, 2007).

So far, most investigations of mechanism of community colonization focused on local habitat conditions as potential selective factors involved in species sorting (van der Gucht, *et al.*, 2007, Logue & Lindström, 2010), probably influenced by the famous statement of Baas-Becking (Baas-Becking, 1934) 'everything is everywhere, but the environment selects'. Evidence were also gained that random processes (Hubbell, 2006) may play an important role in bacterial community colonization (Sloan, *et al.*, 2006, Woodcock, *et al.*, 2007). Recently, it has been demonstrated that both species sorting and neutral processes interact during the assembly of bacterial communities (Langenheder & Szekely, 2011). Therefore, although species sorting appears to be the most important mechanism, the process of community colonization is a complex interaction among environmental conditions and regional factors (Lindström & Langenheder, 2012). Besides colonization such as selective forces or neutral processes, another ecological concept may come into play, the concept of r and K strategy (Douglas, *et al.*, 1990, del Monte-Luna, *et al.*, 2004, Kristufek, *et al.*, 2005, Dorodnikov, *et al.*, 2009). There is evidence for r- and K-strategists, as defined by different growth rates and substrate utilization efficiencies, among bacteria (Chapelle, 2001, Zhou, *et al.*, 2012). Investigations of community establishment on sediment surface have shown that a multitude of physical-chemical factors may affect the attachment of microbes, such as sediment properties, resources availability and hydrodynamics (Fazi, *et al.*, 2008, Kanzog & Ramette, 2009, Augspurger, *et al.*, 2010). Among the various biological factors, the source of the cells colonizing sediment surfaces is of primary interest. The role of the suspended bacterial

compartment as the 'seed bank' for colonization remains poorly studied. For instance, a stochastic colonization or deterministic processes observed during community assembly (Jones & McMahon, 2009, Dumbrell, *et al.*, 2010, Logue & Lindström, 2010), might be explained by a seed bank containing groups of species with similar ecological function (Munday, 2004). Pristine groundwater systems are characterized by carbon and energy limited conditions and lack of light (Goldscheider, *et al.*, 2006), serving as an interesting environment to study the colonization of sediment surfaces 'seeded' by suspended bacteria exhibiting a comparably low abundance and extraordinary low activity. Not only sterile sediment surfaces will provoke microbial colonization but also activity colonized surfaces introduced from another habitat will be subject to community dynamics.

This study explores the bacterial colonization of sterile sediments as well as community changes on active river bed sediments incubated *in situ* in a shallow, sandy and gravely pristine aquifer. Our study is based on two main hypotheses. (1) In relation to the concept of r/K selective environments, we propose a succession of fast growing species followed by others less fast but more competitive. In terms of the systems carrying capacity we expect a fast plateau in attached bacterial biomass (cell numbers) but a delayed climax in community composition, if there is one at all. (2) The initial phase of sediment colonization by bacteria is governed by stochastic processes whereas local conditions control the later composition of attached bacterial communities. In other words, at the early stage of incubation species from the 'seed bank' (suspended bacteria) attach randomly to sterile surfaces, later the growth and establishment of different species is related to the selective factors (e.g. surface properties, substrate availability, physical-chemical conditions, hydrological dynamics). If this is the case, species dominant in groundwater should mainly constitute the attached communities at the early stage of colonization. Community composition at a later phase is expected to be more independent from the community suspended. Bacterial colonization and community composition in sediment were followed by the period of one year and analyzed by 16s rRNA gene pyrosequencing. Growth rates of individual groups and species were semi-quantitatively estimated based on their relative abundance in comparison to total cell numbers.

1.4.3 Microbial food web structure and carbon cycling in a pristine porous aquifer (Mittenwald, Germany)

As mentioned above, terrestrial groundwater ecosystems constitute the largest fresh water biome on earth. They play a crucial role in providing essential ecosystem servies and goods, such as drinking water. At the same time, groundwater ecosystems are least explored and understood (Cullimore, 2007).

It is estimated that more than 50% of all earth carbon is in the subsurface (Whitman, *et al.*, 1998, Kallmeyer, *et al.*, 2012). Exploring the carbon flux within aquifer systems is thus very essential to understand the groundwater microbial biocenoses and *vice versa*. The trophic interactions play an important role not only in channelling organic carbon but also in shaping of community composition. Bacterial community biomass and growth may be controlled by resource availability (bottom-up) and/or predation (top-down), i.e. protozoa and viruses. The comparative importance of top-down and bottom-up effects in groundwater systems received little attention. Studies are generally limited by the precise determination of bacterial growth rates, the abundance of protozoa and viruses as well as grazing rates and rates of viral lysis.

Due to the lack of photosynthesis, groundwater habitats are thought to be controlled almost exclusively by heterotrophic activities (Simon, *et al.*, 2003). Although low in productivity, the large volume gives microorganisms an important role in the storage and cycling of nutrients and carbon (Cooney & Simon, 2009, Griebler & Lueders, 2009). A large body of studies have suggested a close relationship between the organic matter in groundwater ecosystems (mainly dissolved organic carbon) and the microbial biomass and metabolic activities (Baker, *et al.*, 2000, Foulquier, *et al.*, 2011). Therefore, a detailed look at microbial control on carbon flux is necessary to evaluate the microbial food web in groundwater ecosystems.

Since the concept of the ‘microbial loop’ proposed by Azam and colleagues (1983), the idea of a complex food web starting with dissolved organic carbon taking up by heterotrophic bacteria, complemented by autotrophic bacterial production and followed by a series of grazers was developed and evaluated for aquatic ecosystems. Until 1993, the ‘microbial food web’ concept (Rassoulzadegan, 1993) was applied to bacteriasurface waters only. Later, the finding of abundant populations of viruses in aquatic systems significantly influenced the concept of aquatic microbial food webs (Fuhrman, 1999, Wommack & Colwell, 2000, Weinbauer, 2004). Together with heterotrophic nanoflagellaters, viruses (bacteriophages) are generally considered the main cause for bacteria mortality in aquatic systems. Different from the protozoan grazing that channels the organic carbon from bacteria to higher trophic levels,

viral lysis of bacterial cells fuels a considerable part of organic carbon back to the DOC pool, 'viral loop' or 'viral shunt' (Furhman, 1992, Thingstad, *et al.*, 1993, Wilhelm & Suttle, 1999). Consequently, viral-induced bacterial mortality at the same time can promote bacterial growth by releasing easily assimilable carbon and nutrients and reducing the density of competitors (Middelboe, *et al.*, 1996, Noble & Fuhrman, 1999).

Investigations of microbial food webs mainly focused on surface fresh waters (Manage, *et al.*, 2002, Sherr & Sherr, 2002, Thomas, *et al.*, 2011) and marine systems (Riemann & Middelboe, 2002, Unrein, *et al.*, 2007, Fuhrman, 2009). Studies which not only considered bacteria but also protozoa in groundwater are scarse (Sinclair & Ghiorse, 1987, Sinclair, *et al.*, 1993, Novarino, *et al.*, 1997, Kinner, *et al.*, 1998, Kinner, *et al.*, 2002) and only a few papers looked for viruses indigenous to groundwater systems (Kyle, *et al.*, 2008). Papers speculating about groundwater microbial food web dynamics are missing.

Oligotrophic aquatic environments, in particular groundwater systems, are typically considered to be bottom-up controlled, i.e. driven by the availability and composition of organic carbon (Findlay, *et al.*, 2003, Simon, *et al.*, 2003, Wilczek, *et al.*, 2004, Datry, *et al.*, 2005, Foulquier, *et al.*, 2010). The potential top-down control via protozoan grazing is considered to be far less likely. Additional evidence for a bottom-up control in aquifers caused from the much lower protozoa to bacteria ratios obsserved in groundwater, indicating a lower grazing pressure compared to surface waters. Moreover, since the majority of the bacterial biomass (80-99.99%) is found attached to sediment surfaces (Alfreider, *et al.*, 1997, Griebler, *et al.*, 2001, Griebler, *et al.*, 2002) – control of bacterial biomass by grazers may be limited.

The aim of the present study was to get a first look at the structure of the microbial food web in an oligoalimonic hydrologically dynamic porous aquifer in Mittenwald (Southern, Germany). Main objectives of our research were (1) to test the hypothesis of bottom-up control in oligotrophic groundwater systems; (2) to estimate the carbon flux through the groundwater microbial food web; and (3) to shed some light to the composition of protozoan communities. In this context, the availability and degradability of DOC is hypothesized to be the main controlling factor, while regulates the productivity of the different trophic levels (bacteria, protozoa, virus). We monitored the abundances of bacterial cells, protozoa and virus-like particles in groundwater every two months over the period of one year, and estimated the carbon flux within the food web on basis of concentrations of dissolved organic carbon (DOC), bacterial carbon production (BCP) and assimilable organic carbon (AOC) data complemented with carefully selected rates from literature for bacterial grazing by

heterotrophic nanofagellate (HNF) and mortality induced by viral lysis. In addition, the composition of bacterial and protozoan communities in groundwater was analyzed.

2 MATERIALS AND METHODS

2.1 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. 2). 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.

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 grassland near the foot of a mountain. Well MIT052 was located on a mountain pasture in a recreational area (Figure 2.1).

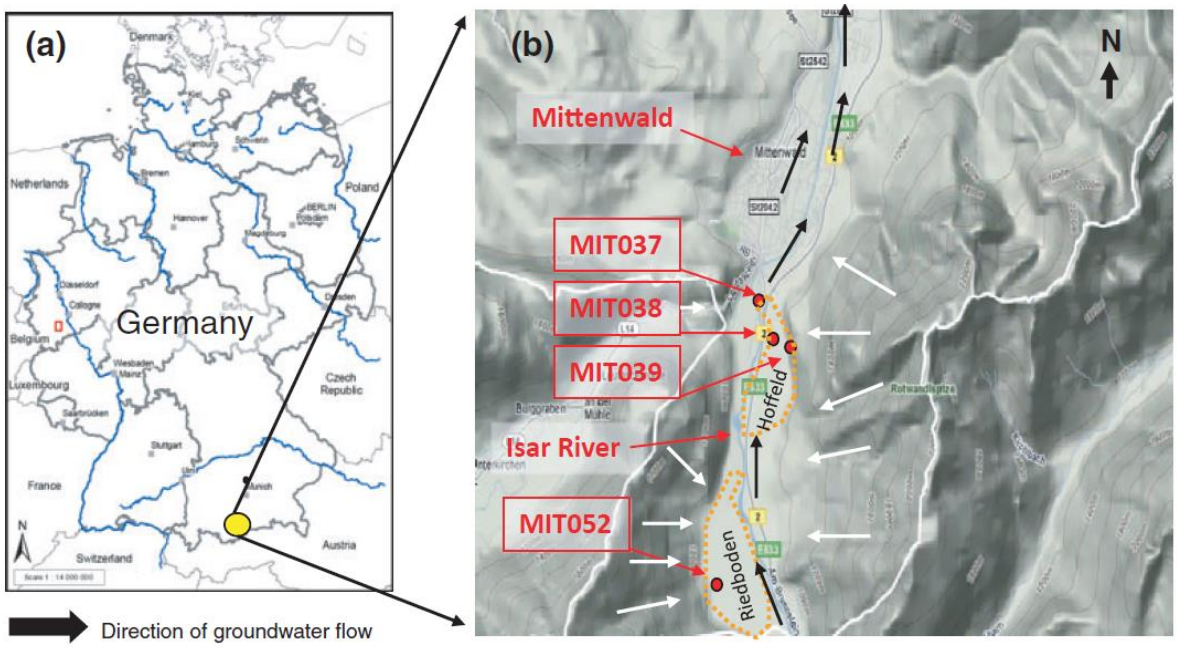


Figure 2.1 Land scale of sampling site. (a) Geographic position of the study area. (b) Topographic map highlighting the location of the selected groundwater monitoring wells and the Isar River. Arrows indicate the general direction of groundwater flow. Wells MIT037, MIT038, and MIT039 are distributed in the area of 'Hoffeld' close to the village of Mittenwald, while well MIT052 is located in the mountain pasture 'Riedboden'.

2.2 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 0.22µm filter membranes (Millipore, MA) to concentrate suspended bacteria for subsequent DNA extraction. Filters were shock-frozen on dry ice and stored at -20 ℃. For physical–chemical analysis, groundwater was collected in 500-mL sterile Schott bottles and stored at 4 ℃ till further processing. Due to the strongly hydrologically dynamics, the velocity of groundwater flow was fast. Consequently, well water undergoes similar annual seasonal dynamics. Moreover, the wells were completely protected from the possibly contamination by closed cover. Therefore we assumed that groundwater is similar to well water. Water samples for DOC measurement were filtered through 0.45µm pore size cellulose-nitrate filters (Millipore)

and acidified with HCl to a pH 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 ℃ by repeatedly changing the water. Afterward, the columns were stored at 4 ℃ 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 ℃ in the dark until further processing. Aliquots for DNA extraction were shock-frozen on dry ice and stored at -20 ℃ till further analysis. All samples were carried out by biological duplicates.

2.3 Physical–chemical conditions

Key physical and chemical parameters such as electric conductivity (EC), temperature, water table, pH, redox potential (EH), 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 CO2 (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.

2.4 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 ℃ for 5 h under constant shaking and for $\delta^{2}H$ values via reaction with U at 800 ℃. 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 $\delta^{2}H$.

2.5 Microbiological variables

2.5.1 Counts of bacterial cells, protozoa and virus-like particles

Total bacterial cell counts. 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 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).

Counts of virus-like particles by flow cytometry. Virus-like particles (VLP) were stained with SYBR Green I (10,000 × concentrate in DMSO; Invitrogen, Molecular Probes; storage at –20 ℃) and water samples were prepared as described in detail by Marie (1999) and by Brussaard (2004). In brief, 5 µL working stain solution (1:200 diluted in sterile Milli-Q water) was added to 500 µL sample. The samples were incubated at 80°C for 10 min in the dark, followed by a cooling period at room temperature in the dark for 5 min before analysis. In

case of high VLP concentrations, samples were diluted 1:10 or 1:100 to increase the accuracy of the analysis. Control blanks, consisting of TE-buffer with autoclaved 0.2-µm-filtered sample with the same dilution factor as the natural samples used were measured first. Samples were measured in biological duplicates. Counting was determined using a flow cytometer (FACSCalibur, BD Sciences, San Joes, CA, USA), equipped with an air-cooled laser, providing 15mW at 488 nm with the standard filter set-up. Population of VLP was identified on the plots of the side scatter versus green fluorescence at 530 nm wave length. FCM modes were analysed using the CellQuest Pro software (BD Bioscences, version 4.0).

Counts of protozoa by fluorescence *in situ* hybridization (FISH). Enumeration of protozoa was conducted using fluorescence *in situ* hybridization (FISH), following protocols of Glöckner (1996) and Manz (1992), which have been modified as given below. According to the general ratio of bacteria to protozoa (1000:1), the protozoan density was expected to be in the magnitude of 100 cells mL^{-1}. In order to collect enough cells, 300 mL water samples, fixed with formamide, were filtered through polycarbonate filters (0.2 µm pore size; 25 mm diameter; type GTTP; Millipore Corp. Bedford, MA, USA), supported by cellulose acetate filters (0.45 µm pore size; Millipore), to ensure an equal distribution of cells on the filter membranes. Subsequently, filters were dipped in low-gelling-point agarose [0.1% (wt/vol) Biozym, USA; in Milli-Q water], dried upside down on a glass slide at 37 °C, and dehydrated in 96% (vol/vol) ethanol. Cell wall permeabilization was performed by incubation of filters in lysozyme (10 mg mL^{-1} in 0.05 M EDTA, pH 8.0; 0.1 M Tris-HCl, pH 8.0; Sigma, Vienna, Austria) at 37 °C for 1 h. Filters were then washed three times in Milli-Q water and incubated in 0.01 M HCl for 20 min at room temperature for inactivation of endogenous peroxidases. After three times of washing, filters were dehydrated in 50%, 80% and 96% ethanol for 2 min respectively and air-dried at room temperature. Each filter was hybridized with 16 µl hybridization buffer [0.9 mol L^{-1} NaCl, 0.02 mol L^{-1}, 45% (vol/vol) formaldehyde, 0.02% (wt/vol) sodium dodecyl sulfate (SDS)] at 46 °C for 1 h. 2 µl of the oligonucleotide probe EUK 1195 (5'-GGGCATCACAGACCTG-3'), labeled with Cy3 and Cy5, were added to each filter to a final concentration of 3 ng $µl^{-1}$. After hybridization, the filters were transferred to pre-heated washing buffer [20 mmol L^{-1} Tris/HCl, 5 mmol L^{-1} EDTA, 30 mmol L^{-1} NaCl, 0.01% SDS] at 48 °C for 20 min. Afterwards, filters were washed three times in Milli-Q water and air dried on the glass slide at room temperature. Slides were embedded with DAPI/Antifade Solution (Millipore, USA, ready to use) and Glycerol/PBS buffer solutions (Citifluor, London, AF1). The cell counting was performed by epifluorescence microscopy

(Microscopy: Axioplan 2 Zeiss; Fluorescence Illumination: HXP-120 LEj; Microscope camera: Axiocam Zeiss) at a magnification of 63×.

2.5.2 Bacterial carbon production

Samples for bacterial carbon production (BCP) measurements were collected in four replicates. BCP was determined via the incorporation of [^{3}H]-leucine into cellular proteins (Kirchman, 1993), following the protocol of (Fischer & Pusch, 2001). Four replicates of 45 mL and one 50 mL formaldehyde-killed control (4% v/v final concentration) were amended with L-[4,5-^{3}H]-leucine (1000 nM final concentration) and incubated for 14h at *in situ* temperature (10-12 ℃) in the dark. The incorporation of [^{3}H]-leucine was terminated by fixation of the samples with formaldehyde (3.7% v/v final concentration). The extraction procedure, measurement and final calculation of BCP followed Kirchman (1993).

2.5.3 Assimilable organic carbon

Assimilable organic carbon (AOC) was determined following a protocol of Escobar & Randall (2001). 45 milliliters of water, filtered through a 0.22µm 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 fg carbon, was based on the study by Griebler et al. (2002).

2.5.4 ATP concentration

Total and extracellular ATP concentrations were determined following the protocol of Hammes (2010), using the BacTiter-Glo™ Microbial Cell Viability Assay (G8231; Promega Corporation, Dübendorf, CH) and a GloMax® 20/20 Luminometer (Turner BioSystems, Sunnyvale, CA, USA). Intracellular ATP was obtained by subtracting extracellular ATP from total ATP values.

2.6 Microbiological molecular analysis

2.6.1 DNA extraction and amplification

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 ℃ until further processing.

2.6.2 Terminal restriction fragment length polymorphism analysis

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. All 63 samples including water and sediment samples were taken for T-RFLP analysis.

2.6.3 Clone libraries

For clone libraries, the bacterial 16S rRNA genes from 8 water samples in May and July 2010 were amplified via PCR using the primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Reysenbach, et al., 2000). One hundred and eight clones were constructed (16–20 clones per samples); Clone libraries were generated from groundwater samples of May and July 2010. DNA was harvested from 5L water samples DNA filtered through 0.22μm filter membranes (Millipore, Massachusetts, USA). Bacterial 16S rDNA were amplified via PCR using the primer pair 27f and 1492r. Gel electrophoresis of DNA samples was used to qualitatively check the amplicons. PCR products were purified using the QIAquick purification kit (Qiagen) and cloned using the pGEM-T kit (Promega). Complete E.coli cells (TOPO XL PCR Cloning Kit) were transferred and grew on LB-Ampicillin (100mg/ml) plates. 16 to 20 clones per samples were selected and subcloned

on fresh plates overnight at 37 ℃. Plasmid DNA was isolated by boiling single clones in 100 µl PCR water and spinning it down for 5 min at 500 rpm. 50 µl supernatant was taken as template. M13F and M13R primers were used to obtain 108 gene sequences of 16S rRNA. Sequences were assembled and manually trimmed in Seqman (DNASTAR-Lasergene v6). The sequences were automatically pre-aligned in ARB and the alignments were subsequently improved considering the secondary structure of the rRNA molecule. The phylogenetic tree was constructed applying the Maximum likelihood algorithm RaxML. Phylogenetic analyses were performed using the ARB software package (Ludwig, et al., 2004).

2.6.4 Tag sequencing

Extracted DNA was PCR-amplified using primers targeting bacterial 16S ribosomal RNA genes on a 454 GS FLX Titanium system (Roche, Penzberg, Germany), following the protocol of Pilloni *et.al* (Pilloni, *et al.*, 2011). The primer Ba27f (5'-agagtttgatcmtggctcag-3') and Ba519r (5'-tattaccgcggckgctg-3') extended with the respective A or B adapters were used for the amplification for multiplexing, key sequence and multiplex identifiers (MID) as recommended by Roche. PCR amplicons were performed in a Mastercycler ep gradient (Eppendorf, Hamburg, Germany) with the following cycling conditions: initial denaturation (94 ℃, 5 min), followed by 28 cycles of denaturation (94 ℃, 30 s), annealing (52 ℃, 30s) and elongation (70 ℃, 60 s). Each 50 µl PCR reaction contained 1 × PCR buffer, 2 mM $MgCl_2$, 0.06 mM dNTPs, 1.0 U recombinant Taq polymerase (Kit from Fermentas, St. Leon-Rot, Germany), 0.2 µg μL^{-1} bovine serum albumin (BSA) (Roche, Penzberg, Germany), 0.2 µM of each primer (Biomers, Ulm, Germany) and 1 µL of DNA template. Each biological duplicate was amplified in technological duplicates, in total four replicates for each sample. Replicates were purified and combined for further analysis. The PCR amplicons were purified using Agencourt AMPure-XP beads (Beckman Coulter, Brea, CA) and pooled in an equimolar ratio of 109 molecules ml21 as quantified by the Quant-iT PicoGreen dsDNA quantification kit (Invitrogen, Paisley, UK). Emulsion PCR, emulsion breaking and sequencing were performed applying the GS FLX pyrosequencer (Roche). River water and groundwater from well MIT039 and MIT052 on March, May, July and December 2010 together with all sediment samples were selected for tag sequencing. In total 28 samples were analyzed.

2.6.5 Sequence data analysis

Quality filtering of the pyrosequencing reads was performed using the automatic amplicon pipeline of the GS Run Processor (Roche), with a modification of the valley filter (vfScanAll-Flow false instead of TiOnly) to extract sequences. Reads were further quality-trimmed using TRIM function of Greengenes (DeSantis, *et al.*, 2006) with settings of good-quality score 20, window size 40 bp and window threshold 90%. Subsequently, reads were batched per sample based on MID-identifiers with BIOEDIT (Hall, 1999) and reads with inferior read length (< 250 bp) were excluded from further analysis. The total community composition was classified via read affiliation using the RDP classifier (Wang, *et al.*, 2007) at a confidence threshold of 80%. Taxonomic assignments with less than 80% confidence were marked as unknown. Read abundance percentage of classified lineages was recorded. The RDP pyrosequencing pipeline (Cole, *et al.*, 2009) was also used to generate Shannon diversity indices *H*' and rarefraction curve for overall community comparison based on linkage clustering of detected OTUs (97% sequence similarity). The number of operational taxonomic units (OTUs) in a sample is defined by the clusters formed at a given level of sequence difference (Quince, *et al.*, 2009). The number of OTUs is used as an indicator of bacterial richness. Compared to the number of OTUs which stands for the visible richness, the Chao1 index is an nonparametric estimator of the total richness (total number of phylotypes) (Chao, 1984, Chao, *et al.*, 1993). The Shannon diversity index (*H'*) is an estimator of the biodiversity (Hill, 1973, Jost, 2006).

Relative abundance was calculated for the OTUs in each sample and used to calculate pairwise similarities among samples using the Bray–Curtis similarity coefficient. Multiple dimensional scaling (MDS) and cluster analysis was used to explore similarity matric among samples based on Bray-Curtis similarity by R 2.14.1.

2.6.6 Eukaryotic communities profiling by T-RFLP, cloning and sequencing

Eukaryotic communities were analyzed using an optimized PCR/T-RFLP fingerprinting approach (Euringer & Lueders, 2008) with the EUK 20f/502r+3 primer set in combination with Bsh1236I digestion enzyme. Water samples from well MIT052 in October 2010, well MIT037 in May 2010 and Isar river in July 2010 were selected for cloning and sequencing with the EUK20f/502r+3 primer set as previously described (Winderl, *et al.*, 2007). 39, 51 and 53 clones for groundwater from MIT052, MIT037 and river water library were analyzed

respectively. Sequences were identified using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

2.7 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.

2.8 Estimation of bacterial growth rates and mortality

2.8.1 Estimation of growth rates

The abundance of each species is calculated by multiplying the relative abundance with the total cell abundance. Therefore, the average growth rate of each species can be estimated when dividing their abundances by the time in days. For instance, growth rate of a certain species in May 2010 was calculated by (total cell abundance × relative abundance of this species) / 49 days (incubated since March 2010).

2.8.2 Estimation of bacterial mortality

Bacterial mortality was calculated by summed up mortality due to grazing and mortality due to viral lysis. Bacterial mortality due to protozoa grazing was calculated by dividing the grazing rate by the bacterial production rate. Grazing rates were estimated as described by Steward (1996) as the product of the bacterial abundance and protozoa abundance and clearance rates.

The model of Binder (1999) was used to estimate the fraction of bacterial mortality caused by viral lysis (FMVL): $FMVL = FVIC / [\gamma \times \ln(2) \times (1-\varepsilon-FVIC)]$, where $\gamma = 1$(the ratio of the latent period and generation time), $\varepsilon = 0.816$ (the fraction of the latent period during which

viral particles are not yet visible) and FVIC is the frequency of visible infected bacterial cells. FVIC data were collected from comparable aquatic ecosystems (Table 4.1). Lytic viral production = FMVL $\times$ burst size (Bz) $\times$ BP, shown in VLP L^{-1} h^{-1}.

3 RESULTS

3.1 Physical-chemical characteristics

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 (Figure 3.1A and B). Groundwater temperature ranged from 4 to 9 ℃, which was significantly less variable than the temperature dynamics in the river, which peaked at a value of 13.2 ℃ in July 2010 (Figure 3.1C). The electric conductivity was found with an annual mean value of 299 ± 44 µS cm^{-1} for groundwater from all sites investigated (Figure 3.2). 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. The concentrations of dissolved oxygen (DO) always close to saturation, which, corresponding to the low temperature ranged from 8.2 - 11.3 mg L^{-1}.

Table 3.1 Summary of relationships between microbial variables and hydrological characteristics for groundwater and Isar river water provided by Pearson correlation matrix analysis. A P value of < 0.05 was considered 'statistically significant'. T: temperature, DOC: dissolved organic carbon, AOC: assimilable organic carbon. n=24.

	pH	K	nitrate	DOC	richness	Shannon
water table changes			r = 0.468 P = 0.0211			
T	r = -0.667 P = 0.00037	r = 0.665 P = 0.0004				
Ca		r = 0.594 P = 0.0022				r = -0.439 P = 0.032
Mg		r = 0.411 P = 0.046		r = 0.408 P = 0.048	r = -0.588 P = 0.0025	r = -0.608 P = 0.0016
Alkalinity					r = -0.444 P = 0.030	
AOC					r = 0.422 P = 0.040	r = 0.425 P = 0.039

Groundwater and water from the Isar river in the opposite, were found to be low in dissolved organic carbon (DOC concentration = 1 to 3 mg L^{-1}, Figure 3.1E) and nutrients (NO_3^- concentration = 1.7 to 4.7 mg L^{-1}, PO_4^{3-} concentration = b.d. to 0.065 mg L^{-1}). Nitrate concentrations were positively correlated with the water table changes (r = 0.468, P = 0.0211) (Table 3.1). Stable water isotope data, that is, $^2H/^1H$ and $^{18}O/^{16}O$, dropped from March to May before rising again.

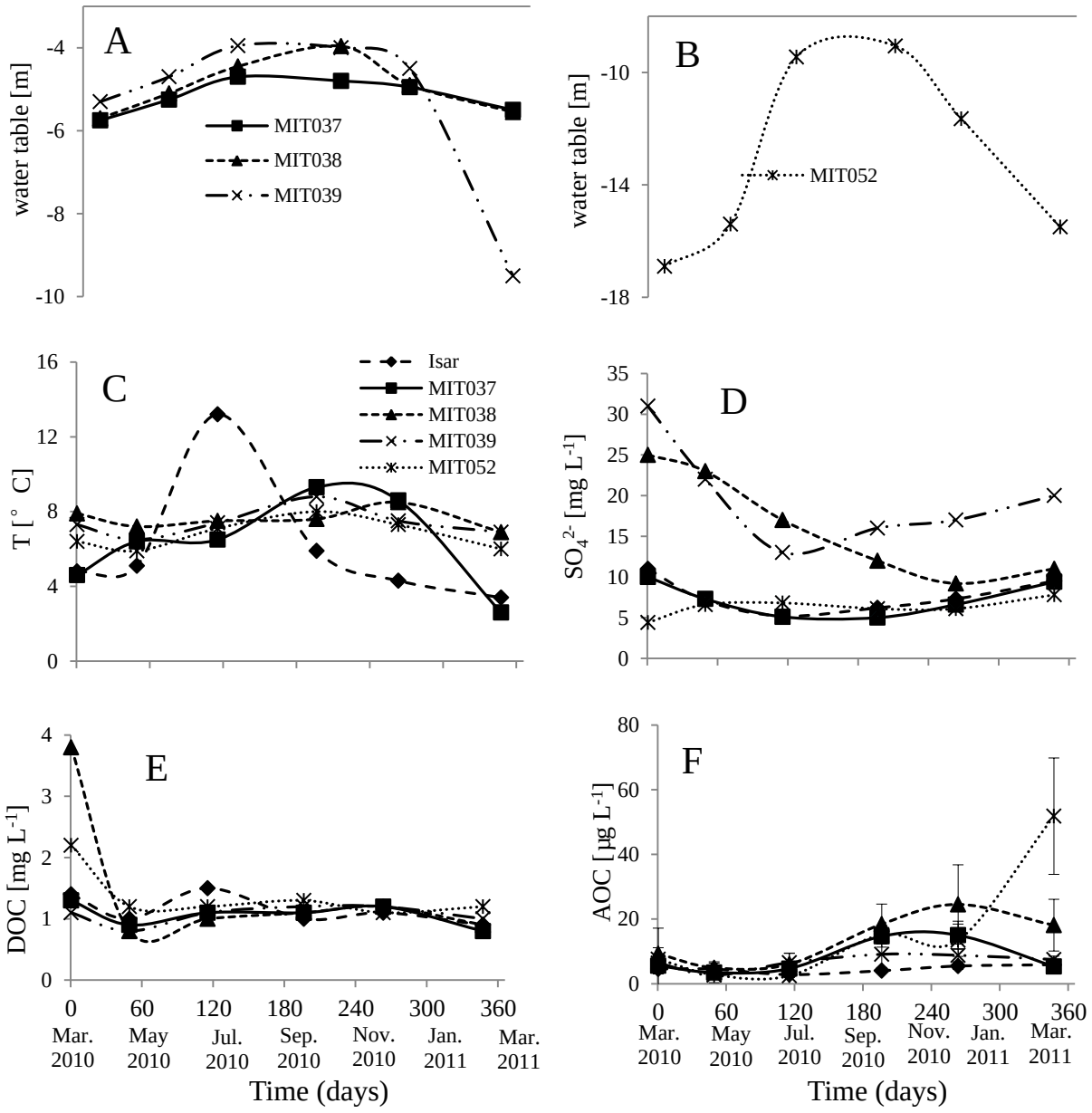


Figure 3.1 Seasonal patterns of selected physical–chemical parameters of groundwater and river water.

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 L^{-1} were observed in March and May 2010, respectively.

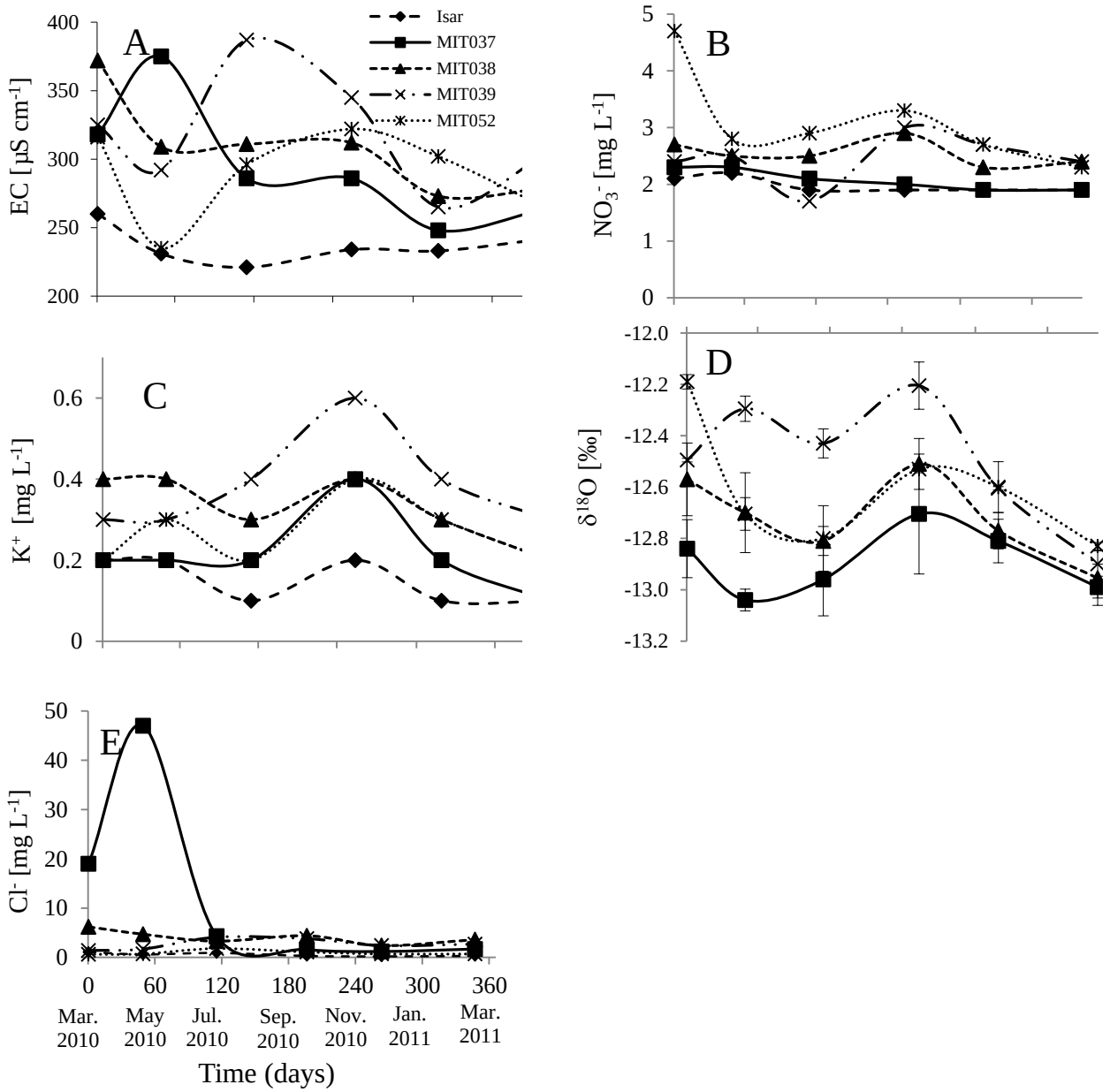


Figure 3.2 Seasonal patterns of electric conductivity (EC), nitrate, potassium, ^{18}O in water and chloride content.

Sulfate concentrations separated the groundwater adjacent to the river from groundwater of the more distant wells in the Hoffeld. Groundwater pumped from below the Riedboden was found most similar to river water (Figure 3.1D). 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. 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 (Figure 3.1). Apart from these extraordinary high concentrations, a slightly elevated DOC level in groundwater was found during summer and winter and lowest values occurred in spring (Figure 3.1). 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 (Figure 3.1).

3.2 Spatio-temporal patterns of bacterial communities

3.2.1 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} (Figure 3.3A). 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. 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.

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 (Figure 3.3B).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 (Figure 3.3B).

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 $H' = 3.64 \pm 0.19$ (Figure 3.3B). 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 (Figure 3.3B).

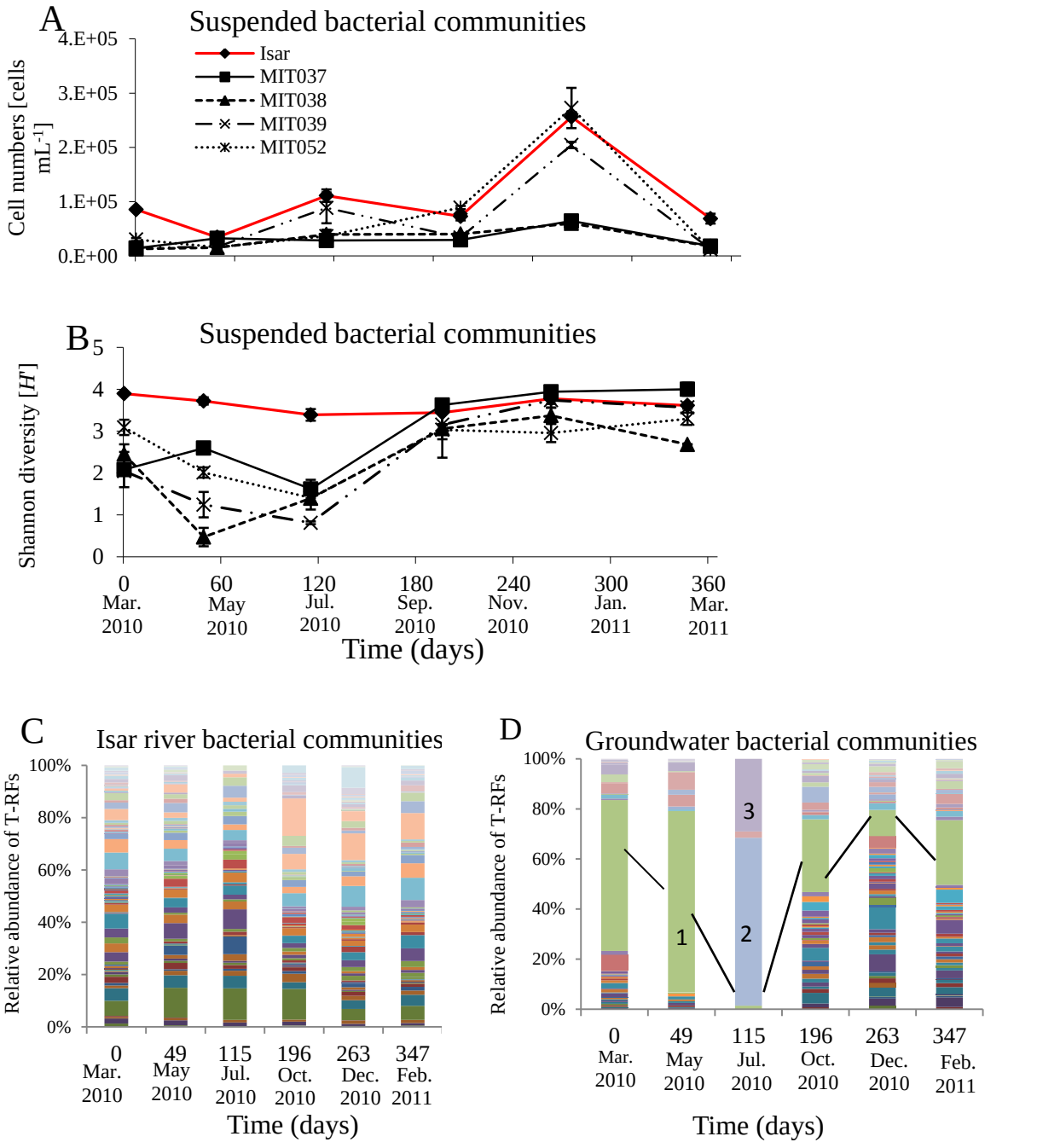


Figure 3.3 Seasonal patterns of suspended bacterial communities. (A) bacterial abundance; (B) bacterial Shannon diversity; (C) relative abundance of T-RFs in water samples from the Isar River and (D) in groundwater samples collected at well MIT039. T-RFs are distinguished by color, and its relative abundance corresponds to bar height. Dominant T-RFs are highlighted: (1) bp 267, (2) bp 313, (3) bp 353.

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 (Figure 3.3B). 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 (Figure 3.3B). 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 $H' = 3.64 \pm 0.19$.

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. 5c 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.

The low diversity of bacterial communities was further supported by clone libraries generated from groundwater samples in May and July 2010 (Figure 3.4). Compared to July, suspended bacterial communities in May were still more diverse with sequences affiliated to *Rhodocyclaceae* (43%), *Comamonadaceae* (6%), *Alphaproteobacteria* (6%), and *Gammaproteobacteria* (6%).

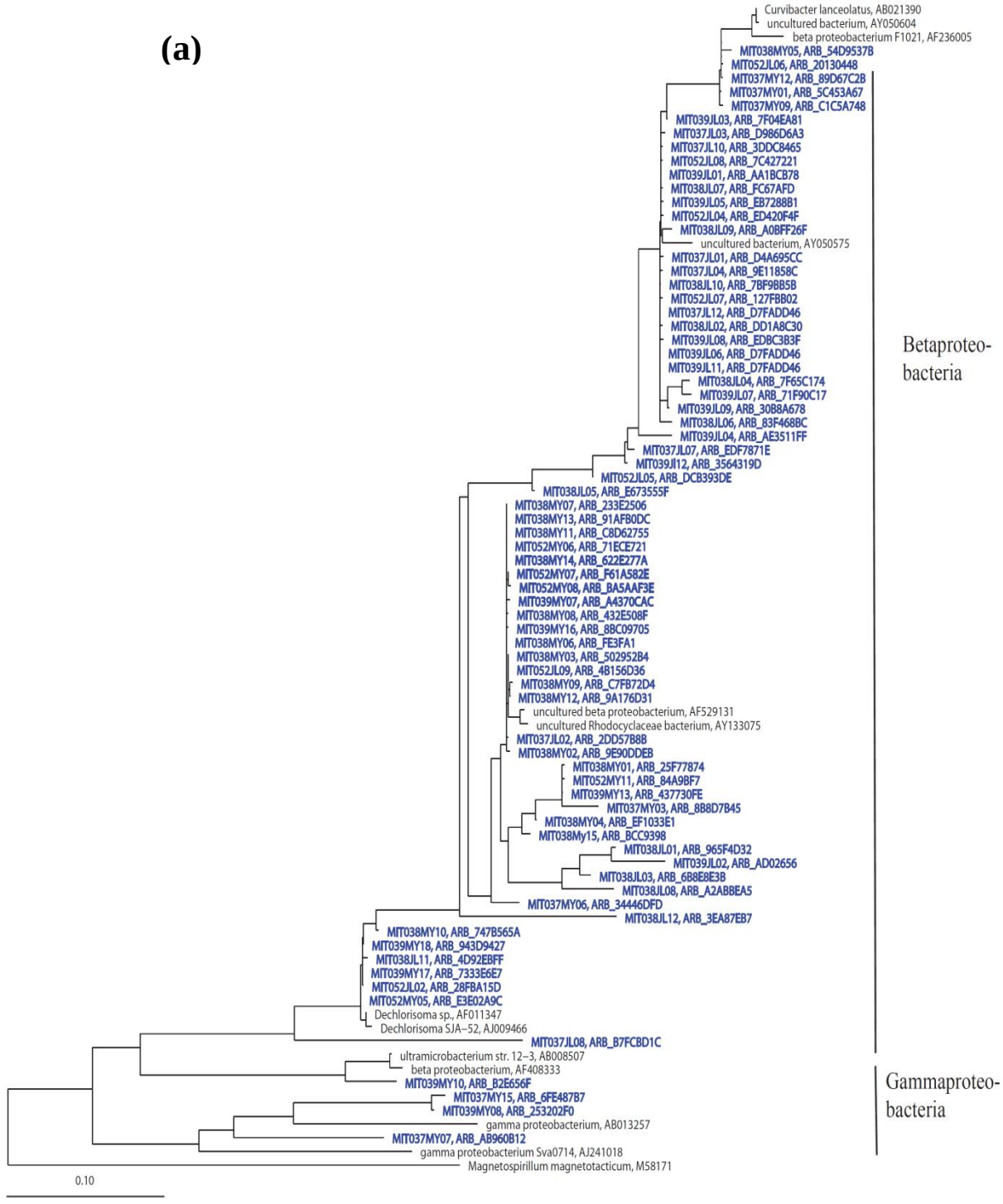


Figure 3.4a Phylogenetic tree of 16S rDNA sequences from groundwater samples collected in May 2010. ARB accession numbers are given as recommended per sequence in ARB. MY = May 2010. The tree was reconstructed for almost full-length 16S rRNA gene sequences with the ARB software environment (Ludwig, *et al.*, 2004) using maximum likelihood algorithms.

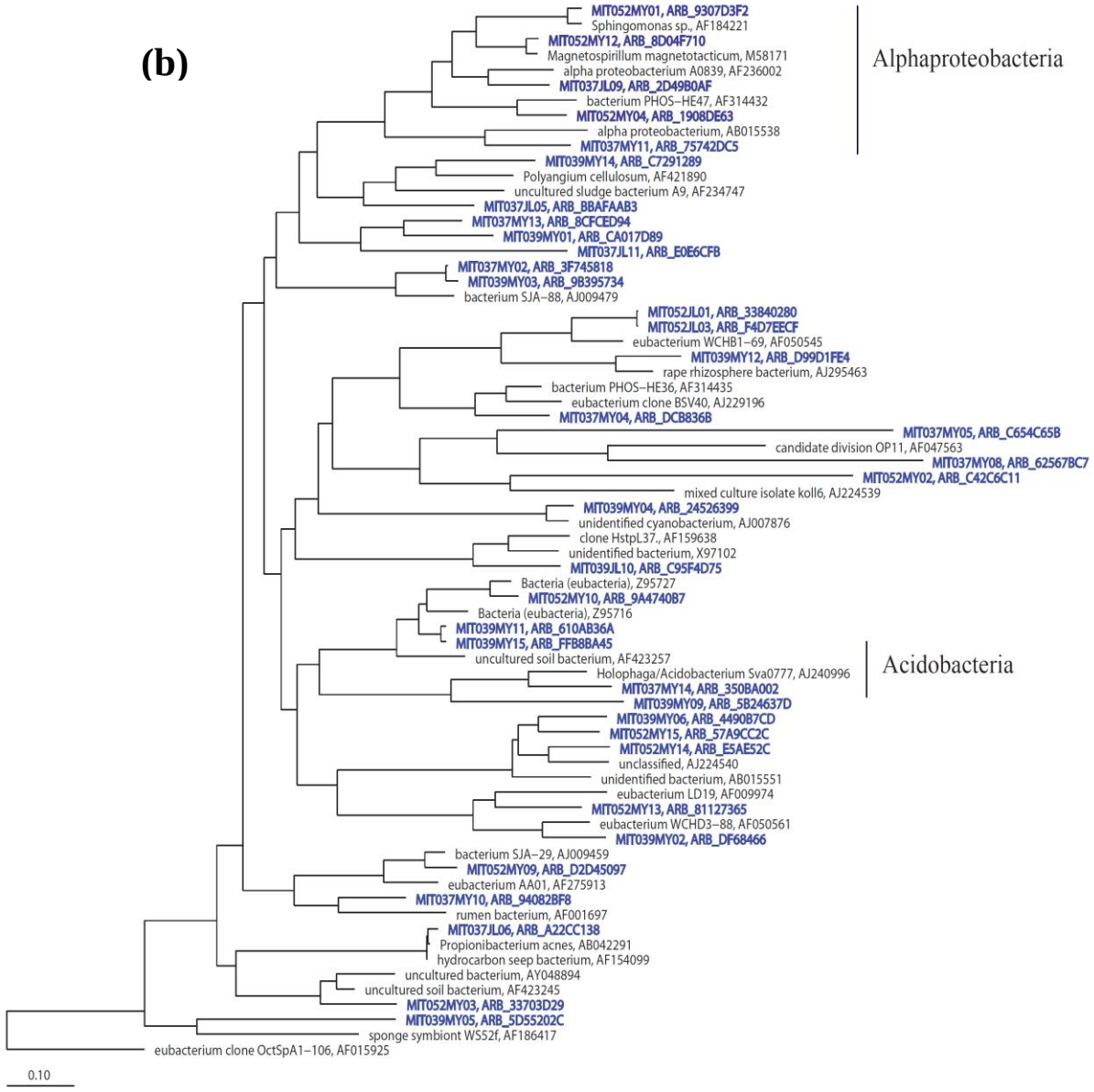


Figure 3.4b Phylogenetic tree of 16S rDNA sequences from groundwater samples collected in July 2010. ARB accession numbers are given as recommended per sequence in ARB. JL = July 2010. The tree was reconstructed for almost full-length 16S rRNA gene sequences with the ARB software environment (Ludwig, *et al.*, 2004) using maximum likelihood algorithms.

On average, bacterial communities in groundwater were characterized by higher cell numbers but lower Shannon diversities than those in well water (Table 3.2). 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 3.2). However, similar to groundwater samples, well

waters displayed a seasonal dynamic in Shannon diversity (Figure 3.5), although less pronounced.

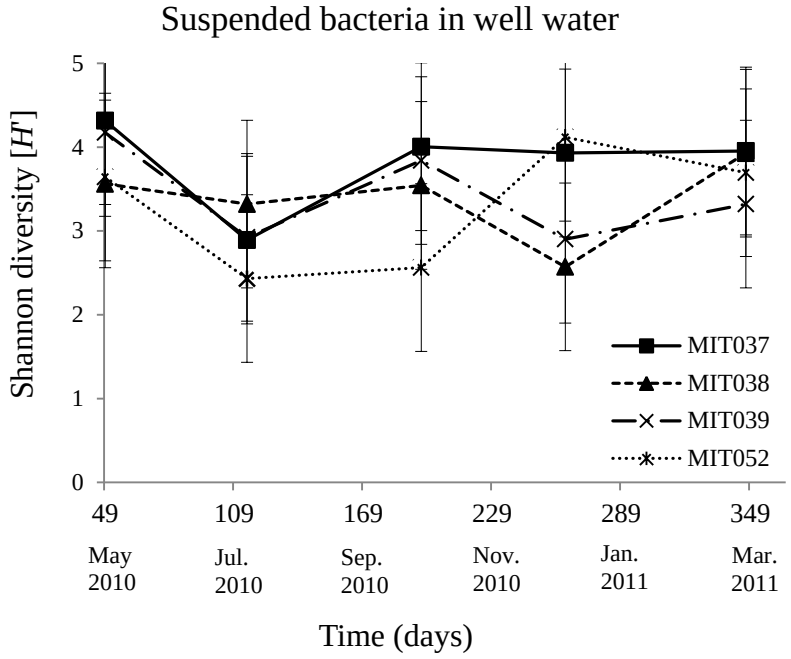


Figure 3.5 Seasonal dynamics of bacterial Shannon diversity in water of the individual groundwater monitoring wells.

Table 3.2 Comparison of suspended bacterial communities of groundwater and well water. 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.

	Groundwater		Well water		Community similarity for groundwater and well water
Wells	TNC ($\times 10^4$ cells mL^{-1})	Shannon Diversity	TNC ($\times 10^4$ cells mL^{-1})	Shannon Diversity	
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 ± 10.8
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

3.2.2 Attached bacterial communities

Total cell counts of the fresh river bed sediments dropped slightly from $1.0^{8} \times 10^{7}$ 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 (Figure 3.6A). Attached bacterial cells on the sterile sediments reached an average cell number of $9.0 \pm 6.5 \times 10^{6}$ cells mL^{-1} after two months incubation and stayed constantly for later incubation period (Figure 3.6A).

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 $H' = 4.19 \pm 0.18$. 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 (Figure 3.6C and D).

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. 9b, 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 high and constant Shannon diversities ($H' = 4.31 \pm 0.32$) and small changes in composition (Bray–Curtis dissimilarity = $21.34\% \pm 3.46\%$). With water samples, a similar constant clustering was only observed for the river water communities ($H' = 3.59 \pm 0.2$; Bray–Curtis dissimilarity = $37.9\% \pm 4.2\%$), while groundwater communities grouped more according to the site and season (Figure 3.7).

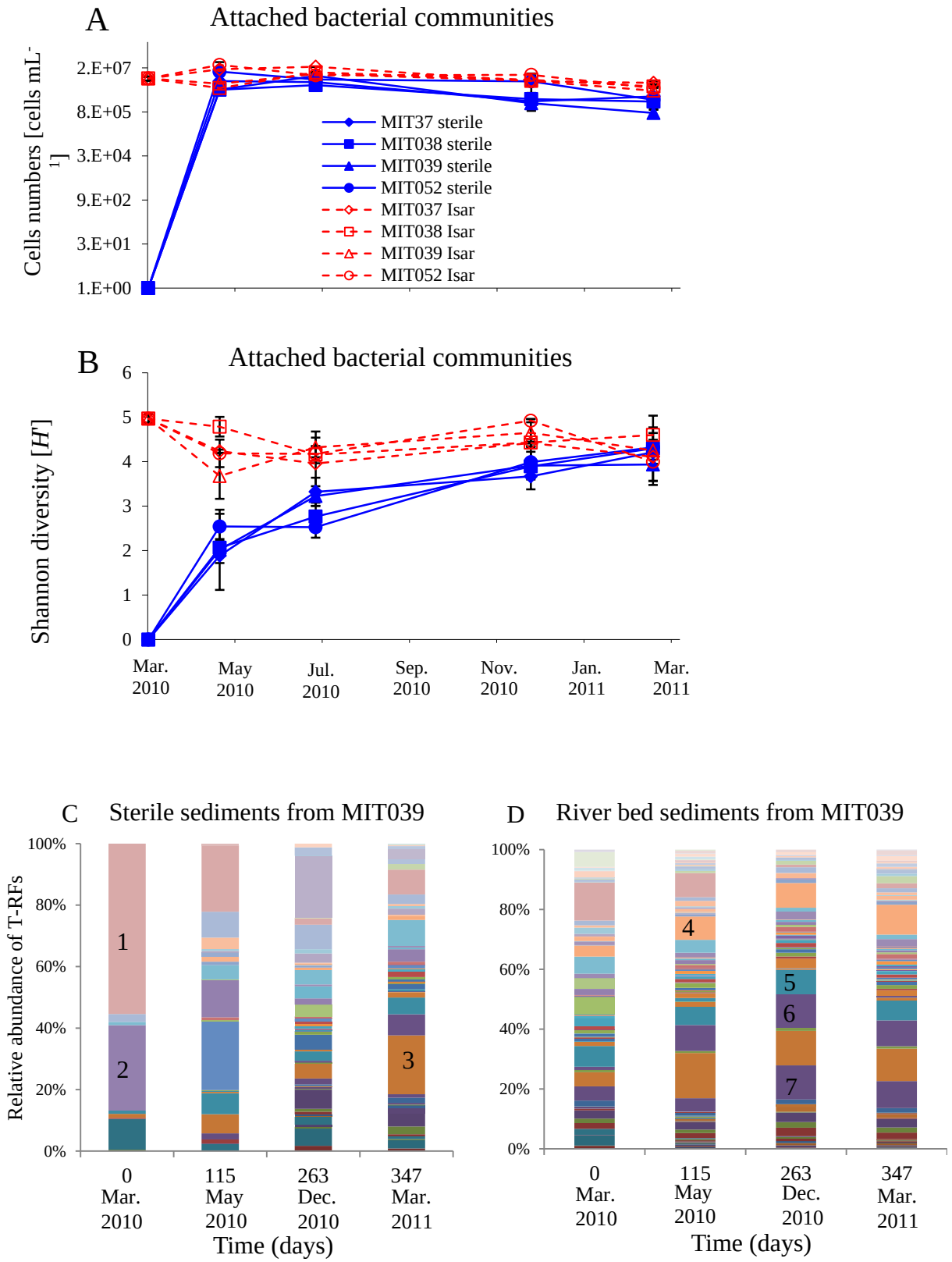


Figure 3.6 Sediment bacterial communities. (a) bacterial abundance; (b) bacterial Shannon diversity; and relative abundance of T-RFs of (c) initially sterile sediment and (d) river bed sediments exposed to water in well MIT039. Dominant T-RFs are highlighted: (1) bp 325; (2) bp 233; (3) bp 126; (4) bp 269; (5) bp 132, (6) bp 130, (7) bp 124.

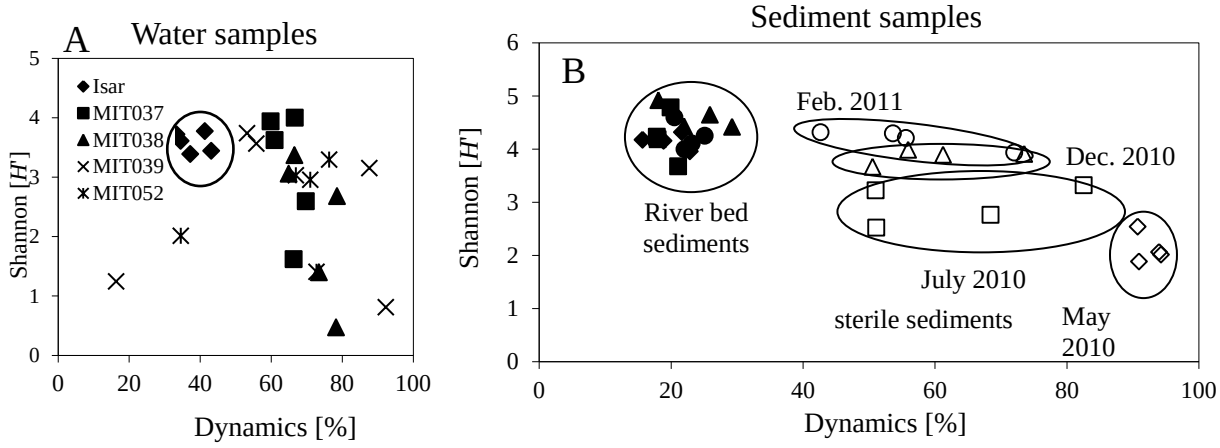


Figure 3.7 Scatter plot of bacterial Shannon diversity vs. the degree of change in community composition [Dy] for (a) water and (b) sediment samples. The dynamics of community composition changes based on the Bray–Curtis dissimilarity index calculated for between consecutive sampling events; hence, the data from March 2010 served as starting point.

3.3 Colonization of bacterial communities revealed by 454 pyrosequencing

3.3.1 Estimation of microbial diversity and richness

In total, 29 samples collected at 5 occasions between March 2010 and Februay 2011, including water and sediment were analyzed. Initially colonized sediments and initially sterile sediments were exposed to wells and subsamples over a period of one year. The 454 pyrosequencing revealed in total 250981 raw reads. After removing low quality and short reads, 240233 bacterial reads were kept for further analysis (Table 3.3). Because richness and diversity was a function of OTU cut-offs (dissimilarity), the number of OTUs, the Chao1 and Shannon diversity index were calculated based on 1%, 3%, 6% and 10% dissimilarity level (Table 3.3). Shaw and his colleagues (Shaw, *et al.*, 2008) found that the 97% cut-off (3% dissimilarity) produced the most consistent rankings. Therefore, the 97% cut-off was used for further comparison.

For suspended bacteria, the number of OTU from river water (3432 ± 4006, from 810 to 2582) was higher than the groundwater (1619 ± 1146, from 1775 to 4883 for well MIT039 and 1694 ±1178, from 901 to 2935 for well MIT052). For attached bacteria, the highest OTU

number was observed in the natural river bed sediment, with OTUs value of 4397. The sediments initially taken from the river bed at any time throughout the incubation cantained higher OTU numbers (with OTU numbers of 2543 ± 430, from 2016 to 2953 for well MIT039 and 1494 ± 394, from 1104 to 2035 for well MIT052 respectively) than the initially sterile sediments (with OTU numbers of 474 ± 269, from 240 to 828 for well MIT039 and 484 ± 277, from 155 to 823 for well MIT052 respectively). Chao1 was about two to three times higher than the number of OTUs for all samples with the exception of the sterile sediment samples (Table 3.3). Shannon diversity index showed a similar pattern to Chao1, with always higher values for the river water (H' = 7.2 ± 0.5, from 6.4 to 7.7) than for the groundwater (H' = 5.4 ± 1.5, from 3.8 to 7.2 for well MIT039 and H' = 5.4 ± 1.4, from 3.8 to 7.0 for well MIT052) and a higher diversity found in river bed sediments (with Shannon diversity index of 6.9 ± 0.2, from 6.6 to 7.0 for well MIT039 and 6.4 ± 0.2, from 6.3 to 6.6 for well MIT052 respectively) than the initially sterile sediments (with Shannon diversity index of 4.4 ± 0.9, from 3.5 to 5.7 for well MIT039 and 4.7 ± 0.6, from 3.5 to 5.4 for well MIT052 respectively).

Table 3.3 Similarity-based OTUs and diversity estimations. SS: initially sterile sediment. RS: initially river sediment. Values were expressed as average ± SD of all seasonal samples from each well. The OTU, Chao1 and Shannon diversity index (*H'*) were presented for a dissmilarity of 1%, 3%, 6% and 10%.

Sample	Reads	1% dissimilarity			3% dissimilarity			6% dissimilarity			10% dissimilarity		
		OTU	Chao1	*H'*	OTU	Chao1	*H'*	OTU	Chao1	*H'*	OTU	Chao1	*H'*
water													
Isar	51765	5068 ±1538	12825 ±6346	7.9 ±0.4	3432 ±4006	7115 ±4006	7.2 ±0.5	2414 ±2094	4038 ±2094	6.6 ±0.5	1525 ±884	1998 ±884	6.1 ±0.5
MIT039	34467	2373 ±1044	4937 ±1927	6.3 ±1.2	1619 ±1146	2838 ±1146	5.4 ±1.5	1230 ±764	1795 ±764	5.0 ±1.6	844 ±453	1040 ±43	4.6 ±1.5
MIT052	25840	2334 ±1079	5789 ±2394	6.3 ±1.1	1694 ±1178	3361 ±1178	5.4 ±1.4	1272 ±565	2036 ±565	5.1 ±1.3	850 ±327	1103 ±327	4.7 ±1.2
sediment													
IsarS	19461	7306	14657	8.2	4397	7180	7.2	2822	3931	6.6	1651	1989	5.9
MIT039SS	23609	1096 ± 279	1619 ±369	5.7 ±0.5	474 ±269	622 ±325	4.4 ±0.9	324 ±202	397 ±215	4.0 ±0.9	225 ±145	251 ±157	3.6 ±0.9
MIT039RS	40744	4353 ±685	8757 ±1526	7.8 ±0.1	2543 ±430	4140 ±885	6.9 ±0.2	1709 ±285	2393 ±432	6.3 ±0.2	1056 ±157	1256 ±166	5.8 ±0.2
MIT052SS	19130	1063 ±592	1545 ±907	5.8 ±0.8	484 ±277	631 ±338	4.7 ±0.6	317 ±195	387 ±233	4.1 ±0.7	223 ±146	253 ±163	3.7 ±0.8
MIT052RS	25217	2579 ±767	4879 ±1320	7.3 ±0.2	1494 ±394	2281 ±520	6.4 ±0.2	1010 ±248	1352 ±251	5.8 ±0.2	653 ±161	767 ±172	5.3 ±0.2

3.3.2 Colonization patterns of bacterial communities on sterile sediments

3.3.2.1 Colonization patterns of relative abundances of dominant groups

A clear succession of species colonizing the sterile sediments over time was found (Figure 3.8). It can be seen that there are groups readily colonizing the sterile sediment establishing a high relative abundance. These prominent fast colonizers consecutively loose in dominance with time of incubation (Figure 3.8A), and are replaced by others which are less prominent at the beginning or even not deteded after two months of incubation. The dominant groups attached to sediments in well MIT039 after two months of incubation belonged to *Brevundimonas*, *Oxalobacteraceae*, *Flavobacterium*, *Pseudomonas* and *Undibacterium* with a relative abundance each of 5% and more. After two more months, their abundance decreased by several folds, while the initially minor groups *Acidovorax*, *Bacteriovorax* and *Hydrogenophaga* started to become more prominent. Until December 2010, the community had further changed with *Aquabacterium*, *Burkholderiales incertae sedis* and *Nocardia* being most dominant, with relative abundances of 21.4%, 9.0% and 9.1% respectively.

The sediment incubated in well MIT052 showed very similar colonization patterns as found for sediments in well MIT039 which are separated by a distance of almost 0.5 km. A fast colonization dominanted by only a handful of groups took place. *Flavobacterium*, *Pseudomonas*, *Oxalobacteraceae* and *Massilia* were among the pioneer groups, while *Brevundimonas* exhibited an only low relative abundance and *Undibacterium* obviously had a relative abundance of less than 1% (Figure 3.8B). However, the early colonizers with time lost in dominance and were replaced by new groups, i.e. *Aquabacterium*, *Burkholderiales incertae sedis* and *Nocardia* which at the end of incubation exhibited a relative abundances of 17.4%, 11.3% and 10.1% in February 2011, respectively. Similar to well MIT052 sediments, in well MIT039 the early colonizers such as *Brevundimonas*, *Oxalobacteraceae* and *Flavobacterium* were finally replaced by *Aquabacterium*, *Burkholderiales incertae sedis* and *Nocardia* which were found to constitute the dominant groups attached to sediments.

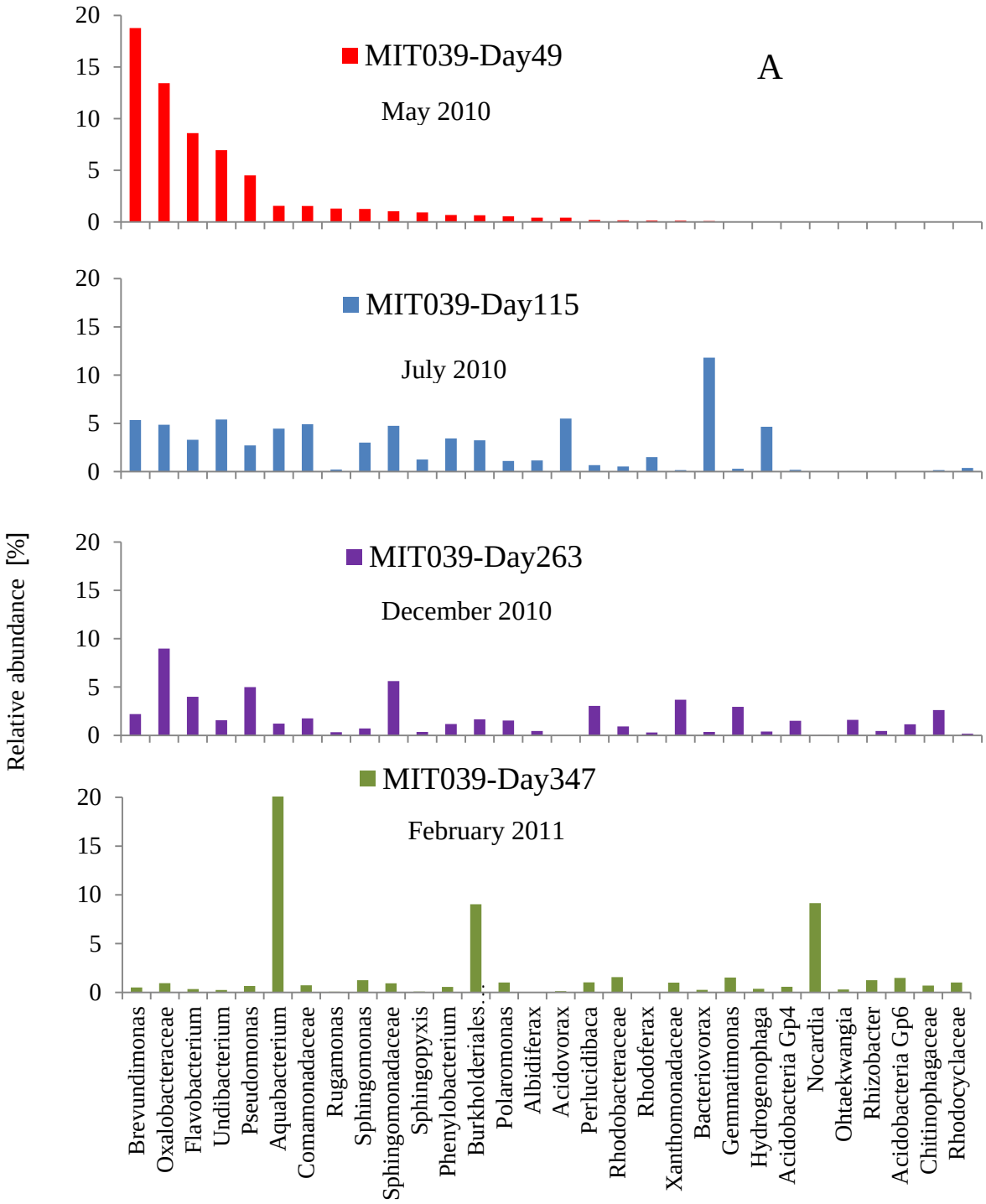


Figure 3.8A Colonization patterns of bacterial communities on initially sterile sediments exposed to groundwater in well MIT039. Only the most dominant genera are shown.

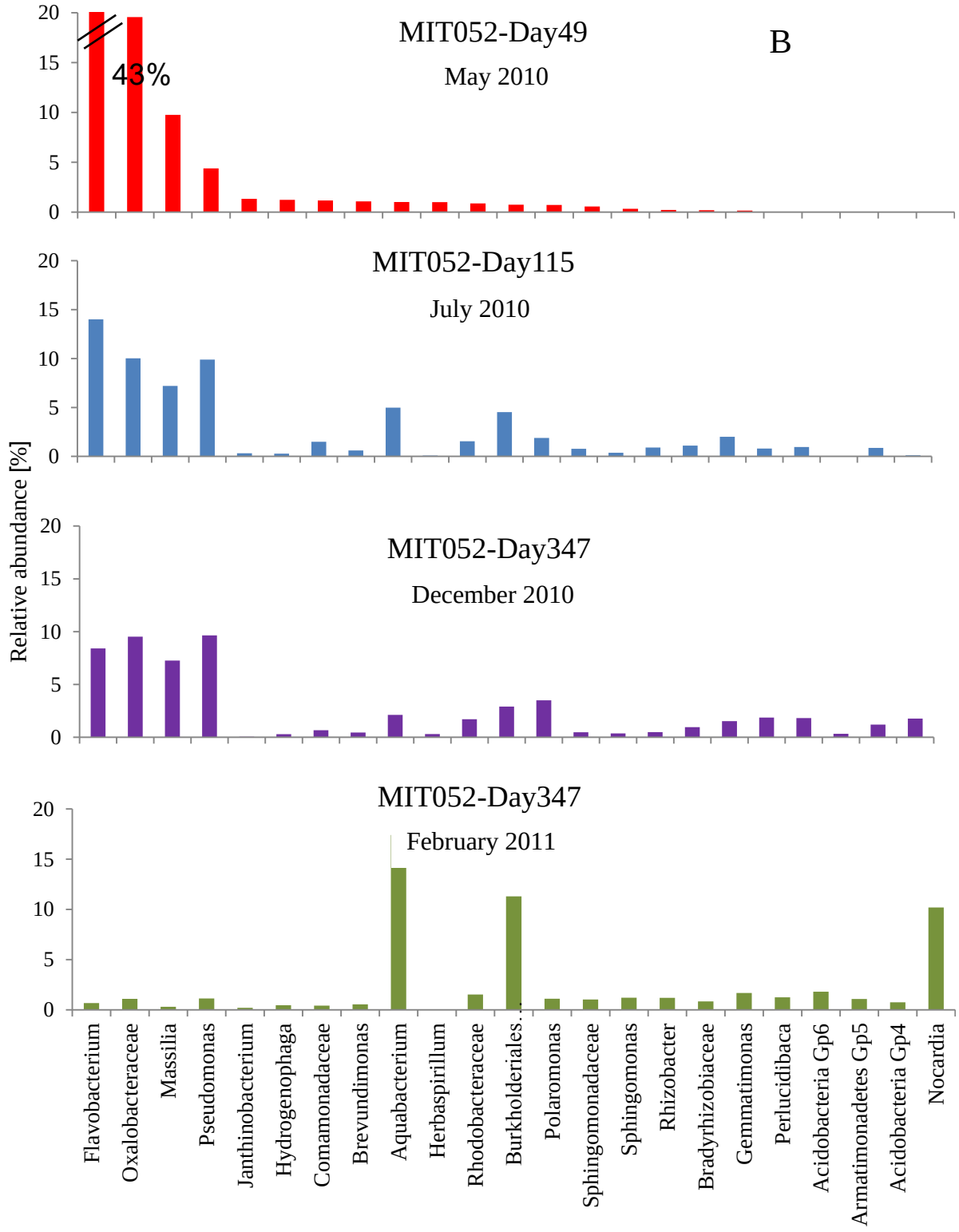


Figure 3.8B Colonization patterns of bacterial communities on initially sterile sediments exposed to groundwater in well MIT052. Only the most dominant genera are shown.

3.3.2.2 *Colonization patterns of cell abundances of dominant groups*

In order to better understand changes in the dominant colonizers, the relative abundance of individual groups was converted to their cell density by multiplying with the total cell counts (Figure 3.9). Compared to groups based on their relative abundances, clear patterns get partly lost. Based on cell abundance of each group, they were grouped into three different clusters according to the trend of their cell abundances. For well MIT039, *Brevundimonas*, *Oxalobacteraceae Flavobacterium* and *Pesudomonas* fell into cluster 1 as their abundances were found very high at the beginning of incubation (May 2010) and after four months the abundances of these species kept decreasing. Most groups (23 out of the 30 most dominant groups) fell into cluster 2, such as *Sphingomonas*, *Acidovorax* and *Burkholderiales incertae sedis*. Their abundances were found very low in the first two months of incubation, then started to increase and reached highest values in July 2010. Afterwards, their abundances started to decrease constantly (Figure 3.9A). Few groups such as *Nocardia* in cluster 3 were found highest abundances during the late incubation period. Similarily, at sediments in well MIT052 groups in cluster 1 exhibited highest abundance at beginning and started to decrease after 115 days of incubation (Figure 3.9B). The cluster 2 includes groups with highest abundance after two months of incubation (May 2010) such as *Aquabacterium*, *Burkholderiales incertae sedis* and *Acidobacteria*. *Nocardi*a in cluster 3 was found highest abundance at late incubation.

However, the patterns of abundance did not reflect the patterns of growth rates. For instance, in well MIT039 *Undibacterium* was found to be high abundant after 49 days incubation and kept increasing its abundance until after 115 days. In order to calculate their growth rates, abundance of each group was devided by the incubation time in days as below.

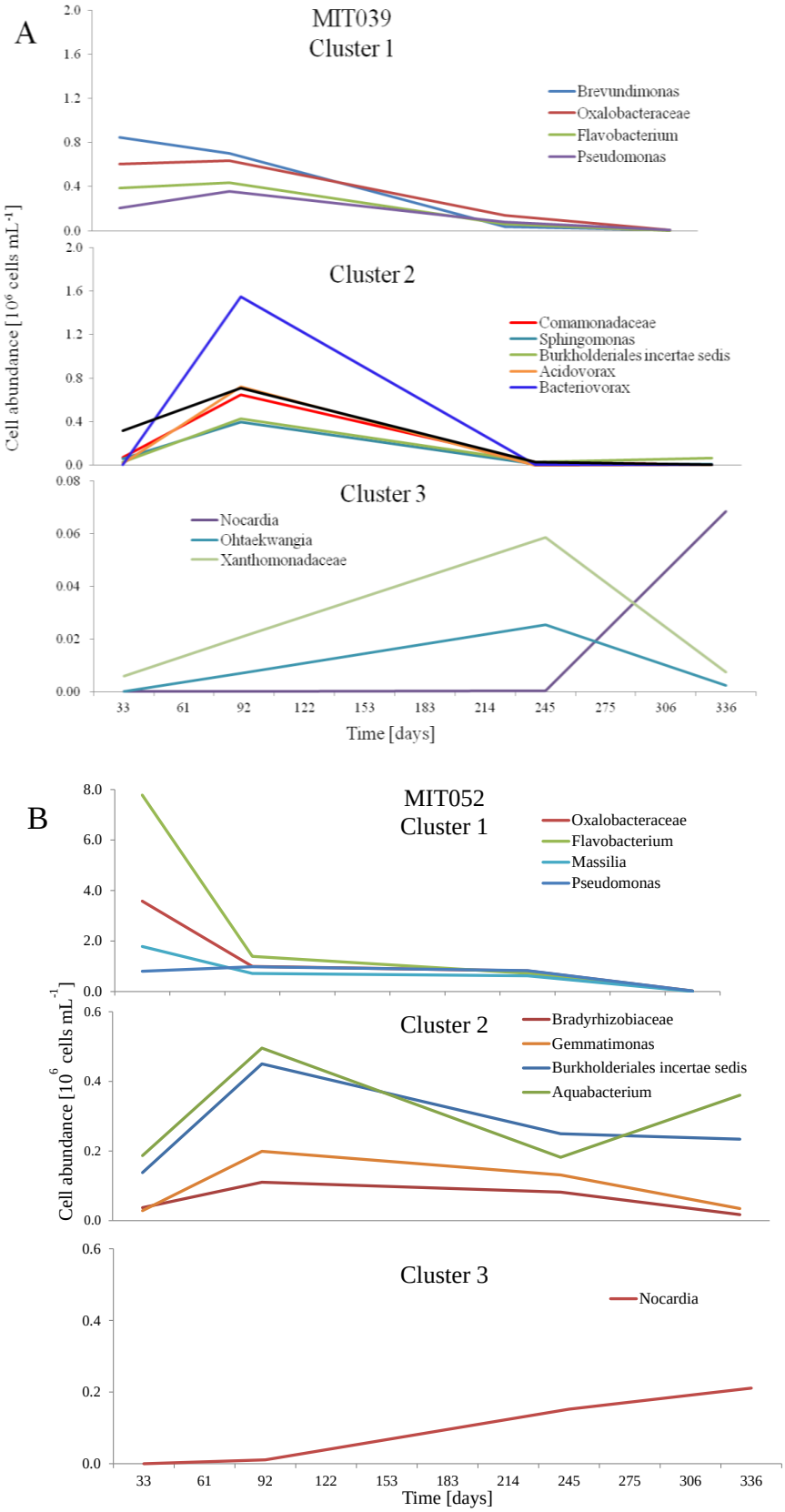


Figure 3.9 Total abundance profiles for selected dominant genera attached to sediments in well MIT039 and well MIT052.

3.3.2.3 Growth patterns of dominant groups

The growth patterns of individual groups were esitmated at four sampling times (Table 3.4). Early colonizers and successors are clearly grouped according to their growth rates. For instance, growth rates of *Brevundimonas*, *Oxalobacteraceae* and *Flavobacterium* at sediments in well MIT039 were found to be highest at beginning (from day 0 to day 49) while the growth rate of *Sphingomonas*, was highest at middle of incubation (from day 49 to day 115 or from day 115 to day 263). At sediments in well MIT052 *Flavobacterium*, *Oxalobacteraceae* and *Pseudomonas* were found to have highest growth rates from day 0 to day 49 and *Burkholderiales incertae sedis*, *Nocardia* and *Acidobacteria* showed highest growth rates at middle or late time of incubation. Interestingly, few groups exhibited different growth patterns in two wells, such as highest growth rate of *Comamonadaceae* was found at beginning in well MIT039 but at middle time in well MIT052.

As seen in Figure 3.10 and Table 3.4, the successors colonizers do not necessarily characterized with low growth rates which are expected to be defined as K-strategists. The early colonized groups with high growth rates such as *Flavobacterium*, *Brevundimonas* and *Oxalobacteraceae* were found to be typical r-strategitis. K-strategy as *Nocardia* was characterized by low growth rates but continuously increased relative abundances. Others seems to be important in early colonization which are not distiguished by their growth rates such as *Sphingomonas* and *Rugamonas*. Conversely, some late comers were found with very high growth rates such as *Burkholderiales incertae sedis* and *Aquabacterium*. It suggested that the identification of r and K-strategists in microbiology should take not only the growth rate but also other criteria.

The average relative abundance of most dominant groups together with variation range are shown in Figure 3.10 which reveals a combination of colonization patterns and seasonal dynamics. It becomes clear that sediments in well MIT052 colonized few very dominant bacterial groups, with *Flavobacterium* reaching a maximum relative abundance of 35%. In sediments from well MIT039 further groups, such as *Brevundimonas*, *Bacteriovorax* and *Undibacterium* play a significant role in colonization dynamics.

Table 3.4 Growth patterns of selectied bacteria genera (cells mL^{-1} day^{-1}) attached to initially sterile sediments exposed to well MIT039 and well MIT052 for different incubation periods. Growth rates were calculated by (cell density at Day_b– cell density at Day_a) / (Day_b - Day_a). Only dominant groups (with relative abundacnes at least once bigger than 5%) are shown in table 5 according to their highest growth rates. And only their highest growth rate isdepicted with the respectove oncubation phase.

MIT039					MIT052				
Days / OTUs	49	115	263	347	Days / OTUs	49	115	263	347
Brevundimonas	17334				*Flavobacterium*	158728			
Oxalobacteraceae	12399				*Oxalobacteraceae*	73056			
Flavobacterium	7938				*Massilia*	36440			
Undibacterium	6418				*Pseudomonas*	16372			
Pseudomonas	4157				*Janthinobacterium*	4988			
Rugamonas	1203				*Hydrogenophaga*	4636			
Bacteriovorax		23438			*Brevundimonas*	4049			
Acidovorax		10670			*Comamonadaceae*	4401			
Hydrogenophaga		9231			*Herbaspirillum*	3755			
Comamonadaceae		8718			*Rhodobacteraceae*	3286			
Sphingomonadaceae		8741			*Polaromonas*	2699			
Aquabacterium		7801			*Sphingomonadaceae*	2112			
Phenylobacterium		6376			*Sphingomonas*	1291			
Burkholderiales incertae sedis		6021			*Rhizobacter*	822			
					Burkholderiales incertae sedis		4729		
Sphingomonas		5116			*Gemmatimonas*		2581		
Rhodoferax		2892			*Acidobacteria Gp6*		1399		
Albidiferax		2017			*Acidobacteria Gp4*		1268		
Sphingopyxis		1879			*Perlucidibaca*		1126		
Polaromonas		1820			*Bradyrhizobiaceae*		1106		
Perlucidibaca		1200			*Aquabacterium*		4677		
Rhodobacteraceae		960			*Nocardia*			955	
Rhodocyclaceae		748			*Armatimonadetes Gp5*			179	
Gemmatimonas		569			*Planctomycetaceae*			316	
Acidobacteria Gp4		365							
Xanthomonadaceae			252						
Ohtaekwangia			124						
Acidobacteria Gp6			123						
Nocardia				810					

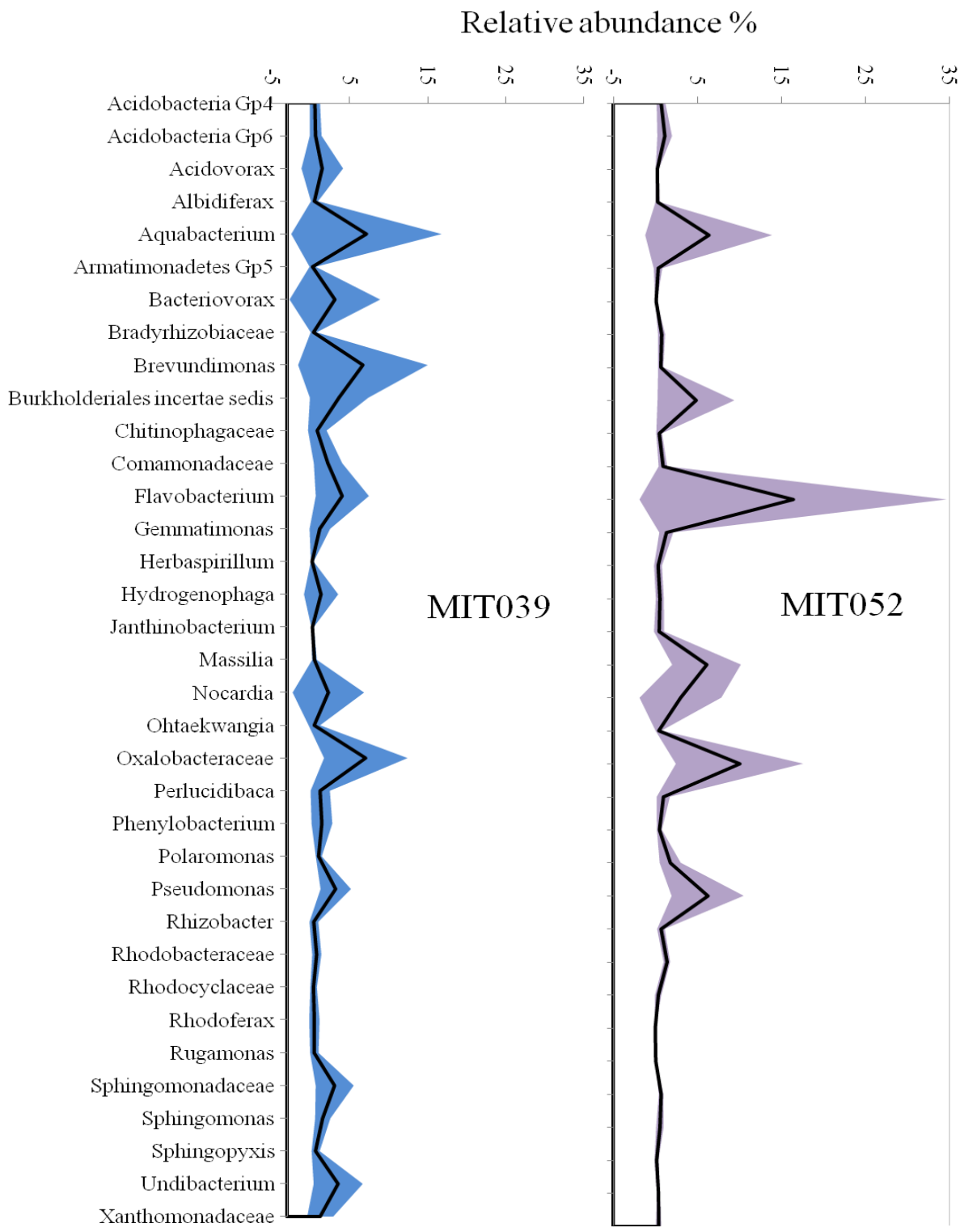


Figure 3.10 The average relative abundances of dominant groups (with relative abundances of at least once > 1%) on genus level found attached to sediments exposed to groundwater. The black line represents mean value of four samplings points and the shadow area depicts the standard deviation.

In order to estimate the percentage of biomass established by the early and late colonizers respectively in present study, the percentage of biomass of individual groups was calculated by (relative abundance × the total cell counts measured at the different time points) ⁄ (highest total cell count out of the different time points). Assuming the highest total cell count as 100% of biomass, Groups with highest percentage value on May of 2010 are clustered as early colonizers and the rest are clustered as K-strategists (Figure 3.11).

In general, the colonization patterns of groups observed during exposed of sediments to groundwater follow two main trends. First, early colonizers readily colonize the sediment surfaces and approaching 70% to 80% of the total biomass. This initial bacterial community is expected to be characterized by a low biodiversity (*H'* = 2.0 for attached bacterial communities in well MIT039 and *H'* = 2.5 for attached bacterial communities in well MIT052) but high growth rates. Subsequently, the 'initially less abundant' species together with new ones start to increase their biomass and the formerly dominant ones were replaced in dominance or disappeared towards the end of the incubation period. The community at this stage of incubation is found with a high biodiversity (*H'* = 3.9 for attached bacterial communities in well MIT039 and *H'* = 4.0 for attached bacterial communities in well MIT052) but lower growth rates of the individual OTUs. The maximum level of the total biomass established is supported by the energy availability and the respective sediment surface. In combination with growth rates of individual groups estimated (Table 3.4) the some members of early colonizers were found with high growth rates such as *Brevundimonas*, *Flavobacterium*, *Massilia* and *Oxalobacteraceae* and some late colonizers were characterized with low growth rates such as *Nocardia*. However, the late dominant groups, *Burkholderiales incertae sedis* and *Aquabacterium* were also found very high growth rates. It indicates that growth rates and the succession of groups can not absolute distinguish the r and K-strategists for bacterial community.

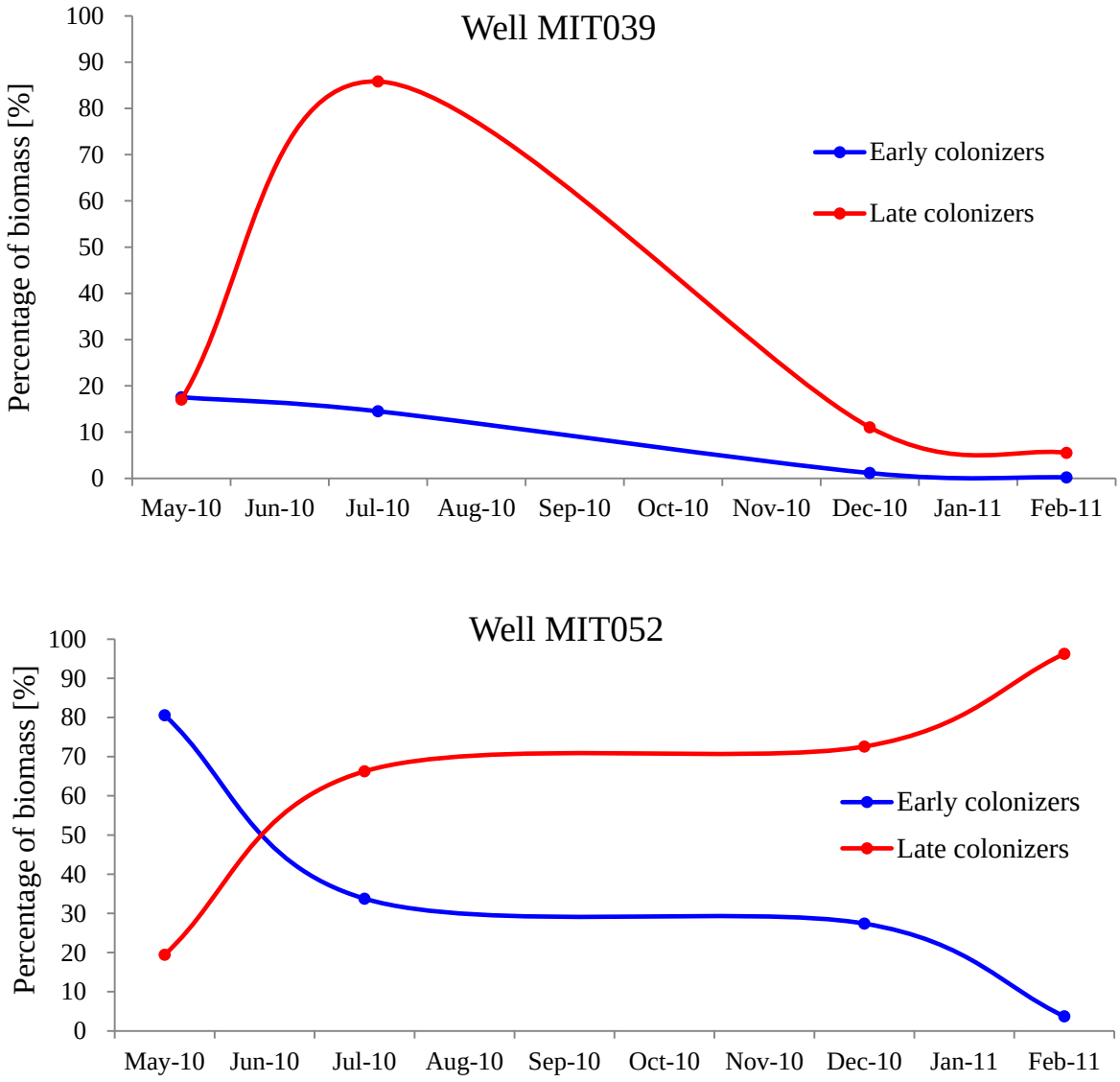


Figure 3.11 Schematic illustration of the succession of community early and late colonizers during colonization from two wells

3.3.3 Comparison of bacterial community composition from groundwater and sediments

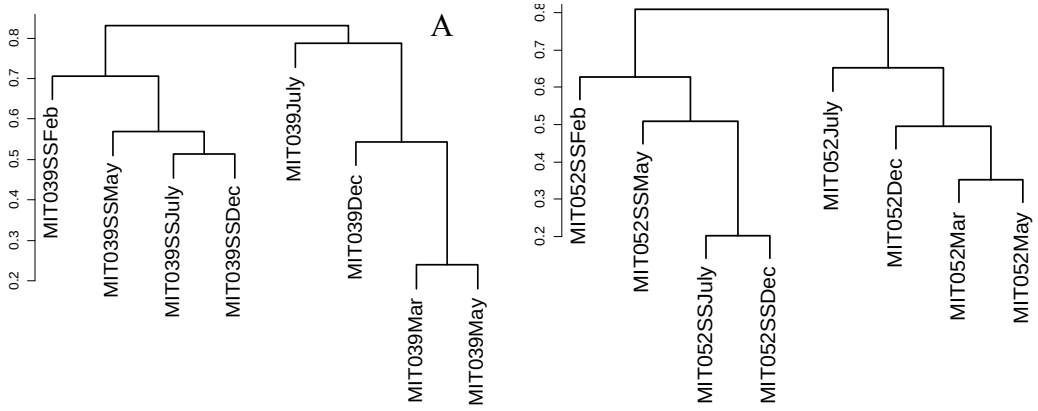
3.3.3.1 Comparison of suspended and attached bacterial communities

To compare suspended and attached bacteria, we carried out bacterial community cluster analysis based on OTUs patterns, which are shown in Figure 3.12. Patterns in well MIT039 and in well MIT052 (Figure 3.12A) were found very similar.Cluster analysis clearly seperated samples into two groups, i.e. suspended bacteria and attached bacteria.

On genus level, the dominant groups found in water did not show a high relative abundance in sediment samples (Figure 3.12B). *Dechloromonas* and *Azospira* were found to be dominant

in water samples while *Flavobacterium* and *Brevundimonas* were dominant in sediment samples. On a class level, within the *Betaproteobacteria, Comamonadaceae* were found to be dominant in groundwater while *Burkholderiales incertae sedis* and *Oxalobacteraceae* were found prominent in sediments. For the groups belonging to the *Alphaproteobacteria,* patterns were similar for the sediments of the two wells with a dominant abundance of *Caulobacteraceae* and *Sphingomonadaceae*. Within the *Gammaproteobacteria*, sequences assigned as *Pseudomonadaceae* were dominating for all samples while the *Legionellales* dominated in the water samples only.

On phylum and class level, the most dominant groups present in water were also found to be dominant on the sediment (Figure 3.12B). The largest group of sequences obtained were related to the phylum *Proteobacteria*. Within *Proteobacteria,* the OTUs that belong to phylum of *Beta-*, *Alpha-* and *Gammaproteobacteria* were found to be dominant across groundwater and sediments at both sites, taking up average percentage abundances of 35.5%, 18.6% and 9.3% in well MIT039 and 40.7%, 8.3% and 9.0% in well MIT052. The similarity between suspended and attached bacterial communities increased over the time in both wells (Figure 3.12D), from 6.5% and 10.2% in May 2010 to about 25% in February 2011.



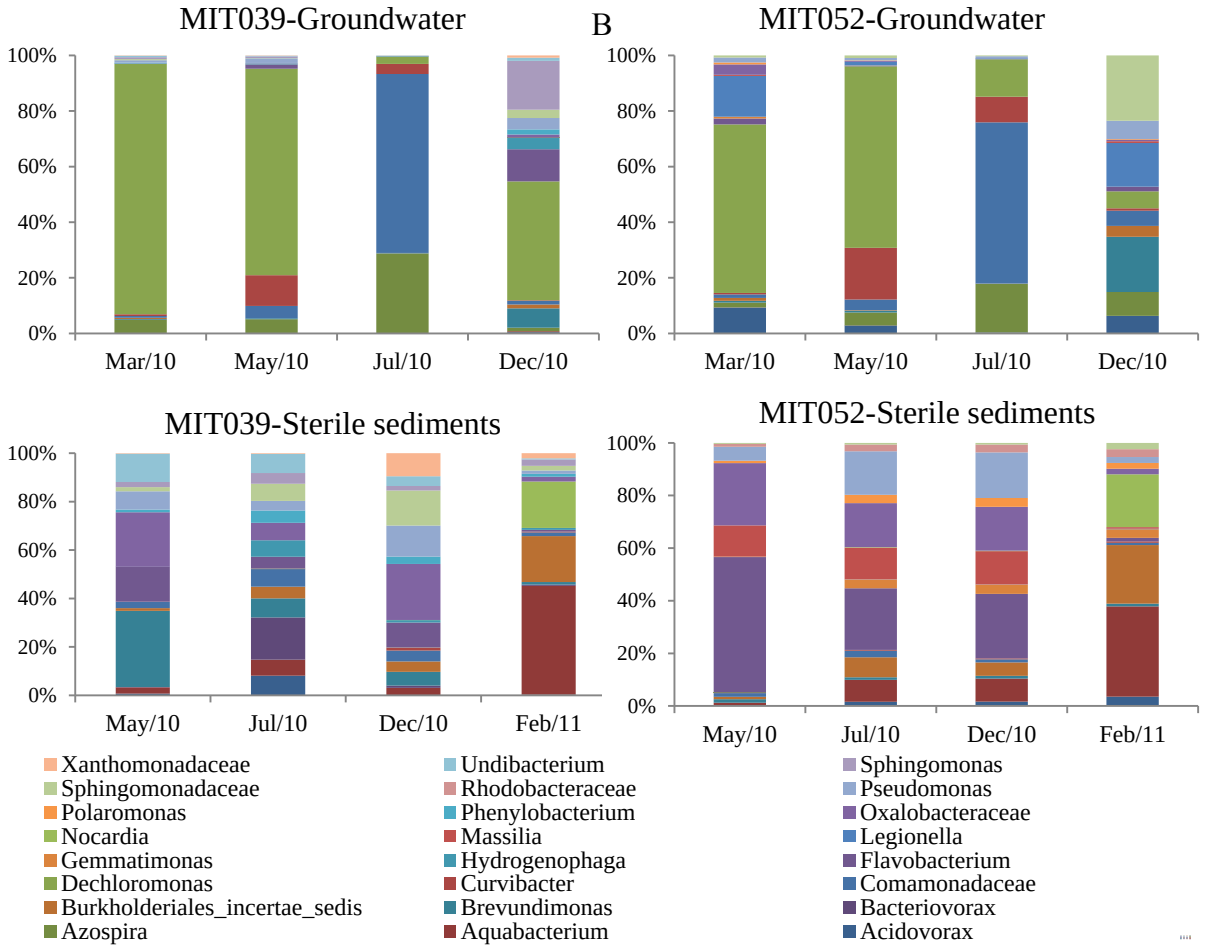
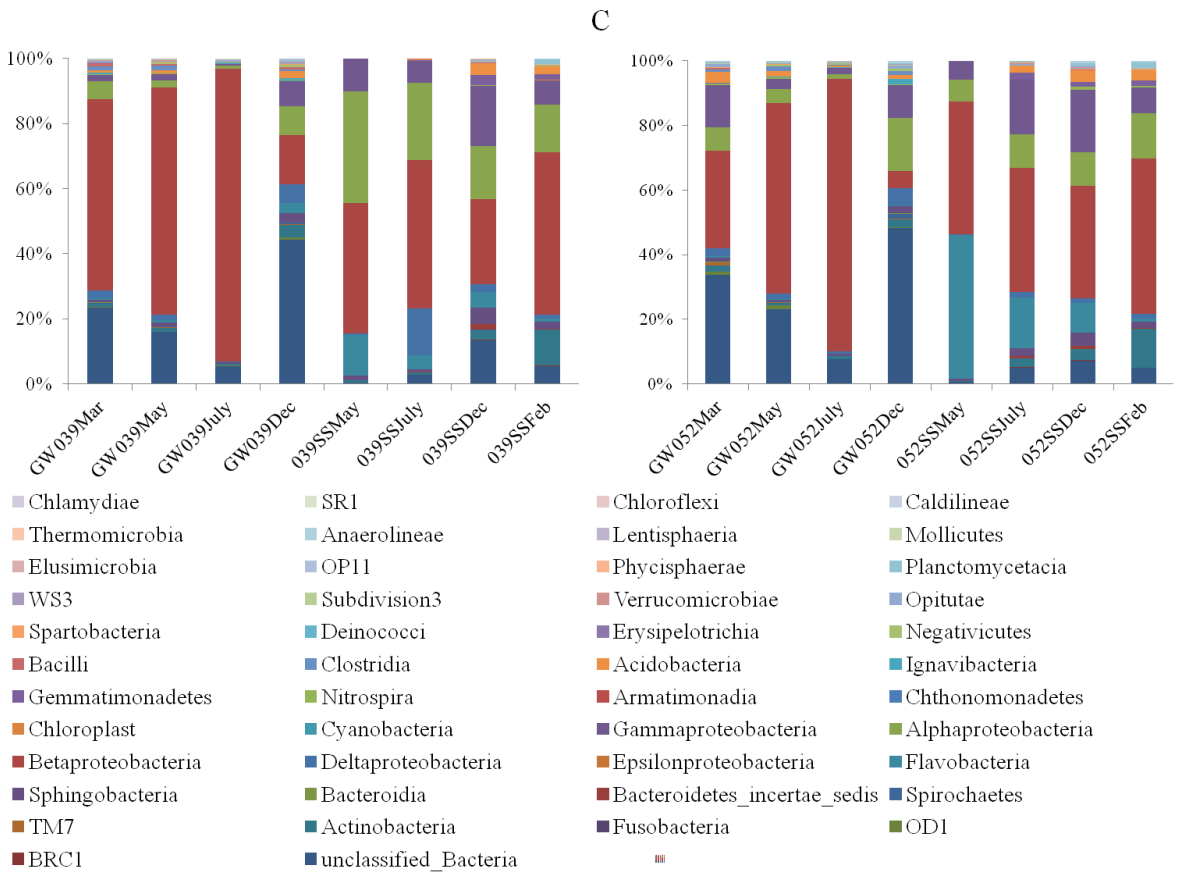


Figure 3.12 A and B. Comparison of suspended and attached bacterial communities attached to sediments in well MIT039 and MIT052. (A) Cluster analysis using Bray-Curtis similarity. Sample codes depict the well (MIT039 or MIT052), sampling season (March, May, July, December of 2010 and Feburary of 2011), and type of samples (GW for groundwater and SS for sterile sediments) on genus level. (B) Relative abundance of OTUs on genus level.



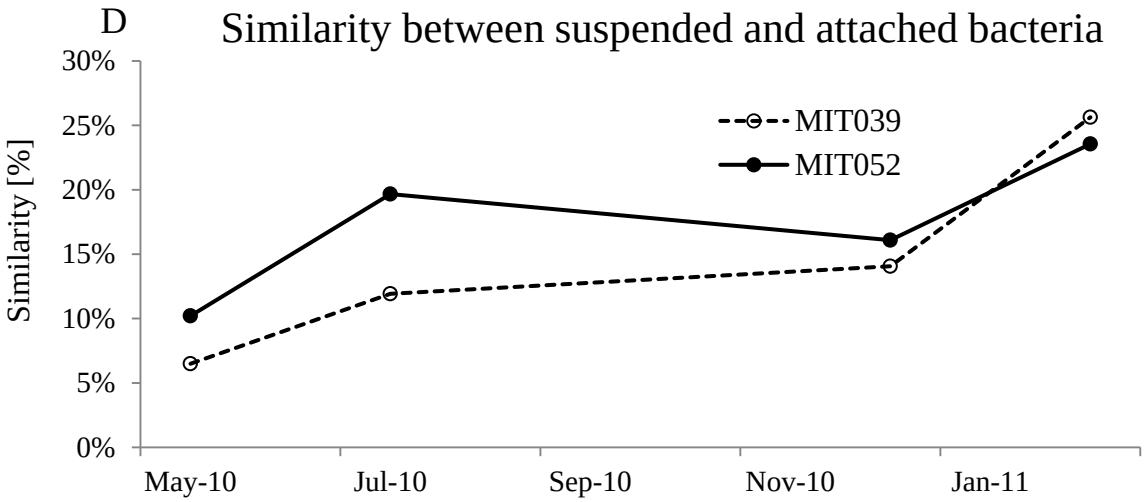


Figure 3.12 C and D Comparison of suspended and attached bacterial communities attached to sediments in well MIT039 and MIT052. (C) Relative abundance of OTUs on phylum and class level. (D) Similarity between suspended and attached bacterial communities.

3.3.3.2 Comparison of bacterial communities on initially river bed sediments and initially sterile sediments

Attached bacterial communities on active sediments taken from the river bed were clearly distinct from the communities which established on the sterile sediments. At the end of incubation attached bacterial communities on initially sterile sediments showed highest similarity to the river bed sediments exposed to groundwater (Figure 3.13A). Although exposed to groundwater over the period of one year, the attached bacterial community composition on river bed sediments did not change much, with *Alpha-*, *Beta-*, *Gammaproteobacteria* and *Acidobacteria* dominating (Figure 3.13C). Bacterial communities on the initially sterile sediments exhibited a lesser diversity but with the same dominant groups. Similar patterns were observed for the analysis on genus level (Figure 3.13B). *Acidobacteria Gp4* and *Nitrospira* were most dominant on river bed sediments and stable constituents in terms of community composition during one year. The initially sterile sediments, as expected showed a seasonal succession of colonizers. In well MIT039, *Brevundimonas*, *Pseudomonas* and *Untibacterium* were dominant during the first four months of incubation. In well MIT052, *Flavobacteriu*m and *Massilia* were dominant at beginning of incubation. A similar bacterial composition established on those two sediments by the end of incubation.

It is well known that one advantage of 454 pyrosequencing is capable of recovering large amount of bacterial taxanomy information. We would lost the information of groups with individual minor relative abundances but core communities only analyzing on genus level. Therefore, we compared the the bacterial community composition across samples on phylum and class level. (Table 3.5). For example, the value derived among IsarW samples was to compare the suspended bacterial community composition in Isar river water at different time point (seasonal dynamics), while the value derived between 039W and IsarW samples was to compare the suspended bacterial community composition between well MIT039 and river water at the same time point (temporal dynamics).

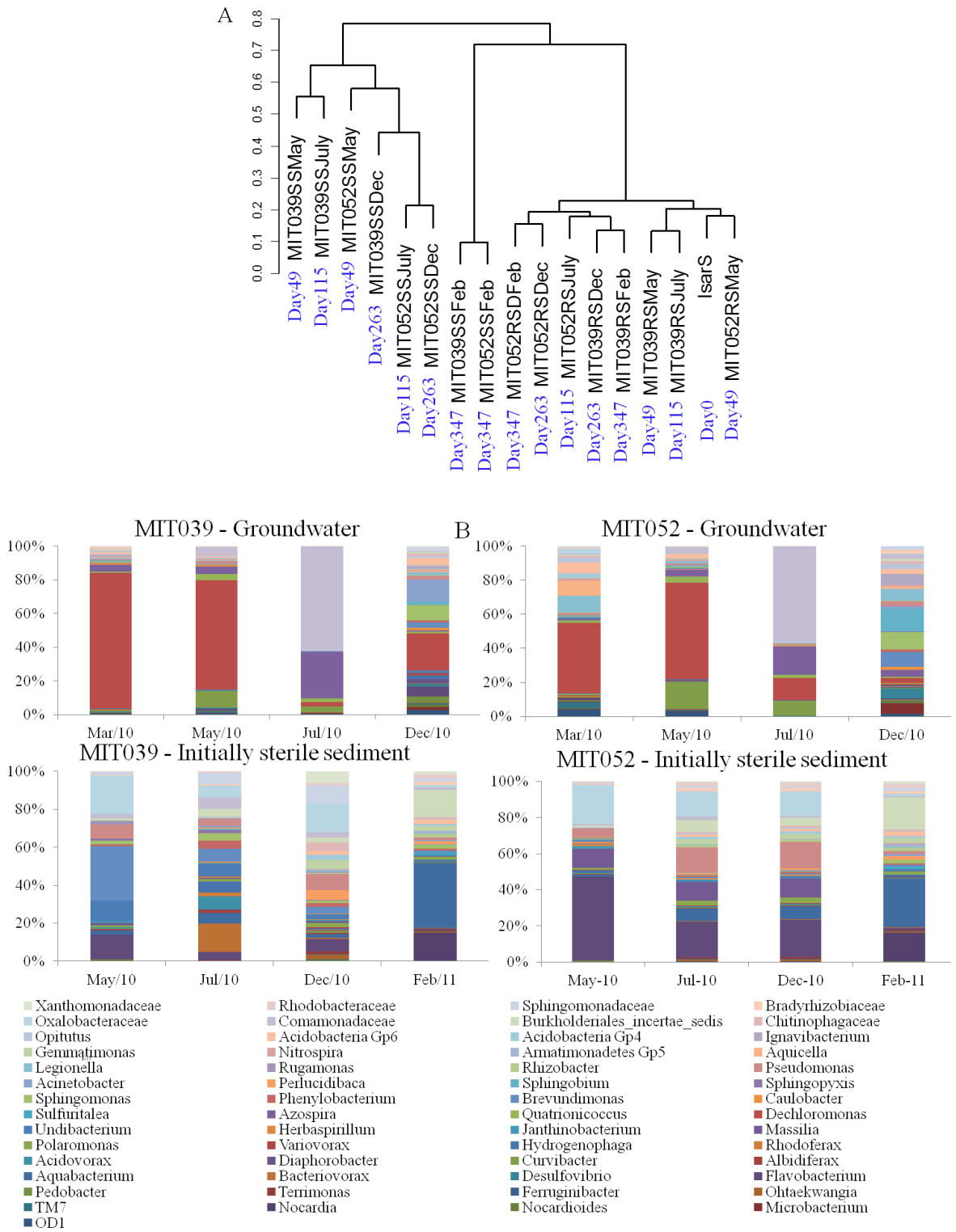


Figure 3.13 A and B Comparison of bacterial communities attached to initially sterile sediments and on active river bed sediments exposed to groundwater in wells MIT039 and MIT052. (A) Cluster analysis using Bray-Curtis similarity. Samples code are composed of well name (MIT039 and MIT052), sampling season (March, May, July, December of 2010 and Feburary of 2011), and type of samples (RS for initially river sediments and SS for initisly sterile sediments) on genus level. (B) Relative abundance of OTUs on genus level.

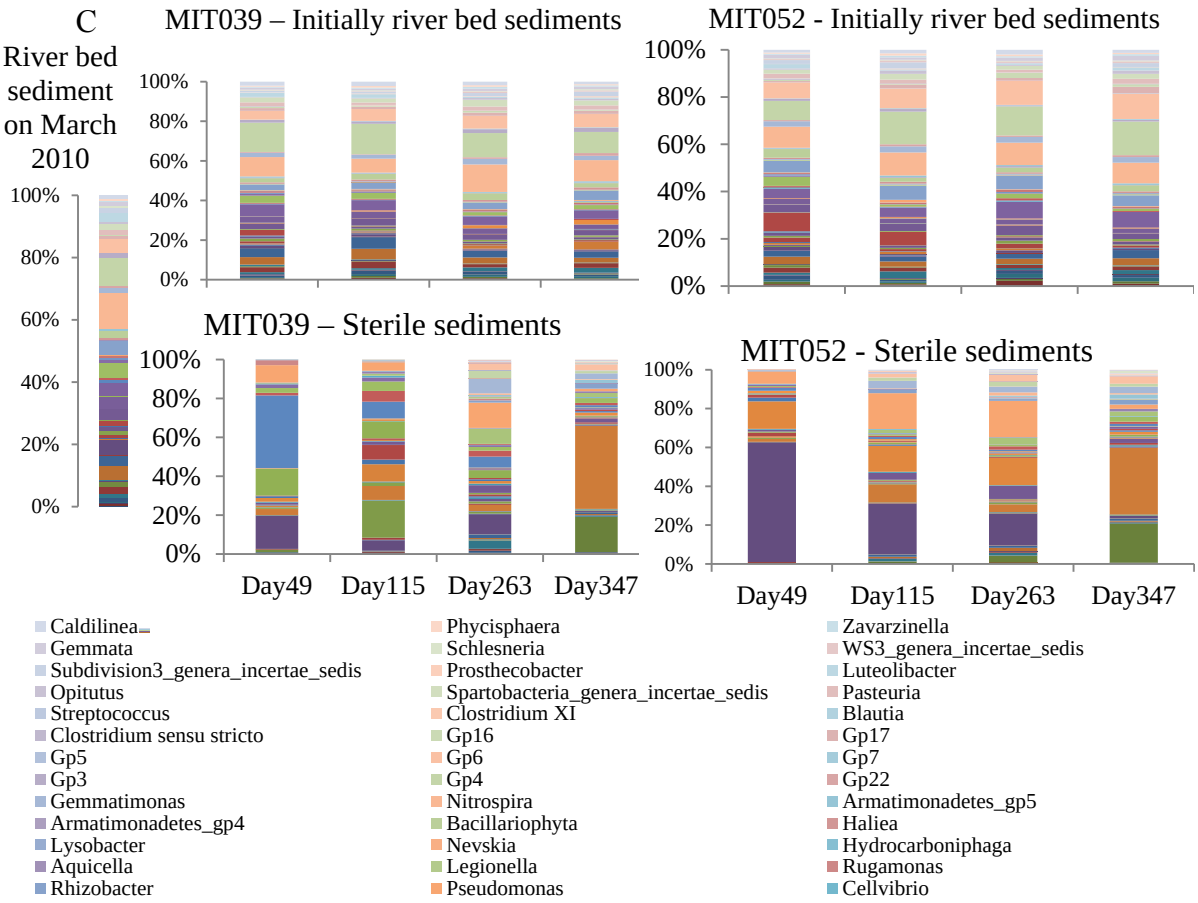


Figure 3.13C Comparison of bacterial communities attached to initially sterile sediments and on active river bed sediments exposed to groundwater in wells MIT039 and MIT052. Relative abundance of OTUs on phylum and class level.

An intersting pattern was found when comparing the bacterial communites suspended in river water and groundwater. Similarity within the Isar river water samples from four sampling points (60.0% ± 10.1%) was higher than the similarity between Isar river water and groundwater. In groundwater, similarity was higher when comparing the water from two wells at the same time point (70.0% ± 6.7%) than water from one well at different time points (38.6% ± 22.1% for wel MIT039 and 45.0% ± 15.7% for well MIT052). With sediment samples, the river bed sediments when exposed to groundwater showed very stable community composition within seasons (> 80% similarity) and were also found to be quite similar to the natural river bed sediment (81.7% ± 4.6% for well MIT039 and 78.9% ± 4.7% for well MIT052). Although dominant early or later colonizers of the initially sterile sediments exhibited strong temporal dynamics, 40% of the early bacterial community stayed at the

sediments till the end. A very high similarity between well MIT039 and MIT052 was found (60.2% ± 22.0%).

In general, the similarity between the bacterial community in water and at the initially sterile sediment samples from the individual wells were low (16.8% ± 8.7% in well MIT039 and 19.0% ± 7.9% in well MIT052), especially low at the beginning of incubation, with similarities of 6.5% and 10.2% for well MIT039 and MIT052 repectively. Compared to groundwater, river water exhibited a higher similarity to the attached bacterial communities exposed to wells.

Table 3.5 Average similarity ± SD in percent within and between groups determined by Bray-Curtis similarity coefficient. IsarW, 039W and 052W represents water samples from the Isar river, well MIT037, MIT038, MIT039 and MIT052. IsarS represents the river bed sediments sampled in March 2010. 039SS and 052SS represents the initially sterile sediments incubated in well MIT039 and MIT052. 039RS and 052RS stands for the river sediments incubated in well MIT039 and MIT052. Similarity values > 60% were marked in bold.

%	IsarW	039W	052W	IsarS	039SS	039RS	052SS	052RS
IsarW	**62.0±10.1**							
039W	26.5±6.2	38.6±22.1						
052W	29.4±4.2	**72.0±6.7**	45.0±15.7					
IsarS	**63.3±18.4**	23.3±6.0	26.4±5.4					
039SS	33.9±11.2	16.8±8.7	18.9±9.2	37.1±15.5	44.6±4.4			
039RS	58.9±13.4	25.2±6.1	28.8±7.2	**81.7±4.6**	36.3±17.7	**84.1±4.1**		
052SS	34.7±9.5	16.3±6.7	19.0±7.9	37.4±14.3	**60.2±22.0**	37.9±15.7	**60.0±17.4**	
052RS	56.7±12.3	24.6±5.9	28.2±7.0	**78.9±4.7**	34.3±18.4	**81.7±2.9**	37.5±15.7	**82.7±3.1**

3.3.3.3 *Multi-dimensional scaling analysis of all samples for the genus level data*

The multi dimensional scaling of all 29 samples based on Bray-Curtis similarity reveale four goups into which the bacterial communities clustered, i.e. groundwater, river, initially river sediments and initially sterile sediments. (Figure 3.14). The distribution of samples exhibited seasonal patterns along dimension 1 and spatial patterns along dimension 2. A strong correlation was shown between Dimension 1 and the groundwater table with a Spearman's rho value of 0.63 (P = 0.009) as well as the total number of prokaryotes (r = -0.67, P = 0.001).

Dimension 2 was found to be significantly correlated to bacterial Shannon diversity ($r = 0.73$, $P < 0.001$). Groundwater samples were scattered to two subgroups, summer samples and spring / winter samples. River water samples formed a separate group except river water from March of 2010 which fell into the group of initially river sediments. Compared to initially river sediments, initially sterile sediments exhibited an increasing similarity of bacterial communities with time series.

The distribution and relative abundance of most dominant groups on genus level is shown in individual multi dimensional scaling (Figure 3.14B). For instance, *Nitrospira* were dominant on river bed sediments only. *Oxalobacteraceaea, Massilia* and *Pseudomonas* on the other hand were dominant on initially sterile sediments only. *Flavobacterium* was abundant in river water and initially sterile sediments. *Burkholderiales incertae sedis* and *Planctomycetaceae* were dominant on sediments and showed pattern of increased abundance on sterile sediments along incubation time series. *Sphingomonas* was dominant in groundwater and sediments. *Brevundimonas, Undibacterium* and *Aquabacterium* were found related to the incubation time on intially sterile sediment.

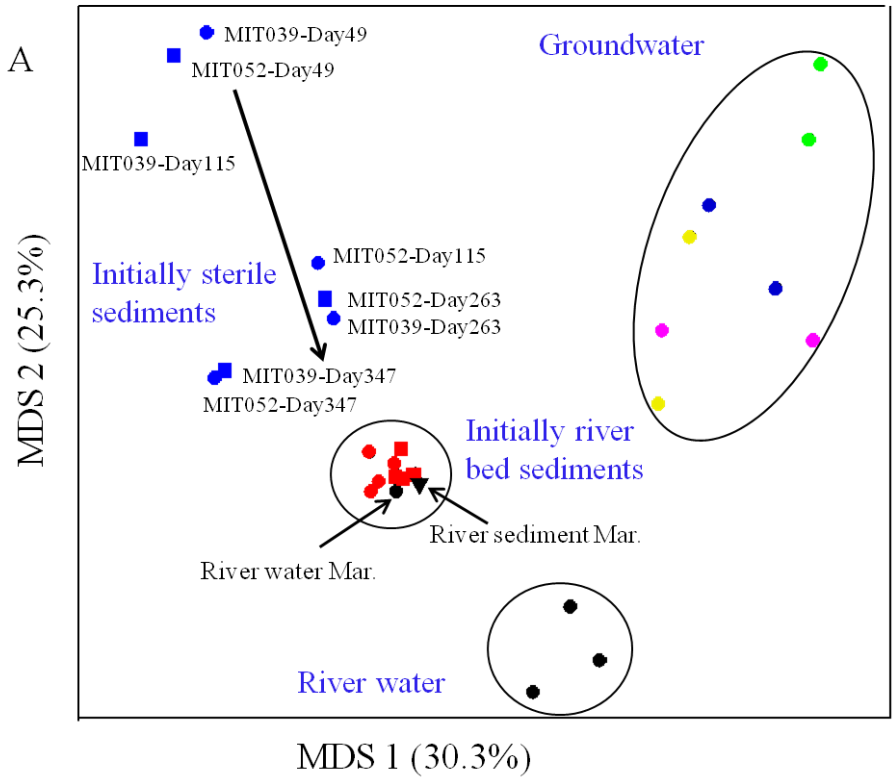


Figure 3.14A Seasonal distribution of bacterial communities based on similarity of composition. (A) Multi-dimensional scaling of groundwater and sediments samples based on composition of bacterial communities.

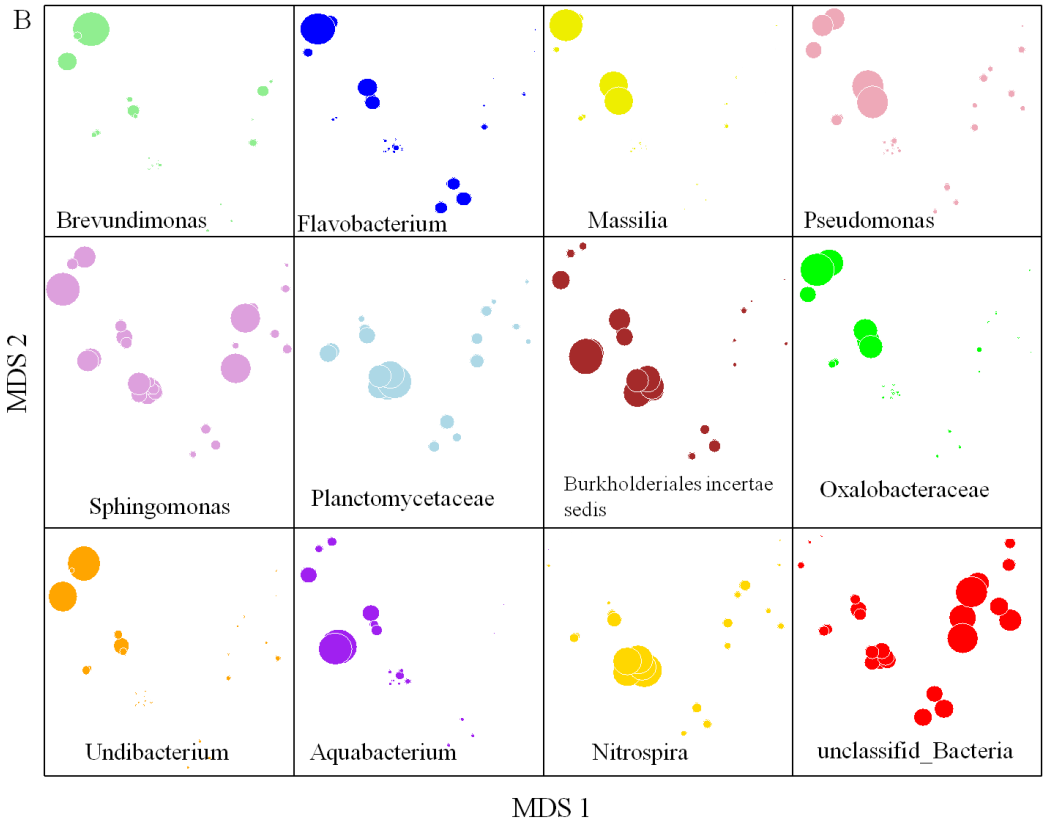


Figure 3.14B Seasonal distribution of bacterial communities based on similarity of composition. (B) Multi-dimensional scaling of selected dominant groups. The size of circles represents the relative bacterial abundance based on OTUs, determined by the RDP classifier with an 80% confidence threshold.

3.4 Microbial food web structure and carbon cycling

3.4.1 Bacterial carbon production, AOC and concentrations of ATP

Bacterial carbon production. The heterotrophic bacterial biomass or carbon production in groundwater, as determined via ^{3}H-leucine incorporation ranged from 0.01 to 0.3 ng C L^{-1} h^{-1}. Compared to groundwater, the river water showed much higher carbon production rates between 0.3 to 7.7 ng C L^{-1} h^{-1}. Lowest production rates were observed during the dry season in winter characterized by relative low groundwater tables, and low temperatures, especially in river water (Figure 3.15C). Highest carbon production rates (0.06 to 1.0 ng C L^{-1} h^{-1}) in groundwater occurred during summer (May – July 2010).

ATP concentration of planktonic microbes. The microbial ATP concentration (intracellular

ATP) in river water was 3 to 6 folds higher than in groundwater with an average value of 1.8 pmol L^{-1} (Figure 3.15A). In groundwater, ATP concentrations were considerably low, in the range of 3.5×10^{-3} and 3.3 pmol L^{-1}. For all sampling sites, lowest ATP concentrations were observed in summer (0.035 to 9.5 $\times 10^{-1}$ pmol L^{-1}) and winter (1.4 to 28 $\times 10^{-1}$ pmol L^{-1}), whereas the highest ATP values were found in autumn (7.6 to 59 $\times 10^{-1}$ pmol L^{-1}). Estimated from measurements of cellular adenosine triphosphate (ATP), the active microbial biomass was highest in autumn and lowest in winter.

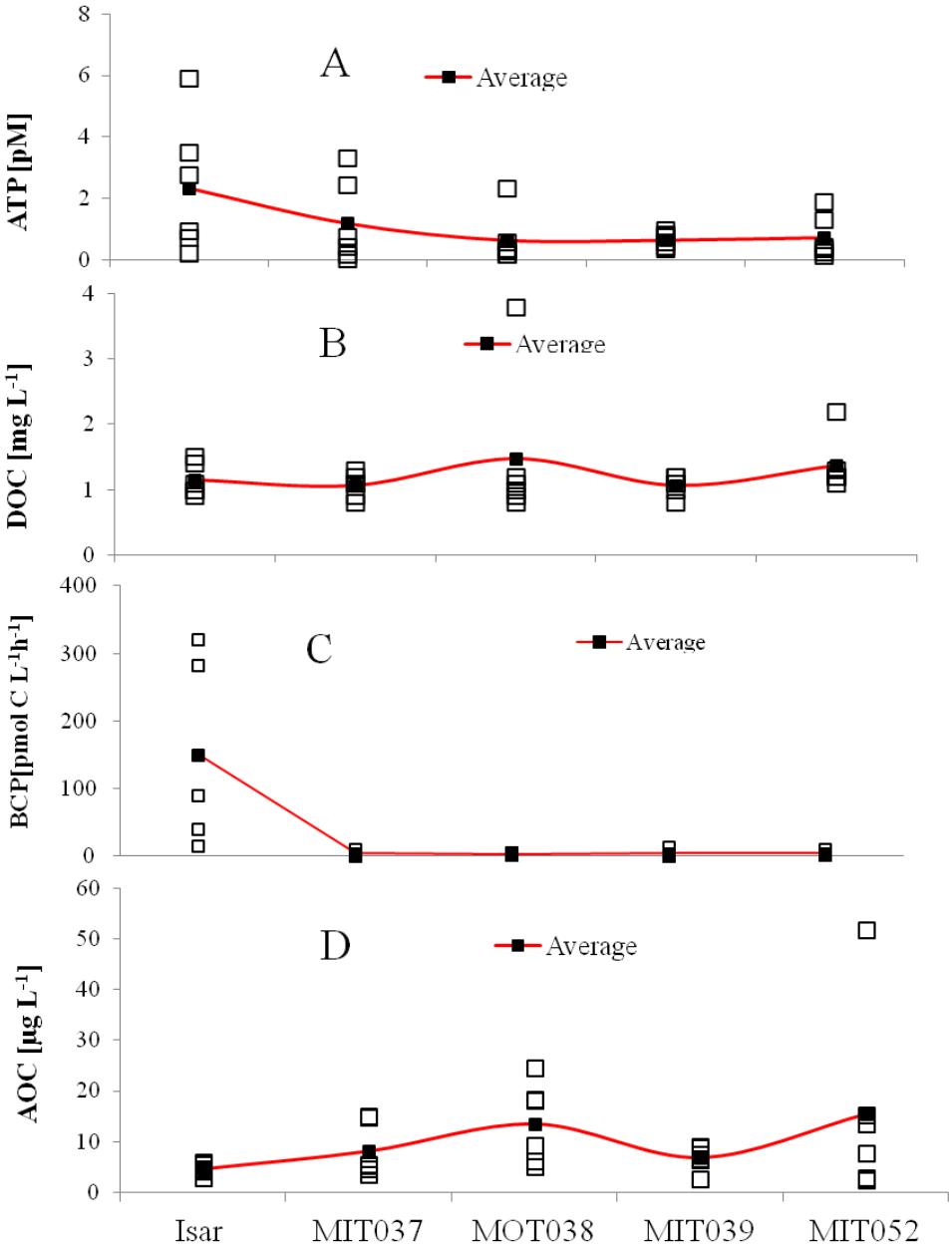


Figure 3.15 Concentration of ATP, DOC, BCP and AOC from groundwater and river water over one hydrological cycle. The average value from each site is shown as a black square connected by red trend line.

3.4.2 Abundance of bacterial cells, virus-like particles and protozoa

The overall densities of bacterial cells varied from 1.23 $\times 10^4$ to 2.73 $\times 10^5$ cells mL^{-1}.The relative higher cells abundances were found in summer (2.85 to 11.4 $\times 10^4$ cells mL^{-1}) and winter (6.0 to 27.3 $\times 10^4$ cells mL^{-1}) and lower in spring (1.3 to 8.6 $\times 10^4$ cells mL^{-1}) in river and groundwater. Compared to groundwater, the abundance of bacterial cells was slightly elevated by a factor of 1.4-3.3 in the Isar river. of the ration of bacteria to protozoa (BPR) ranged from 33 to 2089 (mean, 349) exhibiting seasonal patterns. For most samples, BPR values were comparably high in summer (194-768) and winter (519-2089) and low in spring (151-306) and autumn (97-653) (Figure 3.17B). The concentration of virus-like particles (VLP) ranged from 6.74 $\times 10^4$ to 5.96 $\times 10^6$ mL^{-1}, with highest values in the Isar river and the adjacent groundwater well MIT037 in March 2010 (Figure 3.16). For river water and groundwater, the VLP concentrations showed strong seasonal patterns (One Way ANOVA, P = 0.037), which was also true for the VLP to bacteria ratio (Figure 3.17B).

The virus to bacteria ratio (VPR) were in the range of 2 to 230 (36 $\pm$ 41). In groundwater, the highest VPR values (27-230) were found in spring (March 2010 and March 2011) and lowest values (2-25) occured in summer (July 2010) and winter (December 2010), An extreme VPR, of 230 was found in water from well MIT037 in March 2010. Other samples always revealed ratios less than 70. In river water, VPR were found in the range from 6 (summer and winter) to 70 (spring), respectively.

The total number of protozoa were relative constant over the year varying from 70 to 412 cells mL^{-1}. The number of protozoa in the Isar river water were always at the upper range of the values from groundwater with an average concentration of 317 cells mL^{-1}. Relative highest values of protozoa in groundwater and river water were found in winter (124 to 343 cells mL^{-1}) and lowest (82 to 290 cells mL^{-1}) in May 2010. The seasonal patterns of protozoan numbers were found relative less dynamics (One way ANOVA, P = 0.012).

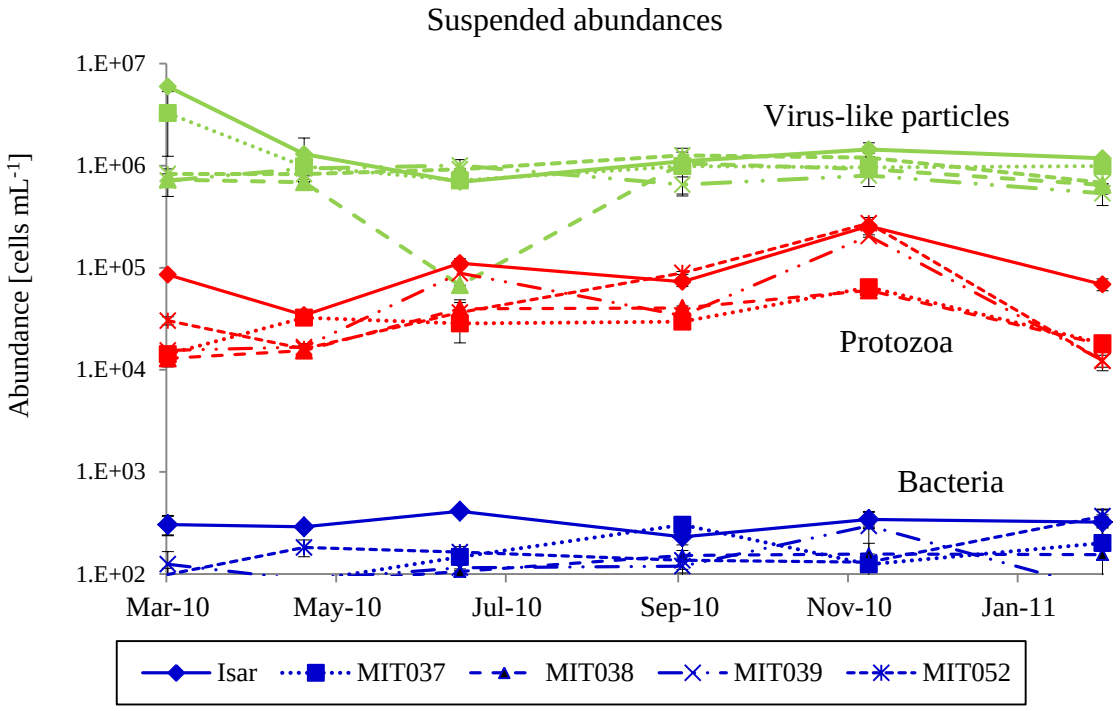


Figure 3.16 Total abundance of virus-like particles, bacterial cells and protozoa over one hydrological cycle. Values are mean ± SD.

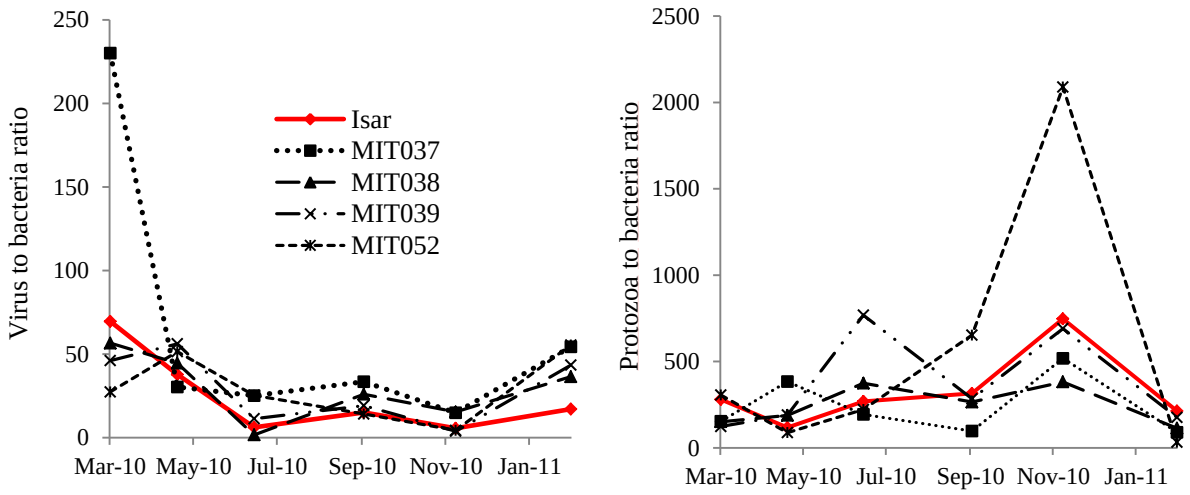


Figure 3.17 Seasonal patterns of (A) virus to bacteria ratios and (B) bacteria to protozoa ratios in water from the Isar river and groundwater from different sites.

Numbers of protozoa were negatively correlated with the electric conductivity (r = -0.77, P = 0.000), nitrate (r = -0.40, P = 0.027), and chloride (r = -0.64, P = 0.000) and positively correlated with pH (r = 0.61, P = 0.000). A positive correlation was found between the

abundance of protozoa and bacterial Shannon diversity (r = 0.576, P = 0.001). Bacterial abundances were found to be positively correlated with ATP concentration (r = 0.37, P = 0.04) and strongly positively correlated with BCP (r = 0.68, P = 0.000). Significant positive correlation was found between bacterial and protozoan abundances. The VLP was positively correlated with the abundance of bacterial cells (r = 0.48, P = 0.008), bacterial carbon production (r = 0.42, P = 0.037) and cellular ATP concentration (r = 0.51, P = 0.004), while negatively correlated to temperature (r = -0.38, P = 0.039) (Table 3.6).

Table 3.6 Spearman rank correlation analysis of selected biotic and abiotic variables monitored in groundwater for one hydrological cycle. Siginificant correlations are given in bold. TNC: total numbers of bacterial cells, VLP: abundance of virus-like particles , Protozoa: protozoan abundances. *Significant correlation ($P \leq 0.05$). **Highly significant correlation ($P \leq 0.01$).

	VLP	Protozoa	BCP	Nitrate	pH	Cl	EC	ATP
TNC								
r	0.478	0.361	0.678	-0.196	0.295	-0.357	-0.148	0.372
P	**0.007****	**0.050***	**0.000****	0.296	0.112	0.052	0.432	**0.043***
VLP								
r		0.274	0.419	-0.302	0.398	-0.335	-0.186	0.513
P		0.142	**0.037***	0.104	**0.030***	0.070	0.320	**0.004****
Protozoa								
r			0.315	-0.403	0.609	-0.638	-0.769	-0.046
P			0.124	**0.027***	**0.000****	**0.000****	**0.000****	0.805

3.4.3 Estimation of bacterial mortality induced by protozoan grazing

The following models and equations were used for the calculation of bacterial mortality by grazing:

1. Bacterial mortality by grazing = grazing rate / bacterial production
2. Ingestion rate = clearance rate × protozoan density
3. Grazing rate = Ingestion rate × bacterial density

The protozoan grazing results listed below are based on the minimum clearance rate. The maximum clearance rate applied is 10 times higher the minimum value. Consequently, the esitmated ingestion rate and grazing rate were 10 folds higher based on the maximum clerance rate than on the minimum clearance rate.

Ingestion rates estimated for groundwater ranged from 0.0037 to 0.033 bacteria protozoa^{-1} h^{-1} (on average, 0.012 bacteria protist^{-1} h^{-1}) using the low clearance rate. Consequently, the grazing rates calculated on the basis of the protozoan abundance were in the range of 2.6 to 13 $\times 10^2$ bacteria L^{-1} h^{-1} (on average, 25.2 $\times 10^2$ bacteria L^{-1} h^{-1}) for groundwater. Due to the extremely low bacterial carbon production (8.1 and 23 pg C L^{-1} h^{-1} respectively), protozoan grazing in groundwater from well MIT037 and MIT052 in winter accounted for 317.6% and 218.6% of BCP. Excluding the two values of low BCP in winter, the bacterial mortality induced by protozoan grazing in groundwater samples varied from 9.6% to 108.9% (on average 48.7%) of BCP. Compared to these later values, grazing of bacterial cells by protozoa was slightly lower in the river water than in groundwater, accounting for 2.2% to 128.2% (on average, 44.4%) of BCP (Table 3.7).

3.4.4 Estimation of bacterial mortality induced by viral lysis

The models and equations used for calculation of viral lysis are as follows:

1. FMVL = FVIC / $\gamma \times \ln 2 \times (1 - \varepsilon$ - FVIC)
2. Lytic viral production = FMVL $\times$ burst size (Bz) $\times$ bacterial production

where Υ = 1(the ratio of the latent period and generation time), ε = 0.816 (the fraction of the latent period during which viral particles are not yet visible), FMVL is the fraction of bacterial mortality caused by viral lysis and FVIC is the frequency of visible infected bacterial cells.

Taking the minimum and maximum burst size (10 and 23, respectively) and frequency of visible infected bacteria (0.5% and 5.2%, respectively) reported for oligotrophic aquatic systems into account, the calculated fraction of bacterial mortality caused by viral lysis (FMVL) was 0.9% to 9.8%. Lytic viral production in groundwater ranged between 0.2 and 9.4 $\times 10^2$ viruses L^{-1} h^{-1} (on average, 2.6 $\times 10^2$ viruses L^{-1} h^{-1}) using a low Bz and FVIC and between 0.6 and 23.8 $\times 10^3$ viruses L^{-1} h^{-1} (on average, 6.7 $\times 10^3$ viruses L^{-1} h^{-1}) using a high Bz and FVIC, respectively. Comparable higher lytic viral production was found in the river water with viral production from 0.6 to 13.8 $\times 10^3$ viruses L^{-1} h^{-1} using a low Bz and FVIC (on average, 6.4 $\times 10^3$ viruses L^{-1} h^{-1}) and from 0.2 to 3.5 $\times 10^5$ viruses L^{-1} h^{-1} (on average, 1.6 $\times 10^5$ viruses L^{-1} h^{-1}) using a high Bz and FVIC, respectively (Table 3.7).

3.4.5 Total bacterial mortality

Total bacterial mortality = mortality induced by protozoan grazing + FMVL

Due to overall low bacterial production measured in pristine groundwater, the maximum clearance rate we selected for estimation of protozoan grazing produced surprising high values. To compare the relative importance of grazing and viral lysis, we chose the minimum grazing rates and maximum viral lysis values.

Excluding the high protozoan grazing in well MIT037 and MIT052 in winter, the impact from viral and protozoan grazing, caused theoretically a total bacterial mortality from 19.4% to 119% of BCP (on average, 59%) in groundwater. Bacterial mortality caused by viral lysis and grazing in river water, removed 6.4% to 184.4% (on average, 42.0%) of BCP. The bacterial motarlity in samples from well MIT037 and MIT052 was found to be highest, even when using the low clearance rate, it accounted for 327% and 238% of BCP, respectively (Table 3.7).

Table 3.7 Bacterial carbon production, lytic viral production, protozoan grazing impact and total bacterial mortality due to viral lysis and protozoa grazing.

	BCP [ng C $L^{-1}h^{-1}$]		Lytic viral production[a] [$\times 10^3$ viruses $L^{-1}h^{-1}$]		Protozoan grazing[b] [%BCP]		Total mortality[c] [%BCP]	
Site	Range	Average	Range	Average	Range	Average	Range	Average
Isar river	0.3-7.7	3.6	16-350	160	2.2-128.2	44.4	6.4-184.4	42
MIT037	0.008-0.3	0.09	0.6-19.4	6.8	9.6-317.6	91.8	194-327.4	101.6
MIT038	0.03-0.1	0.06	2.2-9.5	5	10.4-83.3	41.5	20.2-93.1	51.3
MIT039	0.01-0.3	0.1	0.9-23.8	7.3	17.1-108.9	65.1	26.9-118.7	74.9
MIT052	0.02-0.2	0.1	1.7-18.8	7.5	10.9-228.6	86.1	20.7-238.4	95.9
Average	0.8		37.9		65.8		75.6	

a. Viral production calculated with burst size of 23 and FVIC of 5.2% which was maximum value of literatures.
b. Protozoa grazing calculated with a clearance rate of 0.3 nL protozoa^{-1} L^{-1} which was the minimum value from the literature.
c. Summed mortality calculated as the sum of mortality due to maximum viral control and minimum protozoa grazing.
d. Average of all values of each parameter.

3.4.6 Protozoan community composition

When using fingerprinting methods to investigate protistan communities, the possibility to also cover eukaryotes others than protozoa including fungi and metazoa has to be taken into account. According to Euringer and Lueders (2008), T-RFLP fingerprinting using the EUK20f-FAM/EUK502r+ primer set well covers eukaryotic communities. The eukaryotic richness in river and groundwater were 9 ± 3, 22 ± 11, 18 ± 6, 22 ± 8, 31 ± 12 for Isar river, well MIT037, MIT038, MIT039 and MIT052, respectively (mean of all sampling times ± SD), In order to identify the most dominant groups within the protozoa as well as to evaluate biodiversity, the samples with the highest diversity (MIT037 in May and MIT052 in October) and with the lowest diversity (Isar river in July) were selected for cloning and sequencing. 31 clones from the river water sample and 50 clones from well MIT052 in October and well MIT037 were sequenced.

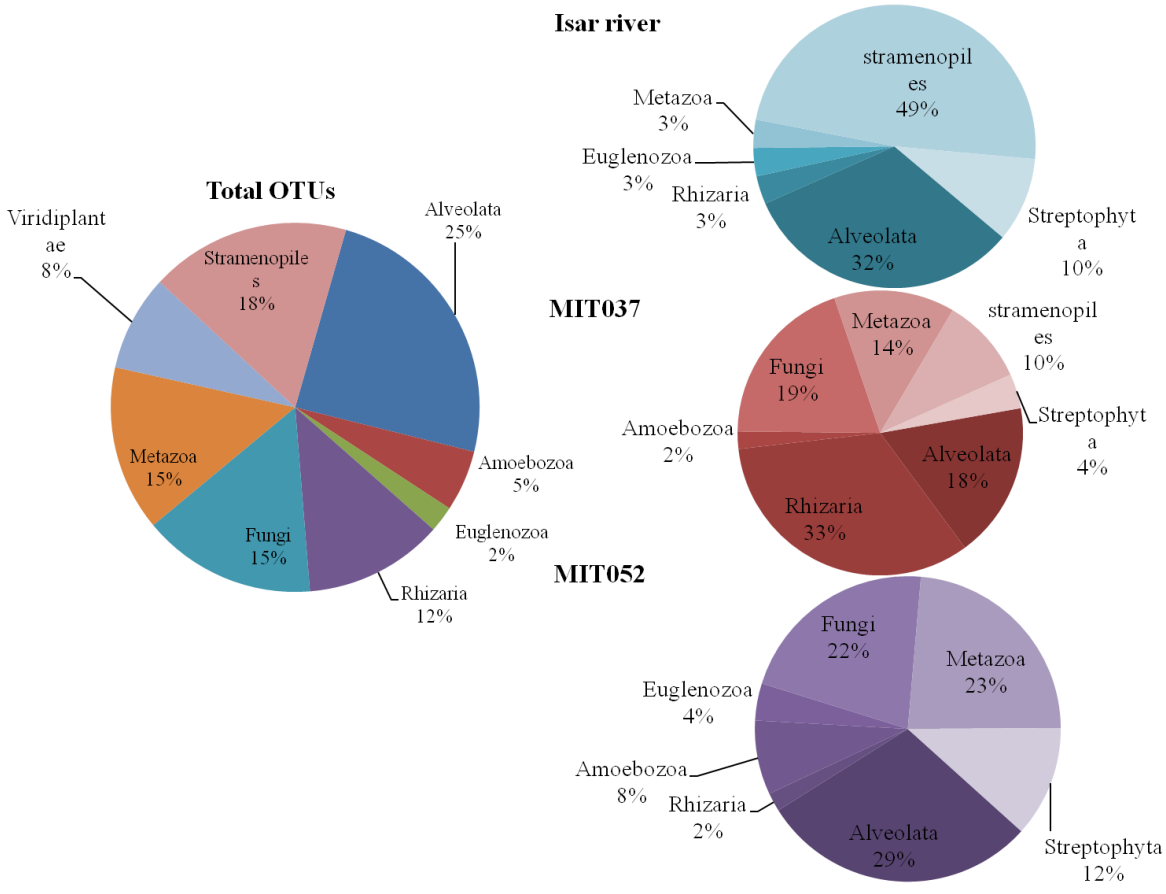


Figure 3.18 Diversity of eukaryotes from well MIT 037, MIT052 and the Isar river.

In total, out of 138 sequences from three samples, 57 sequences were affiliated to protozoan groups. 17 protozoan OTUs fell into 4 lineages, i.e. *Alveolata* (25%), *Rhizaria* (13%),

Amoebozoa (5%) and *Euglenozoa* (2%), while 13 OTUs were identified as fungal (15%), metazoa-derived (15%), algal (18%) and plant-derived (8%) (Figure 3.18). An overview of the phylogenetic affiliation is given in Table 4. The *Alveolata* affiliated sequences were most divers with 6 OTUs, 5 OTUs and 5 OTUs detected in the river, well MIT057 and MIT037, respectively. Only 1 OTU of the river water was identified as cercomonadidae *Cercomonas sp*., two OTUs found in well MIT057 represented the flagellates related to *Petalomonas sp*. and *Procryptobia sp*.. No Fungi and Amoebozoa were detected but 3 clones identified as *Tracheophyta* (plantae, S*treptophyta*) (48%) was found in the Isar river. Non-targeted metazoan clones comprised 13.4% of all analyzed clones and were identified as *Arthropoda, Nematoda*, *Mermithoidea* and *Platyhelminthes* (Table 3.8).

Table 3.8 Clone library composition of eukaryotic 18S rRNA genes amplified with the primer set EUK20f/EUK502r+3 from three selected samples.

Phylogenetic affiliation	**number of clones (OTUs) from**		
	Isar river-July	MIT052-Oct	MIT037-May
Alveolata			
-Eucoccidiorida	3 (1)	2 (1)	
-Gregarinia	5 (2)		
-Colpodea	1 (1)	3 (1)	
-Litostomatea			1 (1)
-Oligohymenophorea		11 (3)	3 (2)
-Spirotrichea	1 (1)		1 (1)
-Gymnodiniaceae			4 (1)
Cerozoa			
-Cercomonadidae	1 (1)	1 (1)	17 (1)
Amoebozoa		4 (2)	3 (1)
Euglenozoa			
-Sphenomonadidae		1 (1)	
-Bodonidae	1 (1)	1 (1)	
Fungi			
-Pezizomycotina		10 (1)	9 (1)
-Pucciniomycotina		1 (1)	
-Entomophthoromycotina			1 (1)
Metazoa	1 (1)	12 (3)	7 (2)
Stramenopiles	15 (4)		5 (1)
Streptophyta	3 (1)	6 (1)	2 (1)
clones (OTUs) in total	31 (13)	51 (16)	53 (13)
diversity estimate *H'*			
T-RFLP	0.39	2.97	2.86

4 DISCUSSION

4.1 Spatio-temporal patterns of bacterial communities in a pristine porous aquifer

4.1.1 Hydrological dynamics and environmental conditions

Groundwater is the most important fresh water resource playing a pivotal role for connected ecosystems, terrestrial as well as surface aquatic habitats. Besides, it is an important source for human being as it serves us with drinking water. The investigation on the activity and distribution of microorganisms in aquifers which are the key players of water purification is essential to understand its natural attenuation potential. Research on spatio-temporal patterns of bacterial communities in groundwater systems so far mainly focused on karst systems and contaminated aquifers (Haack & Bekins, 2000, Haack, *et al.*, 2004, Roling, *et al.*, 2004, Farnleitner, *et al.*, 2005, Anneser, *et al.*, 2008, Winderl, *et al.*, 2008, Wilhartitz, *et al.*, 2009). Investigations of pristine porous aquifers are scarce and most data back to before the 'molecular tool box' was developed (Hirsch & Rades-Rohkohl, 1983, Bone & Balkwill, 1988, Phelps, *et al.*, 1989, Kölbel-Boelke & Nehrkorn, 1992, Rheims, *et al.*, 1996, Dojka, *et al.*, 1998). Therefore, one aim of this thesis was to start filling this gap of knowledge. Moreover, since groundwater systems are perceived to be very stable in environmental conditions we selected a hydrologically dynamic pristine porous aquifer to uncover patterns of suspended and attached bacterial communities as well as groundwater microbial food web dynamics.

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'. 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. Groundwater temperature was significantly less variable than the temperature dynamics in the river. Only moderate seasonal changes in water chemistry were indicated by several physical–chemical parameters such as the electric conductivity. High concentrations of dissolved oxygen all year-round at all sampling sites showed that the shallow aquifer was fully oxygenated. More interestingly, individual parameters indicated the different origin of

water in spring and autumn. Small peaks of nitrate and potassium concentrations in autumn indicate water from summer precipitation percolating through the soil layers before reaching the aquifer. Stable water isotope data clearly indicate snow melting water flushing the aquifer in late spring to early summer; snow is characterized by significantly lower 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 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.

Extraordinary high chloride concentrations were observed in March and May 2010, respectively, at well MIT037 that is located in a 10 m distance to a main road. These exceptional high values are explained by the application of salt (NaCl) for deicing the road during winter. 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. Highest values in October again point at soil water from summer precipitation recharging the aquifer. The higher fraction of assimilable DOC in autumn and winter 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, indicating that at higher temperatures more DOC is converted into bacterial biomass.

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 period of one year 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. Phase II is then characterized by the return of the groundwater table between November 2010 and March 2011. 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 (Figure 2.1) 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.

4.1.2 Suspended bacterial communities

Bacteria suspended in pristine groundwater are generally small (< 1 µm), morphologically simple (spherical cells or small rods) and of low activity or even inactive (Griebler, *et al.*, 2002, Goldscheider, *et al.*, 2006). Results from the Mittenwald aquifer clearly support these findings (Zhou, *et al.*, 2012). Total numbers of bacterial cells (from 10^4 to 10^5 cells mL^{-1}), rates of bacterial carbon production (from 10 pg to 1 ng C $L^{-1}h^{-1}$) and microbial ATP concentrations (from 0.2 to 6 pM) found in this study were in a range comparable to other pristine aquifers (Marxsen, 1982, Griebler, *et al.*, 2002, Detmers, *et al.*, 2004, Farnleitner, *et al.*, 2005, Flynn, *et al.*, 2008, Wilhartitz, *et al.*, 2009, Flynn, *et al.*, 2010). Cell numbers in the groundwater were generally lower than in the river water, which followed the surface water trend overriding surface water numbers slightly. The active microbial biomass estimated from measurements of cellular adenosine triphosphate (ATP) was highest in autumn and lowest in winter, pointing at a higher proportion of active cells within the bacterial communities in autumn.

The really surprising and exciting finding was the pronounced dynamics of bacterial diversity in groundwater of the Mittenwald aquifer triggered mainly by the transient hydraulic conditions. This aquifer, a porous quaternary aquifer in the Alps, regularly receives substantial amounts of snow melting water in spring, which kind of flushes the highly conductive subsurface sediments mainly composed of gravel and sands. Indicated by the groundwater

electric conductivity, the physical-chemical conditions surprisingly did not significantly change during the time of melting water recharge. Different from the physical-chemical parameters, the bacterial Shannon diversity dropped dramatically from $H' = 3.22 \pm 0.28$ in autumn and winter to $H' = 1.31 \pm 0.35$ in summer although total cell numbers did not change much. The dramatic drop of the groundwater bacterial diversity went along with the increase in the groundwater table. The extreme low richness of bacterial communities (8.5 ± 3.5 OTUs) in summer clearly suggested that suspended bacterial communities collapsed during the period of recharge of melting water. Until autumn, with the return of the groundwater table to its original low position, the diversity fully recovered even exceeding the spring values.

As evaluated by T-RFLP fingerprinting. *Rhodocyclaceae*, *Comamonadacea*e, *Alphaproteobacteria*, and *Gammaproteobacteria* were found to be dominant in May and July. 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. The origin of these 'resistant' species or 'newcomers' is still unclear. We may speculate that they either were present already in the snow or were detached from surface soil and unsaturated sediments along with the groundwater recharge.

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 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. 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). 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.

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. 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. 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) (Hirsch & Rades-Rohkohl, 1988, Hirsch & Rades-Rohkohl, 1990, Hirsch & Rades-Rohkohl, 1992, McNabb & Mallard, 1994, Griebler, *et al.*, 2002).

In the future, the bacterial communities from snow, surface soil and springs in the catchment as well as from the flooding river need to be analyzed to further explore seasonal patterns of the community dynamics on a catchment scale.

4.1.3 Patterns of attached bacterial communities

For porous groundwater systems it is well known that the attached bacterial community always dominate in terms of cell numbers (Hazen, *et al.*, 1991, Brockman, *et al.*, 1992, Alfreider, *et al.*, 1997, Pedersen, 2000), activity (Phelps, *et al.*, 1994, Ringelberg, *et al.*, 1997,

Lehman & O'Connell, 2002), and diversity (Hirsch, 1992, Kölbel-Boelke & Nehrkorn, 1992, Flynn, *et al.*, 2008). Since it was not possible to obtain fresh sediments from the Mittenwald aquifer by drilling (area strictly protected for drinking water production), we incubated sediments from the nearby Isar River, either freshly (still carrying the river benthic microbial community) and after sterilization (several times of autoclaving) in groundwater wells for up to 12 months. After one year of exposure to groundwater, the total cell number was 100 – 300 times higher and the ATP concentration was 300 to 600 times higher than the average seasonal values in groundwater. It was shown in the past that the differences in bacterial numbers, biomass and activity between sediment and water, become less pronounced with the favourability of environmental conditions, such as increased concentrations of DOC and nutrients (Griebler, *et al.*, 2002) or organic contamination (Griebler, *et al.*, 2002, Anneser, *et al.*, 2010).

The sterile sediments were quickly colonized within the first 2 months reaching an average cell number similar to fresh river bed sediments, and then leveling-off at a more or less constant cell density for the rest of the incubation period. The trend of bacterial diversity of the fresh river bed sediments and initially sterile sediments showed that, after 1 year, a similar Shannon diversity established for both types of sediments. However, 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.

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.

4.1.4 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. 5c), 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, 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 inter-specific 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 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.

4.2 Colonization patterns of bacterial communities on sterile sediments

4.2.1 Colonization of sediments

As a novel high-throughput and sensitive sequencing technology, 454 pyrosequencing has been revealed a tremendous microbial diversity in various habitats (Acosta-Martínez, *et al.*, 2010, Andersson, *et al.*, 2010, Kirchman, *et al.*, 2010, Pommier, *et al.*, 2010, Fortunato, *et al.*, 2012, Zhang, *et al.*, 2012). It provides reliable recovery of bacterial abundances and the overall community structure within natural microbial communities (Pilloni, *et al.*, 2012), which enabled us to semi-quantitatively estimate the abundances of each identified OTU. Based on that, it allowed us to estimate the minimum growth rates of species for a long term *in situ* observations. To evaluate the colonization patterns of bacterial communities in a pristine aquifer, we analyzed changes in groundwater bacterial communities colonizing sterile sediments using 454 pyrosequencing. Moreover, we compared the bacterial communities of groundwater and sediments to explore the mechanisms of colonization patterns.

Since the theory of r/K strategy was proposed by MacArthur and Wilson (1967), together with some expansions such as the triangular C-S-R theory (Grime, 1988) and the quadrangular habitat templet (Greenslade, 1983), it was broadly used in ecology to explain the population dynamics along with environmental selection pressures. In microbiology, the concept of r-/K- strategy has been tackled in lab experiments by supplementing nutrients in agar media (Kristufek, *et al.*, 2005, Panova-Karadzhova & Dimkov, 2009), in soils exposed to

elevated CO_2 concentrations (Dorodnikov, *et al.*, 2009), as well as in a few field studies, e.g. in a receding glacier (Sigler, *et al.*, 2002, Sigler & Zeyer, 2004). In the present study, we evaluated the r-/K- strategy in a pristine aquifer limited by organic carbon and nutrients supply.

Classically, r- and K- strategists can be, among many other criteria, separated by growth rates. According to Pianka (1970), during the colonization of new habitats, r-strategists growing quickly on easily available substrates and K-strategists follow utilizing resources slowly but more efficiently. The differences in life strategies of r- and K-species lead to different growth rates. Evidence shows the differences in growth rates between the species fast-growing but short life span (r-strategist) and the species utilizing substrates slowly but more efficiently (K-strategist). Due to different living strategies, r- and K-strategists have been reported to be present sequentially and eventually establish maximum biomass (Christensen & Pauly, 1998, Hartvigsen, 2001).

The incubation of sterile sediment allowed us to evaluate if there is a certain carrying capacity level (Hartvigsen, 2001, del Monte-Luna, *et al.*, 2004) reached after a certain time period. Moreover, following the succession in colonization enabled us to evaluate the concept of 'r' and 'K' strategy with bacteria in groundwater. The maximum attached biomass supported by the ecosystems energy level was formed after two months of incubation and was maintained for all along the incubation. The colonization showed a clear succession of different groups of bacteria. Within the early pioneer groups we found *Oxalobacteraceae, Flavobacterium, Massilia, Udibacterium, Brevundimonas* and *Pseudomonas*, which were followed by *Sphingomonas*, *Acidobacteria* and *Burkholderiales incertae sedis*. At the end of the incubation, dominance, i.e. highest relative abundance, were taken over by *Aquabacterium* and *Nocardia*.

There are some indications for the succession of r- and K-strategists derived from established differences in growth rates. The primary microbial colonizers during the first two months were identified as *Oxalobacteraceae, Flavobacterium, Massilia, Janthinobacterium, Udibacterium, Brevundimonas, Pseudomonas, Rugamonas. Flavobacteriales,* another dominant species, was reported to be both free-living and attached to organic aggregates (Crump, *et al.*, 1999, Kirchman, 2002). *Bradyrhizobiaceae* was mainly found related to nitrate fixation strains and root colonizing bacteria, e.g. (Belser, 1979, Vessey, 2003). *Oxalobacteraceae* were found to be a main group of root colonizing bacteria (Ofek, *et al.*, 2012). On the other hand, successors were characterized by high abundances afterwards, which related to their living strategy. For instance, *Planctomycetaceae* is known as an aerobic

budding bacterium with low growth rates (Ward, *et al.*, 1995, Glöckner, *et al.*, 2003). *Sphingomonas* was reported to be an aerobic heterotrophy with a soil-based life-style and capable of degrading extraordinarily recalcitrant carbon sources with low growth rate (White, *et al.*, 1996). *Acidobacteria*, one of the most abundant phyla found in soil microbial communities (Meisinger, *et al.*, 2007, Kielak, *et al.*, 2009, Ward, *et al.*, 2009), are mostly regarded as K-strategists and oligotrophic bacteria with low growth rates and seem to be favored under resource limited conditions because of high substrate affinities (Fierer, *et al.*, 2007). Interestingly, the globally distributed species such as *Pseudomonadaceae*, *Flavobacteria*, *Comamonadaceae* (Tamames, *et al.*, 2010) were found high abundant not only at the beginning of incubation but also dominant for another two months, probably due to their opportunistic growth and fast growth rates (Pernthaler & Amann, 2005, Fierer, *et al.*, 2007). Worth to notice, most dominant species were also found to show some seasonality. A continuously succession of bacteria during the period of incubation is found.

However, a Chao1 index higher than the number of OTUs indicated that the actual total richness of the community may be twice or even threefold higher than the OTU numbers. Therefore we miss some information of the actual community composition. Nevertheless, it is always an issue of the scale of sampling and detection limit of the analytical and molecular technique which await to beimproved in the future.

4.2.2 Neutral processes and species sorting

In the present study, we found evidence strongly pointing at selective forces to influence the bacterial colonization on the initially sterile sediment surfaces exposed to groundwater in the wells MIT039 and MIT052. According to the statement of Baas-Becking (1934) and discussed by Holyoak (2005), the concept of species sorting assumes that the global microbial diversity is high and microbes grow rapidly, but local habitat conditions finally determine the community composition. In our study, although exposed to groundwater in two different wells, almost 0.5 Km apart, the attached bacterial communities from well MIT039 and well MIT052 are more similar than the similarity of communities on sediments from the individual wells over the season. The similarity of the attached bacterial communities increased over time and approached its highest value of 86.7% at the end of incubation. Furthermore, the similarity between initially sterile sediments and the initially actively colonized river sediments increased over time of incubation. Similar observation were reported by Findlay *et al.* (1992)

and Kanzog and Ramette (2009) in a controlled water flow and deep sea experiment, respectively. They both observed a trend that microbial communities colonizing on artificial sediments exhibited a structure and biomass similar to the communities present in natural sediments, regardless of sediment type. This indicates selective factors such as sediment properties, physical-chemical as well as biotic variables to be active. It has been reported that the grain size and grain shape may influence the final bacterial community composition (Nickels, *et al.*, 1981, Llobet-Brossa, *et al.*, 1998, Köster, *et al.*, 2005).

While most groups of early colonizers and successors at sediments in wells MIT039 and MIT052 overlap, others such as *Brevundimonas* and *Undibacterium* and *Massilia* were found dominant in well MIT039 and in well MIT052, respectively. None of these later groups were dominant in water. Hydrological dynamics leading to changes in abiotic and consequently biotic conditions are considered as another selective factor. Augspurger and colleagues (2010) found that hydrodynamics and biological properties influence the attachment processes as well as biofilm assembly and biodiversity. Well MIT052 and well MIT039 were subject to a varying degree of hydrological dynamics (Zhou, *et al.*, 2012). Consequently, DOC and nitrate concentrations were found different in groundwater of these two wells. The DOC concentrations ranged from 0.8 to 1.2 mg mL^{-1} in well MIT039 and from 1.2 to 2.2 mg mL^{-1} in well MIT052 and nitrate concentrations ranged from 1.7 to 3 mg mL^{-1} in well MIT039 and from 2.3 to 4.7 mg mL^{-1} in well MIT052. Differences in physical-chemical parameters may of course influence the bacterial community composition (Judd, *et al.*, 2006, Kritzberg, *et al.*, 2006, Logue, *et al.*, 2012).

On the other hand, according to Sloan *et al.* (2006), the theory of neutral processes predict that the relative abundance of a taxa which occurs in the source communities (suspended communities in groundwater) should be found similar in the settled communities (bacteria attached to sterile sediments). In our study, on phylum and class level the dominant species in groundwater were also found to be dominant in the sediment but this was not the case when increasing the taxonomical resolution to genus level. Considered as the 'seed bank' for the sterile sediments, the composition of the suspended bacterial community in well MIT039 was found to be quite similar to that in well MIT052 (with a similarity of 72.0% ± 6.7%). Seeded by similar suspended bacterial communities, the bacterial communities attached to sediments in the two wells were also found relatively similar (with similarity of 60.2% ± 22.0%). However, the similarity between groundwater and sediments were quite low, especially low after the first period of incubation. The dominant groups in groundwater were not observed to be dominant on sediments. This suggests selective forces to play a more important role than

neutral processes, at least what was seen after two months of incubation.

Still, some groups such as *Acidobacteria*, *Sphingomonas* and *Planctomyces*, characterized by a relative high abundance in sediment samples, were found dominant in water as well. Some groups showed independent colonization to the suspended community, such as *Oxalobacteraceaea*, *Massilia* were dominant only on the initially sterile sediments. Worth to notice, at sediments incubated in well MIT052 *BRC1*, *Epsilonproteobacteria* and *Caldilineae* taking up small proportion of the total abundance were found to be present in sediment samples but not detectable in groundwater.

We need to keep in mind that more than one mechanism is involved in the assembly of bacterial communities as reviewed by (Lindström & Langenheder, 2012). Besides a multituale of abiotic forces biotic variables may shape bacterial community structure. Food web interactions such as predation and viral infections (Simek, *et al.*, 2003, Kent, *et al.*, 2004, Salcher, *et al.*, 2005, Kent, *et al.*, 2007) significantly contribute to changes in community composition. Fazi *et al.* (2008) observed a succession of bacterial genotypes which indicated a selective top-down control not only on bacterial abundances but also on the initially formed opportunistic microbes. Wey *et al.* (2008) found a selective effect of protozoan grazing within river biofilms. A significant correlation between bacterial Shannon diversity and protozoan abundance ($r = 0.58$, $P = 0.001$) found in this study indicates a link between protozoan grazing and bacterial community composition during bacterial colonization.

4.3 Microbial food web structure and carbon cycling in a pristine porous aquifer

4.3.1 Bottom-up versus top-down control

The trophic interactions within microbial food webs have been well-studied in lake and marine environments (Gasol & Vaque, 1993, Rivkin & Legendre, 2001, Samuelsson & Andersson, 2003), however, almost no data are available for groundwater systems. As standing stocks and mortality rates are usually extremely low, it is rather difficult to reliably count protozoa and viruses in samples from pristine aquifers. To the best of our knowledge, this is the first study investigating the seasonal trend of virus-like particles, bacterial cell numbers and protozoan abundances simultaneously in samples from a porous pristine aquifer.

It is generally believed that in oligotrophic aquatic systems, activity and growth of bacterioplankton is controlled by DOC concentration and quality and the availability of

nutrients (Sanders, *et al.*, 1992, Dufour & Torreton, 1996, del Giorgio & Cole, 1998, Berdjeb, *et al.*, 2011). Characterized by very low DOC and AOC concentrations, groundwater ecosystems are thought to be bottom-up controlled (Baker, *et al.*, 2000, Datry, *et al.*, 2005, Foulquier, *et al.*, 2011). It has been repeatedly reported that an increase of the DOC flux resulted in a stimulation of the microbial productivity in aquifers (Baker, *et al.*, 2000, Foulquier, *et al.*, 2010). Similar evidence can be gained from our results. In the present study, boosting of the bacterial abundance in association with elevated AOC concentrations in winter hint control of bacterial growth by the availability of organic carbon (Mermillod-Blondin, *et al.*, 2010, Foulquier, *et al.*, 2011). The higher DOC concentrations induced by snow melting water in early spring 2010 neither increased the bacterial density nor the bacterial carbon production. As it contained only a small fraction of AOC, which can be seen for water from well MIT037 located close to the river, no stimulation occured. Bacterial production as well as growth efficiency, without doubt, not only depend on DOC concentrations but composition (del Giorgio & Cole, 1998). Interestingly, the correlation between ATP and the total numbers of bacteria, which is repeatedly reported for shallow and deep groundwater samples (Eydal & Pedersen, 2007, Pedersen, *et al.*, 2008, Hammes, *et al.*, 2010) could not be confirmed in the present study. We think, this is at least partly related to the transient environmental conditions, that cause transient ratios of active vs inactive portion of cells and various levels of cell activities.

The abundances of protozoa and virus-like particles, *i.e.* the two major bacterial antagonists, exhibited an interesting coupling to bacterial density. Protozoan grazing on bacteria, especially by heterotrophic nanoflagellates (HNF), has been shown to be a significant factor for the loss of bacterial biomass. However, the density of planktonic protozoa in the oligoalimonic aquifer was found low ($1.5 \pm 0.8 \times 10^2$ cell mL^{-1}). The ratio of planktonic bacteria to protozoa ranged from 33 to 2089 (on average, 349 ± 385). The strong variations found in the present study can be related to the seasonal hydrological dynamics. A significant positive correlation between protozoan abundance and the pH value ($r = 0.61$, $P = 0.000$), as well as a negative correlation with chloride concentration ($r = -0.64$, $P = 0.000$) and electronic conductivity ($r = -0.77$, $P = 0.000$), point at the strong influence of the transient hydraulic conditions on protozoan density. Although higher numbers of bacteria theoretically support higher numbers of protozoa, the exceptional high bacterial abundance in winter 2010 were not accompanied by elevated numbers of protozoa. Of course it can not be excluded that a delayed reaction of the protozoan density to the elevated numbers of bacteria was missed with the temporal resolution of sampling chosen. However, a postive correlation between

bacterial abundance and protozoan numbers (r = 0.36, P = 0.05) was found. Moreover, it is known that, some flagellates are able to feed on virus particles (Gonzalez & Suttle, 1993, Pesan, *et al.*, 1994, Manage, *et al.*, 2002, Miki & Jacquet, 2008, Berdjeb, *et al.*, 2011) or high molecular weight DOC (Sherr, 1988), mechanisms which might be of increased relevance in groundwater and further weaken the link between protozoa and bacteria.

As another top-down control factor, viruses can influence the mortality and composition of bacterial and eukaryotic communities, and therefore can regulate energy flow, food web dynamics and microbial diversity (Wommack & Colwell, 2000, Weinbauer, 2004). A significant virus-induced bacterial mortality was repeatedly reported for lake ecosystems (Weinbauer & Höfle, 1998, Almeida, *et al.*, 2001, Simek, *et al.*, 2001), and marine systems (Fuhrman & Nobel, 1995, Guixa-Boixareu, *et al.*, 1996, Steward, *et al.*, 1996, Guixa-Boixereu, *et al.*, 1999). Viral lysis has been shown to be as significant as grazing (Fuhrman & Nobel, 1995, Wommack & Colwell, 2000, Weinbauer, 2004) and or may even exceed it (Fischer & Velimirov, 2002, Pradeep Ram, *et al.*, 2005). The total number of bacterial cells (TNC) and virus-like particles (VLP) as measured in our study were in the range of 10^4-10^5 TNC mL^{-1} and 10^5-10^6 VLP mL^{-1}, which correspond to a previous study (Kyle, *et al.*, 2008). Overall, the VLP showed a positive correlation to the bacterial abundance (r = 0.47, P = 0.007). Similar correlations between VLP and bacterial cell density was found in deep sea water, surface waters (Kepner, *et al.*, 1998) and in granitic groundwater (Kyle 2008). The ratio of virus-like particles to bacterial cells (2-230) showed considerable seasonal fluctuations. Similar variations were also found in oligotrophic marine systems (ratios from 3 to 96) (Christaki, *et al.*, 2011) and a temperate lake (ratios from 8 to 121) (Maurice, *et al.*, 2010) indicating different phases of infection and release of host cells (Personnic, *et al.*, 2009) and/or a connection to bacterial activity (Middelboe, 2000, Danovaro, *et al.*, 2002, Middelboe, *et al.*, 2003, Motegi & Nagata, 2007). The significant correlation between VLP and ATP (r = 0.51, P = 0.004) as well as VLP and BCP (r = 0.41, P = 0.037) provide support that viral production is directly linked to active bacterial biomass. At the same time, the overall low BCP and celullar ATP hint at a low metabolical status of the planktonic bacteria in the energy-limited aquifer. Since viruses may not only derive from suspended bacteria infected and lysed but from cells attached to sediment surfaces, the stable bacterial biomass on sediment grains (Zhou, *et al.*, 2012) may explain the less dynamic VLP numbers in groundwater.

4.3.2 Viral lysis and protozoan grazing in groundwater

Since the investigations of the Mittenwald aquifer did not include a direct measure of bacterial mortality by viral lysis and protozoan grazing, we carefully selected rates and conversion factors from the literature to derive a first estimation of protozoa and virus induced bacterial mortality in groundwater (Table 4.1). Since reports on protozoan grazing in groundwater are available only from contaminated aquifers (Kinner, *et al.*, 1997, Kinner, *et al.*, 1998, Kinner, *et al.*, 2002) (with clearance rates from 0.1-1.4 nL flagellate^{-1} h^{-1}), we used values from other oligotrophic aquatic ecosystems, i.e. a maximum and minimum clearance rate of 2.6 nL flagellate^{-1} h^{-1} and 0.3 nL flagellate^{-1} h^{-1}, respectively.

Table 4.1 Values on protozoan grazing and viral lysis from different oligotrophic aquatic ecosystems. FVIC = fraction of visible viral infected bacterial cells.

Aquatic environment	clearance rate nL flagellate^{-1} h^{-1}	Ingestion rate bacteria ind^{-1} h^{-1}	lytic viral production *10^{8} viruses L^{-1} day^{-1}	FVIC %	Reference
ultraoligotrophic lakes			0.5-7.2	1.7-5.2	(Säwström, *et al.*, 2007)
oligotrophic lake	0.7-11.5	1.8-41.7		0.5-3.5	(Bettarel, *et al.*, 2003)
oligotrophic sea	2.4		0.4-13.7	1.2-2.2	(Hwang & Cho, 2002)
marine				3.2	(Proctor & Fuhrman, 1990)
oligotrophic sea				1.3-1.5	(Weinbauer, *et al.*, 2002)
oligotrophic alpine lake				0.9-2.3	(Hofer & Sommaruga, 2001)
Oligotrophic coastal system		4			(Unrein, *et al.*, 2007)
River	0.2-8.9	1.1-90			(Barcina, *et al.*, 1991)
Oligotrophic atoll lagoon		0.26			(Sakka, *et al.*, 2000)
Oligotrophic eastern Mediterranean	2.6	1- 4			(Christaki, *et al.*, 1999)

In the present study we estimate that the impact of protozoan grazing on bacterial mortality generally prevails over viral lysis (by the factor of 0.1-18.7 with mean value of 3.7) in groundwater, using the minimum protozoan grazing rate and maximum viral lysis rate. Similar results were previously reported from a coastal site in the NW mediterranean Sea (Bettarel, *et al.*, 2002), a mesoeutrophic reservior (Simek, *et al.*, 2001), two solar salterns

located in Tarragona, Spain (Guixa-Boixareu, *et al.*, 1996) and lake Pavin (Bettarel, *et al.*, 2003). However, recently an increasing number of studies indicate a more significant importance of viral control of the bacterial production in marine ecosystems (Steward, *et al.*, 1996, Guixa-Boixereu, *et al.*, 1999), in ultraoligotrophic lake (Säwström, *et al.*, 2007) and even in experimental river benthic sediments (Fischer, *et al.*, 2006). Weinbauer and Höfle (1998) reported depth-correlated changes in the control of the bacterial mortality in an eutrophic lake. The minor influence of viral-induced lysis on bacteria found in our study might be explained by the extreme low activity of cells in groundwater, indicated by low bacterial production and bacterial growth rate. It has been frequently argued that viruses preferrably infected active bacterial cells (Danovaro, *et al.*, 2002, Middelboe, *et al.*, 2003). For protozoan grazing, the metabolic stage of individual cells or functional/phylogenetic groups may be of less importance than for viral infection. Bohannan and Lenski (2000) modeled that bacterial communities tend to be controlled by phages in high-productivity systems. A viral control of the mortality of bacteria were also found for anoxic environments due to lack of anaerobic protozoa and consequently low grazing pressure (Weinbauer & Höfle, 1998).

4.3.3 Carbon flux within a groundwater microbial food web

Due to the lack of light and substantial primary production, dissolved organic carbon (DOC) in groundwater is generally low and of bad quality. In fact, DOC mainly originates from surface systems (Goldscheider, *et al.*, 2006). Changes in DOC concentrations in the shallow aquifer studied could be related to the recharge of snow melting water and recharge from precipitation during spring and summer. However, due to the overall low biodegradability of DOC in groundwater, a temporal increase of DOC not necessary undergoes along with a stimulation of bacterial activity and production, as is supported by our data. Unfortunately, the carbon flux within and through the microbial food web could be calculated only for the groundwater planktonic community. Thus, the overall carbon flux is considerably underestimated due to the biomass and activity dominance of attached microbes. Moreover, since no *in situ* viral infection and protozoan grazing experiments were so far conducted with oligoalimonic groundwater, values for frequency of visible infected cells, burst size and clearance rates had to be selected from other aquatic environments, such as oligotrophic lakes and deep sea (Wilhelm, *et al.*, 1998, Christaki, *et al.*, 1999, Sakka, *et al.*, 2000, Hofer &

Sommaruga, 2001, Hwang & Cho, 2002, Weinbauer, *et al.*, 2002, Bettarel, *et al.*, 2003, Säwström, *et al.*, 2007).

The average biovolumes of bacterial cells measured were on average 0.20 μm^3 for river water samples and 0.11 μm^3 for groundwater samples. Based on the conversion factor, 1 $\mu m^3 \approx 218 \times \text{biovolume}^{0.86}$ fg C (Loferer-Krossbacher, *et al.*, 1998), biovolume-to-biomass conversion factors equaled 50 fg C per cell for river water samples and 30 fg C per cell for groundwater samples.

To estimate bacterial growth efficiency, we used the model of del Giorgio and Cole (1998) BGE = (0.037 +0.65 × BCP) / (1.8 + BCP), which was developed based on a broad literature survey evaluating the relation between BCP and BGE from different aquatic environments including ultraoligotrophic to eutrophic ones. Unfortunately, groundwater systems have not been considered in this review, since no data have been available, and thus the model may not fit perfectly to our data set. The BGE obtained for our groundwater samples ranged from 2.1% to 2.3%, which is definitely at the absolute lower range reported by del Giorgio & Cole (1998) for ultraoligotrophic systems. It has been reported that, in the most oligotrophic systems, the BGE can be as low as 1% (Pomeroy, *et al.*, 1995, Cherrier, *et al.*, 1996). The low growth effeciency in oligotrophic systems is argued by the low concentration and degradability of organic carbon as well as increased energetic costs for maintainace to keep the cell ready for the next possible pulse of degradable substrate (del Giorgio & Cole, 1998). Calculating, based on the BGE, the total organic carbon uptake via BCP/BGE, values of 0.4 to 15.2 ng C L^{-1} h^{-1} are obtained. Taking into account, that AOC concentration in groundwater ranged between 2.5 – 25 µg L^{-1}, the actual bacterial organic carbon uptake in groundwater is 2-3 orders of magnitude lower, or in other words, the AOC present would be sufficient to fuel BCP for about 1 month. As mentioned above, the groundwater bacterial community only represent a minor fraction of the total bacterial biomass, most of which is attached to sediment surfaces. Including these attached cells in our estimation, AOC available might be consumed within only a few hours. The extreme low ATP concentrations found in groundwater in the present study, indicate that the planktonic cells are of low activity or mainly dormant with a small fraction of cells active.

As mentioned above, protozoan grazing removed 9.6% to 108.9% (average, 48.7%) of BCP in groundwater. By converting bacterial cells grazed by protozoa into carbon (grazing rate multiplied by 30 fg C per cell), the carbon flow through grazing was in the range of 7.8 to 109.3 pg L^{-1} (mean, 38.1 pg L^{-1}). A smaller grazing effect was found in the river water in

which protozoa removed from 2.2% to 128.2% of BCP. The carbon flux through grazing in river water ranged from 149 to 687 pg L^{-1}, using a conversion factor of 50 fg C per cell.

Calculating with the maximun burst size and frequency of visible infected cells measured in a oligotrophic marine system, viral-induced bacterial mortality accounted for 9.8% of BCP. The bacterial carbon mobilized through viral lysis was in the range from 0.8 to 31 pg C L^{-1} h^{-1} (mean, 9 pg C L^{-1} h^{-1}). Lytic viral production revealed 6 - 238 $\times 10^2$ viruses L^{-1} h^{-1} (mean, 67 viruses L^{-1} h^{-1}) from infected bacteria in groundwater. Based on 0.1 fg C virus $particle^{-1}$ (Gonzalez & Suttle, 1993), viral lysis can release 0.9 - 3.6 pg C L^{-1} h^{-1} (on average, 1 pg C L^{-1} h^{-1}) in form of viruses. With respect to bacterial debris, it is assumed that after the viral burst event, approximately 99% of the whole bacterial debris fuels into the DOC pool (Furhman, 1992). Therefore, 99% of bacterial debris (carbon content in bacteria substracted by carbon content in virus) was recycled, which is in the range from 0.7 to 28.3 pg C L^{-1} h^{-1} (average, 8.0 pg C L^{-1} h^{-1}) in groundwater. In river water, viral lysis transformed 30 – 800 pg C L^{-1} h^{-1} (mean, 400 pg C L^{-1} h^{-1}) into new virus biomass. The carbon flow also shown in percentage of carbon uptake by bacteria in groundwater is detailed in Figure 4.1.

Since we used a clearance rate and FVIC and Bz data published for other aquatic systems, our estimations of the carbon flux should be considered with caution. For instance, the low clearance rate of 0.3 nL $protozoa^{-1}$ h^{-1} used for grazing estimation in the present study might still overesitmate (Kinner, *et al.*, 1998) bacterial mortality. According to Unrein and his colleagues (2007), heterotrophic flagellates grazing activities may change seasonally and the clearance rates were found positively correlated with water temperature. The seasonal dynamics can also be true for viral infection in groundwater. The wide variation of the VBR (viruses to bacteria ratio) indicate a seasonal difference of viral impact. Our bacterial calculation using a constant FVIC and Bz value might cover the dynamics of viral-induced mortality. Another issue is the extreme low bacterial growth rate in the oligotrophic groundwater system. As shown above, bacterial growth efficiency was estimated only 0.02. It raises the question how to precisely measure growth rates. Currently, we attempt to establish a sensitive and reliable method to measure bacterial growth rates via stable isotope labelling (Wegener, *et al.*, 2012).

Compared to other aquatic systems, the values of carbon flux among the organic matter pools, bacteria and predators, were found similar to data obtained in other oligotrophic systems. For instant, Hall *et al.* (Hall, *et al.*, 2000) estimated that about 30 mg C m^{-2} day^{-1} (approximately 0.01 kg C m^{-2} $year^{-1}$) of bacterial production was removed by *Chironomidas* in a perennial first order stream. In an oligotrophic lake in mid-Sweden (Andersson & Kumblad,

2006), about 33 kg C $year^{-1}$ (94 ng C L^{-1} $year^{-1}$) was taken by heterotrophic flagellate and 17000 kg C $year^{-1}$ (49 µg C L^{-1} $year^{-1}$) of DOC was utilized by bacteria. In the lake Superior zooplankton was estimated to take about 0.5 to 1.8 Tg C $year^{-1}$ (0.04 – 0.15 ng C L^{-1} $year^{-1}$) and 6 – 38 Tg C $year^{-1}$ (0.5 – 3.1 ng C L^{-1} $year^{-1}$) of DOC was uptake by bacteria (Urban, *et al.*, 2005).

However, because of the following reasons a direct comparison of carbon flow from other systems on a quantitative level is difficult. (1) Due to differences in the interests of ecologists and differences in model systems and ecosystems studied, the food web resolution might dramatically be constructed of different numbers of trophic links. (2) Most estimations of carbon and energy flow were made without consideration of the contribution of viruses (bacteriophages) which have been proven important members in food webs responsible for bacterial mortality and carbon recycling (Weinbauer, 2004). (3) The carbon flux was estimated mostly in surface fresh waters (streams, lakes and rivers) and marine systems (Hansen, *et al.*, 1996, McKenna, *et al.*, 2006, van Oevelen, *et al.*, 2006, Hambright, *et al.*, 2007, Olsen, *et al.*, 2007). Data from groundwater systems are generally missing. However, compared to the complex trophic interactions in these surface aquatic ecosystems, the simplicity and relative stability of food webs in pristine groundwater promised a clear view to the microbial trophic structure in the aquifer.

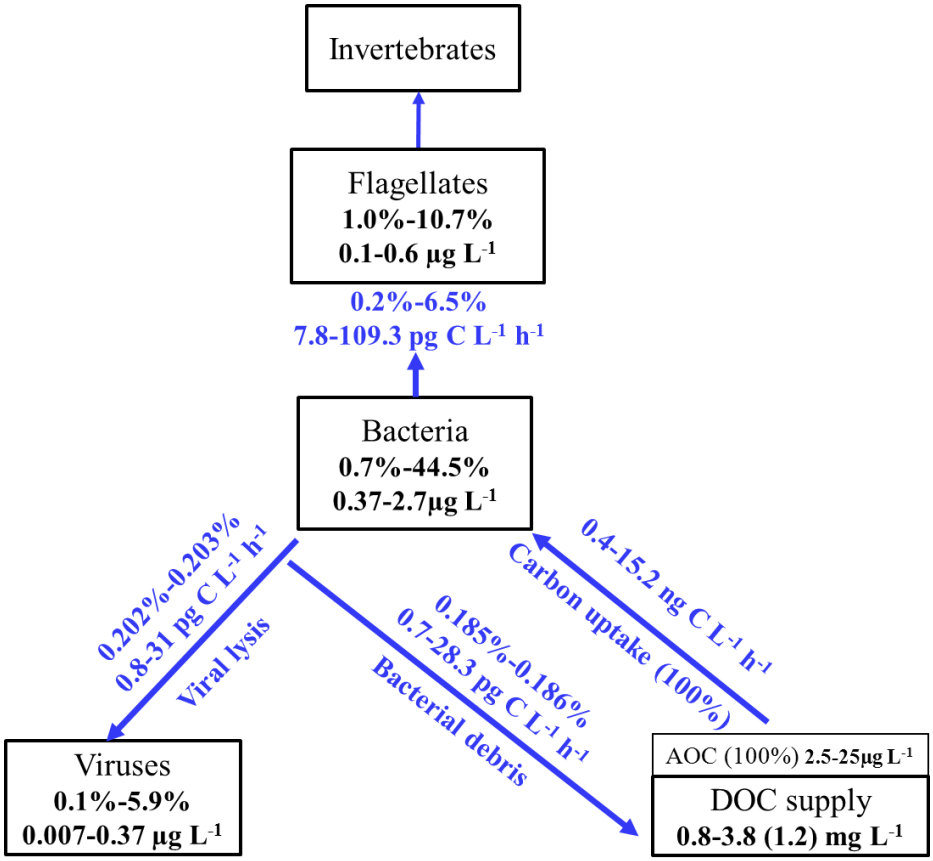


Figure 4.1 Estimation of carbon flux within a microbial food web in pristine, oligotrophic groundwater. The boxes contain the carbon equivalents given in [µg L^{-1}] and [%] with AOC serving as 100%. The arrows show the carbon fluxes between the different trophic levels, given in [ng C L^{-1} h^{-1}] and [%] with the total bacterial DOC uptake serving as 100%.

4.3.4 Seasonal patterns of protistan community composition

The amplicafication of 18S rRNA genes with eukaryote-specific primers followed by cloning and sequencing has been employed to explore the protozoan diversity (Epstein & Lopez-Garcia, 2008). To process samples on higher throughput and monitor the spatio-temporal dynamics of microbial communities, DNA fingerprinting has been frequently applied to analyse protozoan communities (Brad, *et al.*, 2008, Jousset, *et al.*, 2010, Engel, *et al.*, 2012, Tymensen, *et al.*, 2012). However, one has to be cautious using fingerprinting techiques investigating protozoan communities because there is a low number of protistan gene copies and co-amplification of non-targeted genes occurs. Therefore, developed by Euringer and Lueders (2008), the EUK20f/EUK502+3r primer set for 18S rRNA and restriction enzyme

Bsh1236I were used in the present study. Three samples, Isar river water in July 2010, groundwater from well MIT052 in Oct 2010 and from well MIT037 in May 2010, were analyzed by cloning and sequencing, in order to evaluate the distribution of dominant protozoan groups and to distinguish them from possibly detected fungi and algae.

About 50% of clones were affiliated to Fungi, Metazoa and Plants which indicated that the diversity of protozoan communities is present in groundwater considerably overestimated by the T-RFLP assay. The percentage of metazoa and plantae sequences was higher in the river water sample. In groundwater samples, *Rhizaria* were most dominant in water from well MIT037 in May and *Alveolata* were most abundant in water from well MIT052 in October. The site-dependent differences in eukaryotic communities hint at different selection pressures and/or environmental conditions. According to the PhD thesis of Euringer (Euringer, 2008), the protozoan community structure was found depth related in a BTEX contaminanted aquifer. Most ciliates (*Alveolata*) and *Cercozoa* were detected above 8 m depth while *Euglenozoa* was found dominant below 8 m depth. In the study of Brad *et al.* (2008), protozoans related to the *Cercozoan* flagellate *H. globosa* were detected only in sediments polluted with landfill leachate at certain depth (5 m). However, the depth-dependent distribution could be related to the distribution of contaminants. Furthermore, it had been reported that ciliate communities in a shallow groundwater follow seasonal and spatial variations in terms of density and composition (Andrushchyshyn, *et al.*, 2007). It may thus assumed that, in the hydrologically dynamic aquifer studied, protozoan community composition may also be strongly influenced by the seasonal dynamics. Additionally to the data from the contaminanted sites, Valster *et al.* (2009) reported that *Cercozoa, Choanozoa* and *Ciliophora* were found dominant in water from two groundwater supplies. In combination with the study done by Loquay *et al.* (2009) who investigated the protistan habitats from 14 aquifers, so far no clear distribution patterns for protozoan communities in groundwater were found. All information available indicates that groundwater may harbour a considerable diversity of protozoa. The three water samples analyzed can only provide a first glance at the dominant protozoan groups in oligotrophic groundwater.

5 CONCLUSION

(1) A strong spatio-temparal patterns of bacterial communities were found in the hydrologically dynamic pristine porous aquifer investigated. We showed that the suspended bacterial communities in the porous aquifer system were influenced by the hydraulic dynamics. Recharge and discharge of groundwater are therefore important factors for the distribution and composition of suspended bacterial communities. Compared with groundwater, suspended bacteria in the Isar river water exhibited higher cell numbers (by a factor of 1 to 7) but showed a similar seasonality. Microbial communities in the river had a higher diversity (with $H' = 3.6 \pm 0.2$) which was stable all year round. Thus, in our case, the river displayed the 'groundwater-like' stable patterns. These stable seasonal patterns of suspended bacteria in river water might be due to missing the 'flood'.

(2) The attached bacterial communities from the colonized river sediment when incubated in groundwater exhibited very stable biomass and diversity patterns in contrast to the dynamic community suspended in groundwater. The communities colonizing the sterile sediments were found to establish to a composition similar to the initially colonized river sediments. This indicates a common pool of species for the river and the adjacent aquifer. The pioneering of colonization by r-strategists later followed by K-strategists goes more with the concept of 'species sorting', which assumes environmental conditions act as a filter or selective pressure, sorting 'colonizers' from 'non-colonizers'. On genus level, evidence of species sorting was obtained when comparing suspended and attached bacteria. In detail, the early colonization seemed neutral while groups were increasing in relative abundance or popping up at later phases of the incubation might have successfully competed against others. It shed light to the mechanism of bacterial assembly considering the suspended bacterial community as a 'seed bank'.

(3) The coupling between bacterial production and available organic carbon provide evidence for a bottom-up control of bacterial communities in the Mittenwald aquifer. This confirms previous observations in other oligotrophic aquatic systems where the supply of organic carbon and energy controls the bacterial production and consequently controls the biomass of higher trophic levels. Although the major part of microbial activity in aquifers is with the

sediment fraction, the restricted access to fresh aquifer sediment forced us to concentrate our food web analysis to the suspended groundwater community. Surprisingly, the impact of protozoan grazing was found more pronounced than the impact of viral lysis. The total bacterial mortality caused by protozoan grazing together with viral lysis accounted for more than 50% of the bacterial production. The promissing results of the present thesis provide new aspects to be further investigated in pristine groundwater ecosystems. In the future, data for protozoan clearance rates and viral infection rates *in situ* are required. Additionally, methods for measuring the abundance and activity of protozoa and viruses attached to sediments need to be developed to gain the full picture of microbial food webs and carbon flow in a pristine aquifer.

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III. APPENDIX

III.I Physical-chemical parameters

water table [m]	Isar	MIT 037	MIT 038	MIT 039	MIT 052	oxygen [mg L^{-1}]	Isar	MIT 037	MIT 038	MIT 039	MIT 052
0	-	-5.75	-5.7	-5.3	-16.9	0	9.8	9.4	8.8	8.7	9.0
49	-	-5.25	-5.1	-4.7	-15.4	49	10.7	9.6	9.9	10.2	10.4
115	-	-4.7	-4.45	-3.95	-9.45	115	8.8	8.2	8.5	9.9	11.3
196	-	-4.8	-3.96	-4	-9.06	196	10.7	9.3	10.0	10.5	10.8
263	-	-4.95	-4.9	-4.5	-11.65	263	10.4	10.3	11.3	9.5	10.2
347	-	-5.5	-5.55	-9.5	-15.5	347	10.8	10.7	10.8	11.1	10.5
EC-	**[µS/cm]**					**P**	**[mg L^{-1}]**				
0	260	318	372	325	316	0	n.d.	0.005	n.d.	n.d.	n.d.
49	231	375	309	292	235	49	0.005	0.005	0.007	0.005	0.011
115	221	286	311	387	296	115	n.d.	n.d.	n.d.	n.d.	0.01
196	234	286	312	345	322	196	n.d.	0.005	0.05	0.07	0.02
263	233	248	273	265	302	263	n.d.	n.d.	0.006	n.d.	0.01
347	242	264	279	304	264	347	n.d.	0.007	0.007	0.006	0.01
T	**[℃]**					**K**	**[mg L^{-1}]**				
0	4.8	4.6	7.9	7.3	6.4	0	0.2	0.2	0.4	0.3	0.2
49	5.1	6.4	7.2	6.5	5.9	49	0.2	0.2	0.4	0.3	0.3
115	13.2	6.5	7.5	7.4	7.1	115	0.1	0.2	0.3	0.4	0.2
196	5.9	9.3	7.6	8.8	8.0	196	0.2	0.4	0.4	0.6	0.4
263	4.3	8.6	8.5	7.5	7.3	263	0.1	0.2	0.3	0.4	0.3
347	3.4	2.6	6.9	6.9	6.0	347	0.1	0.1	0.2	0.3	0.2
DOC	**[mg L^{-1}]**					**SO_4^{2-}**	**[mg L^{-1}]**				
0	1.4	1.3	3.8	1.1	2.2	0	11	10	25	31	4.4
49	1.0	0.9	0.8	0.8	1.2	49	7.1	7.3	23	22	6.6
115	1.5	1.1	1.0	1.1	1.2	115	5.2	5.1	17	13	6.8
196	1.0	1.1	1.1	1.2	1.3	196	6.2	5.0	12	16	6.1
263	1.1	1.2	1.2	1.2	1.1	263	7.3	6.6	9.2	17	6.1
347	0.9	0.8	0.9	1.0	1.2	347	9.5	9.4	11	20	7.8
pH						**$\delta^{18}O$**	**[‰]**				
0	8.1	7.1	6.9	7.2	7.6	0	-	-12.8	-12.5	-12.5	-12.2
49	8.4	8.1	8.1	8.1	7.7	49	-	-13.0	-12.7	-12.3	-12.7
115	8.4	8.2	7.7	7.8	7.8	115	-	-12.9	-12.8	-12.4	-12.8
196	8.1	7.8	7.7	7.8	7.9	196	-	-12.7	-12.5	-12.2	-12.5
263	8.3	7.8	7.8	7.6	7.9	263	-	-12.8	-12.8	-12.6	-12.6
347	8.4	7.9	7.9	7.7	7.9	347	-	-12.9	-12.9	-12.9	-12.8
Cl-	**[mg L^{-1}]**					**NO_3^-**	**[mg L^{-1}]**				
0	1.1	19	6.2	1.4	0.6	0	1.9	2.2	3.0	3.0	3.3
49	0.6	47	4.7	1.7	0.7	49	2.0	1.9	3.1	2.7	2.8
115	0.9	4.3	3.3	4.1	1.8	115	2.1	2.3	2.7	2.4	4.7
196	0.3	1.6	4.4	3.8	1.1	196	2.2	2.3	2.5	2.5	2.8
263	0.2	1.2	2.4	2.4	0.7	263	1.9	2.1	2.5	1.7	2.9
347	0.3	1.7	3.6	2.7	0.7	347	1.9	2.0	2.9	3.0	3.3

III.II Abundances of bacteria, protozoa and virus-like particles

Water samples

Water samples

viruses mL^{-1}	Isar	SD	MIT 037	SD	MIT 038	SD	MIT 039	SD	MIT 052	SD
Day 0	5.9E+06	9.2E+03	3.3E+06	2.0E+06	7.32E+05	4.0E+03	7.1E+05	2.2E+05	8.3E+05	3.8E+04
Day 49	1.3E+06	5.6E+05	9.8E+05	1.9E+05	6.89E+05	1.1E+04	9.4E+05	6.1E+04	8.3E+05	1.3E+05
Day 115	6.9E+05	3.3E+04	7.2E+05	5.0E+04	6.74E+04	3.4E+03	1.0E+06	1.4E+05	9.2E+05	6.1E+03
Day 196	1.1E+06	5.8E+04	9.9E+05	4.9E+05	1.05E+06	1.0E+05	6.5E+05	1.2E+05	1.3E+06	9.8E+04
Day 263	1.4E+06	2.3E+05	9.6E+05	1.8E+04	9.25E+05	3.0E+05	8.0E+05	2.9E+04	1.2E+06	1.3E+05
Day 347	1.2E+06	4.4E+04	9.9E+05	1.5E+05	6.47E+05	1.4E+03	5.4E+05	1.3E+05	6.8E+05	1.5E+05
Protozoa mL^{-1}	Isar	SD	MIT037	SD	MIT038	SD	MIT39	SD	MIT052	SD
Day 0	3.1E+02	6.6E+01	9.2E+01	3.7E+00	8.54E+01	4.1E+00	1.3E+02	4.1E+01	9.9E+01	1.5E+01
Day 49	2.9E+02	6.3E+00	8.5E+01	2.3E+00	8.21E+01	1.3E+01	8.8E+01	9.4E+00	1.8E+02	3.4E+01
Day 115	4.1E+02	1.3E+01	1.5E+02	4.1E+00	1.06E+02	6.4E+00	1.2E+02	7.2E+00	1.6E+02	2.2E+01
Day 196	2.3E+02	1.4E+00	3.0E+02	5.1E+01	1.52E+02	4.1E+01	1.2E+02	2.7E+01	1.4E+02	3.4E+01
Day 263	3.4E+02	6.3E+01	1.2E+02	8.2E+00	1.57E+02	2.0E+02	3.0E+02	6.9E+00	1.3E+02	6.9E+01
Day 347	3.2E+02	3.7E+01	2.0E+02	2.3E+02	1.55E+02	6.4E+01	7.0E+01	7.9E+00	3.7E+02	1.2E+01
Bacteria mL^{-1}	Isar	SD	MIT037	SD	MIT038	SD	MIT39	SD	MIT052	SD
Day 0	8.6E+04	2.8E+03	1.4E+04	1.6E+03	1.29E+04	2.2E+03	1.6E+04	1.1E+03	3.0E+04	2.7E+03
Day 49	3.4E+04	3.2E+03	3.2E+04	1.8E+02	1.54E+04	1.8E+03	1.7E+04	2.5E+02	1.6E+04	2.1E+03
Day 115	1.1E+05	1.2E+04	2.9E+04	1.0E+04	3.96E+04	8.8E+03	8.9E+04	2.8E+04	3.6E+04	9.3E+03
Day 196	7.3E+04	7.5E+03	3.0E+04	2.2E+03	4.04E+04	1.9E+03	3.4E+04	8.6E+02	8.9E+04	3.0E+03
Day 263	2.6E+05	5.0E+03	6.4E+04	3.5E+03	6.00E+04	8.4E+03	2.1E+05	5.8E+03	2.7E+05	3.7E+04
Day 347	6.9E+04	9.1E+03	1.8E+04	6.3E+02	1.77E+04	6.5E+02	1.2E+04	2.5E+03	1.2E+04	1.7E+03

Sediment samples

Sediment samples	Initially sterile sediment	MIT037	SD	MIT038	SD	MIT39	SD	MIT052	SD
	Day 0	1.0E+00	0.0E+00	1.0E+00	0.0E+00	1.0E+00	0.0E+00	1.0E+00	0.0E+00
	Day 49	8.7E+06	5.3E+06	4.5E+06	1.6E+06	4.5E+06	1.5E+05	1.8E+07	5.3E+06
	Day 115	8.1E+06	8.1E+05	6.4E+06	1.4E+06	1.3E+07	7.8E+05	1.0E+07	5.8E+06
	Day 263	1.8E+06	3.2E+05	2.2E+06	5.1E+05	1.6E+06	7.0E+05	8.6E+06	5.1E+06
	Day 347	2.7E+06	4.9E+04	1.8E+06	8.4E+05	7.5E+05	2.3E+05	2.1E+06	8.3E+05
	Initially river bed sediment	MIT037	SD	MIT038	SD	MIT39	SD	MIT052	SD
	Day 0	1.1E+07	1.7E+06	1.1E+07	1.7E+06	1.1E+07	1.7E+06	1.1E+07	1.7E+06
	Day 49	2.2E+07	1.4E+05	5.1E+06	1.6E+06	7.2E+06	7.7E+04	3.1E+07	7.2E+06
	Day 115	2.7E+07	3.5E+04	1.7E+07	5.6E+05	1.5E+07	1.6E+06	1.4E+07	1.0E+06
	Day 263	8.7E+06	1.2E+05	9.6E+06	9.9E+05	8.9E+06	2.9E+06	1.4E+07	1.6E+06
	Day 347	7.7E+06	8.4E+05	5.8E+06	1.5E+05	4.2E+06	1.1E+06	5.1E+06	4.1E+03

III.III Concentrations of ATP, AOC and BCP in water samples

AOC [µg L^{-1}]	Isar	SD	MIT 037	SD	MIT 038	SD	MIT 039	SD	MIT 052	SD
Day 0	4.6	1.5	5.6	1.2	9.3	1.9	6.4	1.8	7.6	9.6
Day 49	4.5	2.3	3.5	0.5	4.9	1.5	2.7	1.0	2.8	2.4
Day 115	2.8	0.1	4.7	1.0	6.1	3.3	6.5	3.0	2.5	0.5
Day 196	4.0	0.7	14.7	3.4	18.4	6.2	9.1	4.1	15.2	0.5
Day 263	5.5	1.0	15.0	4.3	24.5	12.3	8.8	2.0	13.5	5.0
Day 347	5.9	1.4	5.3	1.2	18.1	8.0	7.5	1.1	51.8	18.0
BCP [pmol $L^{-1}h^{-1}$]										
Day 0	321.5	68.3	2.6	1.4	1.6	1.7	0.7	0.6	10.4	2.1
Day 49	283.1	31.2	10.7	1.9	4.6	1.3	3.2	0.4	3.8	1.1
Day 115	40.1	2.1	2.6	0.3	5.2	1.3	13.1	1.1	3.9	0.8
Day 196	14.4	0.7	0.3	0.4	1.3	0.7	2.6	0.7	1.0	0.2
Day 263	90.0	6.5	2.6	0.9	1.2	0.3	0.5	0.4	1.6	1.2
Cellular ATP [M]										
Day 0	3.5E-12	3.9E+02	7.5E-13	2.4E+02	5.8E-13	5.7E+02	5.7E-13	2.8E+02	1.3E-12	1.3E+02
Day 49	9.2E-13	2.1E+03	4.4E-13	3.5E+02	3.5E-13	3.8E+02	4.1E-13	4.8E+02	3.6E-13	2.3E+03
Day 115	2.0E-13	0.0E+00	3.5E-15	0.0E+00	2.6E-13	0.0E+00	9.5E-13	0.0E+00	4.4E-13	0.0E+00
Day 196	5.9E-12	1.5E+02	3.3E-12	6.7E+01	2.3E-12	5.0E+01	7.6E-13	4.5E+01	1.9E-12	2.6E+02
Day 263	2.8E-12	3.1E+02	2.4E-12	1.9E+02	1.5E-13	1.6E+02	3.5E-13	1.2E+02	1.4E-13	1.8E+01
Day 347	7.0E-13	9.5E+02	1.7E-13	1.3E+02	1.6E-13	1.6E+01	8.1E-13	6.1E+01	2.5E-13	3.2E+02

III.IV Mean bacterial Shannon diversity, richness and evenness in water and sediment samples derived by T-RFLP

Water samples

	Shannon	Isar	SD	MIT037	SD	MIT038	SD	MIT039	SD	MIT052	SD
Groundwater	Day 0	3.9	0.0	2.1	0.4	2.4	0.2	2.0	0.0	3.1	0.2
	Day 49	3.7	0.1	2.6	0.1	0.5	0.2	1.2	0.3	2.0	0.1
	Day 115	3.4	0.1	1.6	0.2	1.4	0.3	0.8	0.0	1.4	0.1
	Day 196	3.4	0.3	3.6	0.1	3.1	0.7	3.2	0.3	3.0	0.1
	Day 263	3.8	0.1	3.9	0.0	3.4	0.2	3.7	0.2	3.0	0.2
	Day 347	3.6	0.1	4.0	0.2	2.7	0.0	3.6	0.0	3.3	0.1
	evenness										
	Day 0	0.8	0.0	0.6	0.1	0.7	0.0	0.5	0.0	0.7	0.0
	Day 49	0.9	0.0	0.6	0.0	0.3	0.1	0.4	0.1	0.6	0.0
	Day 115	0.9	0.0	0.7	0.1	0.7	0.1	0.6	0.0	0.7	0.0
	Day 196	0.9	0.0	0.9	0.0	0.8	0.0	0.8	0.1	0.8	0.0
	Day 263	0.9	0.0	0.9	0.0	0.8	0.0	0.9	0.0	0.8	0.0
	Day 347	0.9	0.0	0.9	0.0	0.7	0.0	0.8	0.0	0.8	0.0
	richness										
	Day 0	98.0	1.4	39.5	6.4	41.0	15.6	42.5	0.7	71.5	2.1
	Day 49	63.5	13.4	65.5	3.5	5.5	2.1	17.5	2.1	33.0	9.9
	Day 115	41.5	7.8	12.5	6.4	9.0	5.7	4.0	0.0	8.5	0.7
	Day 196	58.5	17.7	68.0	7.1	53.5	41.7	47.5	0.7	50.0	0.0
	Day 263	76.5	6.4	95.5	13.4	65.0	29.7	70.0	5.7	40.0	9.9
	Day 347	69.5	10.6	72.5	16.3	41.5	2.1	74.0	2.8	72.0	15.6
	Shannon	MIT037	SD	MIT038	SD	MIT039	SD	MIT052	SD		
well water	Day 49	4.3	0.1	3.6	0.1	4.2	0.1	3.6	0.1		
	Day 115	2.9	0.1	3.3	0.6	2.9	0.4	2.4	0.1		
	Day 196	4.0	0.2	3.5	0.3	3.8	0.1	2.6	0.4		
	Day 263	3.9	0.1	2.6	0.2	2.9	0.3	4.1	0.0		
	Day 347	4.0	0.1	3.9	0.1	3.3	0.0	3.7	0.1		
	evenness										
	Day 49	0.9	0.0	0.9	0.0	0.9	0.0	0.9	0.0		
	Day 115	0.8	0.0	0.8	0.1	0.8	0.1	0.7	0.1		
	Day 196	0.9	0.0	0.8	0.1	0.9	0.0	0.7	0.0		
	Day 263	0.9	0.0	0.8	0.0	0.8	0.1	0.9	0.0		
	Day 347	0.9	0.0	0.9	0.0	0.8	0.0	0.9	0.0		
	richness										
	Day 49	110.0	7.1	56.0	1.4	90.5	21.9	59.0	7.1		
	Day 115	40.0	5.7	58.5	17.7	44.0	0.0	28.0	11.3		
	Day 196	76.5	14.8	72.0	0.0	73.5	10.6	33.5	17.7		
	Day 263	82.0	12.7	31.0	11.3	33.5	4.9	87.0	0.0		
	Day 347	69.5	0.7	73.0	12.7	65.0	0.0	65.5	2.1		

Sediment samples

	Shannon	MIT037	SD	MIT038	SD	MIT039	SD	MIT052	SD
Initially sterile sediments	Day 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Day 49	1.9	0.2	2.1	0.1	2.0	0.9	2.5	0.3
	Day 115	3.3	0.3	2.8	0.3	3.2	0.2	2.5	0.2
	Day 263	3.7	0.3	3.9	0.0	3.9	0.3	4.0	0.4
	Day 347	4.2	0.1	4.3	0.7	3.9	0.5	4.3	0.2
	richness								
	Day 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Day 49	25.5	2.1	21.0	5.7	19.5	0.7	44.0	8.5
	Day 115	44.5	12.0	30.5	14.2	37.0	5.7	23.5	5.5
	Day 263	41.0	11.3	44.3	12.1	45.7	7.1	54.3	14.7
	Day 347	79.0	24.0	75.5	26.2	69.5	24.7	85.5	9.0
	evenness								
	Day 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Day 49	0.6	0.1	0.7	0.0	0.7	0.3	0.7	0.0
	Day 115	0.9	0.0	0.8	0.1	0.9	0.0	0.8	0.0
	Day 263	1.0	0.0	1.0	0.1	1.0	0.0	1.0	0.0
	Day 347	1.0	0.1	1.0	0.1	0.9	0.0	1.0	0.0
Initially river bed sediments	**Shannon**	MIT037	SD	MIT038	SD	MIT039	SD	MIT052	SD
	Day 0	5.0	0.1	5.0	0.1	5.0	0.1	5.0	0.1
	Day 49	4.2	0.0	4.8	0.2	3.7	0.5	4.2	0.3
	Day 115	4.0	0.7	4.2	0.2	4.3	0.2	4.2	0.2
	Day 263	4.4	0.1	4.4	0.0	4.6	0.3	4.9	0.0
	Day 347	4.1	0.5	4.6	0.2	4.3	0.1	4.0	0.0
	richness								
	Day 0	111.0	25.5	111.0	25.5	111.0	25.5	111.0	25.5
	Day 49	81.0	17.0	106.0	29.7	65.5	27.6	82.5	16.3
	Day 115	86.0	26.9	74.5	0.7	74.5	9.2	69.5	0.7
	Day 263	76.5	19.1	68.0	7.1	81.0	25.5	101.0	4.2
	Day 347	69.5	29.9	105.0	12.7	79.5	17.7	76.0	11.3
	evenness								
	Day 0	1.1	0.0	1.1	0.0	1.1	0.0	1.1	0.0
	Day 49	1.0	0.1	1.0	0.0	0.9	0.0	1.0	0.1
	Day 115	0.9	0.1	1.0	0.0	1.0	0.0	1.0	0.0
	Day 263	1.0	0.1	1.1	0.0	1.1	0.0	1.1	0.0
	Day 347	1.0	0.0	1.0	0.1	1.0	0.0	0.9	0.0

III.V Affiliated trimmed reads of pyrosequencing data by RDP (sediments)

	MIT039					MIT052			
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
Acidovorax	0.41	5.50	0.00	0.12	Achromobacter	0.00	0.00	0.00	0.03
Acinetobacter	0.01	0.72	0.10	0.46	Aciditerrimonas	0.00	0.00	0.02	0.00
Aerococcus	0.00	0.03	0.00	0.00	Acidovorax	0.47	0.15	0.27	0.13
Aeromicrobium	0.00	0.03	0.00	0.00	Acinetobacter	0.00	0.07	0.14	0.67
Afipia	0.00	0.00	0.02	0.37	Afipia	0.00	0.28	0.17	0.38
Albidiferax	0.41	1.15	0.45	0.02	Agrococcus	0.00	0.00	0.00	0.01
Alkanindiges	0.05	0.03	0.02	0.00	Albidiferax	0.41	0.24	0.54	0.01
Aminobacter	0.13	0.38	0.00	0.00	Alkanindiges	0.00	0.00	0.09	0.00
Anaeromyxobacter	0.00	0.00	0.00	0.02	Aminobacter	0.08	0.00	0.06	0.01
Aquabacterium	1.55	4.46	1.21	21.40	Anaeromyxobacter	0.00	0.00	0.02	0.00
Aquaspirillum	0.00	0.00	0.00	0.03	Aquabacterium	1.02	4.98	2.12	17.40
Aquicella	0.00	0.00	0.00	0.02	Aquaspirillum	0.00	0.00	0.00	0.01
Arenimonas	0.01	0.00	0.00	0.00	Aquicella	0.00	0.02	0.00	0.00
Armatimonadetes_gp5	0.00	0.03	0.18	0.88	Aquimonas	0.00	0.00	0.02	0.00
Arthrobacter	0.05	0.00	0.02	0.07	Arcicella	0.08	0.00	0.00	0.00
Aspromonas	0.00	0.00	0.08	0.00	Armatimonadetes_gp5	0.02	0.02	0.33	1.08
Bacillariophyta	0.00	0.00	0.02	0.00	Arthrobacter	0.06	0.43	0.30	0.04
Bacillus	0.00	0.00	0.06	0.00	Aspromonas	0.00	0.00	0.02	0.00
Bacteriovorax	0.09	11.81	0.35	0.25	Azohydromonas	0.00	0.04	0.02	0.00
Bdellovibrio	0.03	0.16	0.08	0.02	Bacillus	0.00	0.02	0.00	0.00
Blastobacter	0.00	0.03	0.00	0.00	Bacteriovorax	0.06	0.13	0.06	0.21
Bosea	0.04	0.11	0.00	0.02	Bdellovibrio	0.03	0.09	0.03	0.13
Bradyrhizobium	0.00	0.11	0.02	0.35	Bosea	0.03	0.13	0.03	0.00
BRC1_genera_incertae_sedis	0.00	0.00	0.12	0.24	Bradyrhizobium	0.00	0.02	0.09	0.38
Brevundimonas	18.77	5.34	2.20	0.51	BRC1_genera_incertae_sedis	0.00	0.04	0.08	0.08
Caulobacter	0.12	0.40	0.10	0.10	Brevundimonas	1.08	0.61	0.45	0.54
Cellvibrio	0.14	0.19	0.04	0.00	Byssovorax	0.00	0.00	0.02	0.00
Chryseobacterium	0.01	0.00	0.00	0.00	Caldilinea	0.00	0.00	0.05	0.00
Cloacibacterium	0.01	0.08	0.00	0.08	Castellaniella	0.00	0.00	0.00	0.01
Clostridium sensu stricto	0.00	0.00	0.02	0.00	Caulobacter	0.91	0.37	0.23	0.10

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
Clostridium XI	0.00	0.00	0.02	0.00	Cellvibrio	0.03	0.22	0.00	0.00
Comamonas	0.00	0.00	0.02	0.05	Chondromyces	0.00	0.00	0.02	0.00
Cryobacterium	0.00	0.00	0.10	0.03	Chthonomonas/Armatimonadetes_gp3	0.00	0.00	0.02	0.00
Cupriavidus	0.00	0.03	0.00	0.00	Cloacibacterium	0.00	0.02	0.12	0.08
Curvibacter	0.04	0.03	0.47	0.17	Clostridium sensu stricto	0.00	0.00	0.02	0.00
Dechloromonas	0.00	0.11	0.02	0.00	Clostridium XI	0.02	0.00	0.00	0.00
Devosia	0.04	0.00	0.02	0.19	Comamonas	0.00	0.00	0.05	0.17
Diaphorobacter	0.03	0.24	0.23	0.22	Coxiella	0.00	0.00	0.00	0.08
Dongia	0.00	0.00	0.00	0.19	Cryobacterium	0.00	0.00	0.05	0.04
Duganella	0.07	0.03	0.00	0.00	Cupriavidus	0.00	0.00	0.02	0.00
Elizabethkingia	0.00	0.00	0.00	0.03	Curvibacter	0.06	0.20	0.12	0.28
Emticicia	0.03	0.03	0.00	0.02	Dechloromonas	0.09	0.00	0.06	0.00
Enhydrobacter	0.00	0.00	0.00	0.14	Derxia	0.00	0.00	0.02	0.04
Enterococcus	0.00	0.00	0.02	0.00	Devosia	0.16	0.04	0.05	0.14
Ferruginibacter	0.01	0.05	0.27	0.22	Diaphorobacter	0.00	0.15	0.08	0.32
Finegoldia	0.00	0.03	0.00	0.00	Dokdonella	0.00	0.00	0.03	0.00
Flavitalea	0.00	0.00	0.00	0.05	Dongia	0.00	0.02	0.00	0.41
Flavobacterium	8.60	3.30	4.00	0.34	Duganella	0.00	0.04	0.02	0.00
Fluviicola	0.04	0.03	0.04	0.03	Dyadobacter	0.00	0.00	0.03	0.00
Gemmata	0.00	0.00	0.00	0.10	Emticicia	0.02	0.04	0.03	0.01
Gemmatimonas	0.03	0.30	2.94	1.52	Ferruginibacter	0.02	0.33	0.91	0.24
Gemmobacter	0.00	0.00	0.06	0.00	Flavobacterium	42.49	14.01	8.41	0.67
Giesbergeria	0.09	0.11	0.00	0.00	Fluviicola	0.00	0.09	0.02	0.10
Gp16	0.00	0.00	0.04	0.05	Gemella	0.00	0.00	0.00	0.01
Gp17	0.00	0.03	0.21	0.14	Gemmata	0.00	0.09	0.15	0.20
Gp22	0.00	0.00	0.06	0.00	Gemmatimonas	0.16	2.00	1.53	1.68
Gp3	0.00	0.00	0.08	0.00	Gemmobacter	0.00	0.02	0.02	0.00
Gp4	0.01	0.19	1.50	0.57	Gp10	0.00	0.00	0.02	0.00
Gp5	0.00	0.00	0.02	0.00	Gp16	0.00	0.00	0.02	0.03
Gp6	0.00	0.00	1.15	1.49	Gp17	0.00	0.09	0.14	0.27
Gp7	0.00	0.00	0.06	0.15	Gp22	0.00	0.00	0.06	0.03
Haliea	0.00	0.00	0.06	0.02	Gp3	0.00	0.00	0.05	0.00
Haliscomenobacter	0.00	0.05	0.06	0.12	Gp4	0.02	0.87	1.19	0.75
Herbaspirillum	0.14	0.48	0.23	0.02	Gp6	0.02	0.96	1.82	1.80
Herminiimonas	0.26	0.11	0.31	0.00	Gp7	0.00	0.07	0.11	0.01
Hyalangium	0.00	0.00	0.00	0.03	Haliea	0.00	0.07	0.20	0.04
Hydrogenophaga	0.01	4.64	0.39	0.37	Haliscomenobacter	0.03	0.20	0.39	0.11
Hymenobacter	0.65	0.05	0.06	0.00	Herbaspirillum	1.01	0.09	0.30	0.01

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
Hyphomicrobium	0.00	0.00	0.25	0.39	Herminiimonas	0.27	0.30	0.15	0.01
Iamia	0.01	0.00	0.04	0.02	Hyalangium	0.00	0.00	0.00	0.03
Ideonella	0.01	0.00	0.27	0.03	Hydrogenophaga	1.24	0.28	0.29	0.46
Ignavibacterium	0.00	0.00	0.00	0.02	Hymenobacter	0.02	0.00	0.00	0.00
Ilumatobacter	0.01	0.03	0.12	0.03	Hyphomicrobium	0.02	0.00	0.11	0.52
Janthinobacterium	0.46	0.05	0.35	0.19	Iamia	0.00	0.02	0.03	0.00
Kofleria	0.00	0.03	0.10	0.07	Ideonella	0.02	0.02	0.02	0.04
Lacibacter	0.00	0.00	0.14	0.03	Janthinobacterium	1.34	0.33	0.05	0.21
Leadbetterella	0.07	0.03	0.00	0.03	Kofleria	0.00	0.02	0.03	0.03
Legionella	0.00	0.03	0.35	0.07	Lacibacter	0.00	0.07	0.00	0.04
Leifsonia	0.00	0.03	0.00	0.05	Lactococcus	0.00	0.00	0.00	0.06
Leptothrix	0.00	0.00	0.04	0.00	Legionella	0.00	0.07	0.06	0.10
Lysobacter	0.14	0.00	0.12	0.03	Leifsonia	0.00	0.00	0.02	0.00
Massilia	0.95	0.35	0.51	0.24	Leptospira	0.00	0.00	0.02	0.00
Mesorhizobium	0.00	0.00	0.00	0.10	Leptothrix	0.00	0.02	0.00	0.00
Methylobacillus	0.00	0.00	0.00	0.03	Lysobacter	0.05	0.22	0.29	0.08
Methylotenera	0.00	0.00	0.02	0.02	Magnetospirillum	0.00	0.00	0.02	0.00
Microbacterium	0.01	0.00	0.00	0.03	Massilia	9.75	7.20	7.26	0.31
Microcella	0.00	0.00	0.00	0.02	Mesorhizobium	0.00	0.00	0.00	0.07
Micrococcineae	0.12	0.08	0.37	0.35	Methylobacterium	0.00	0.00	0.02	0.00
Micrococcus	0.00	0.00	0.02	0.00	Methylotenera	0.02	0.00	0.03	0.01
Nakamurellaceae	0.00	0.00	0.02	0.00	Microvirga	0.00	0.00	0.02	0.01
Nitrospira	0.00	0.05	0.31	0.14	Mycobacterium	0.00	0.00	0.05	0.00
Nocardia	0.00	0.00	0.02	9.15	Nitrospira	0.00	0.07	0.85	0.36
Nocardioides	0.38	0.19	0.41	0.22	Nocardia	0.00	0.11	1.77	10.2
Novosphingobium	0.00	0.00	0.02	0.00	Nocardioides	0.00	0.04	0.05	0.25
Ochrobactrum	0.00	0.00	0.02	0.00	Novosphingobium	0.00	0.00	0.02	0.00
OD1_genera_incertae_sedis	0.00	0.00	0.02	0.02	OD1_genera_incertae_sedis	0.00	0.17	0.00	0.00
Ohtaekwangia	0.00	0.05	1.60	0.30	Ohtaekwangia	0.02	0.63	0.76	0.29
Opitutus	0.00	0.03	0.16	0.14	Opitutus	0.02	0.43	0.21	0.13
Ornithinibacter	0.01	0.00	0.00	0.00	Ornithinibacter	0.00	0.02	0.00	0.00
Paracoccus	0.00	0.00	0.00	0.03	Paenisporosarcina	0.00	0.00	0.02	0.00
Pasteuria	0.00	0.00	0.02	0.02	Paludibacter	0.00	0.00	0.02	0.00
Paucibacter	0.01	0.16	0.08	0.12	Pasteuria	0.00	0.00	0.08	0.04
Pedobacter	0.16	0.00	0.00	0.03	Paucibacter	0.03	0.02	0.21	0.01

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
Pelomonas	0.00	0.03	0.00	0.00	Pedobacter	0.53	0.00	0.11	0.00
Peptostreptococcus	0.00	0.00	0.02	0.00	Pedomicrobium	0.00	0.02	0.00	0.01
Peredibacter	0.04	0.67	0.00	0.00	Peredibacter	0.03	0.13	0.09	0.00
Perlucidibaca	0.20	0.67	3.04	1.03	Perlucidibaca	0.03	0.80	1.86	1.24
Petrobacter	0.00	0.00	0.00	0.02	Phenylobacterium	0.25	0.35	0.86	0.59
Phenylobacterium	0.67	3.44	1.17	0.56	Phycicoccus	0.00	0.00	0.00	0.03
Phycicoccus	0.00	0.03	0.04	0.00	Phycisphaera	0.02	0.00	0.05	0.07
Phycisphaera	0.00	0.00	0.04	0.08	Planctomyces	0.00	0.00	0.02	0.06
Planctomyces	0.00	0.00	0.00	0.08	Plesiocystis	0.00	0.02	0.00	0.00
Polaromonas	0.54	1.10	1.54	1.01	Polaromonas	0.72	1.89	3.49	1.10
Prosthecobacter	0.00	0.00	0.00	0.25	Propionibacterium	0.00	0.00	0.03	0.01
Pseudomonas	4.50	2.71	4.99	0.66	Prosthecobacter	0.00	0.37	0.09	0.27
Pseudoxanthomonas	0.09	0.00	0.12	0.02	Pseudomonas	4.38	9.90	9.65	1.13
Ralstonia	0.01	0.00	0.02	0.07	Pseudoxanthomonas	0.00	0.04	0.00	0.06
Rhizobacter	0.00	0.03	0.45	1.25	Psychrobacillus	0.00	0.02	0.00	0.00
Rhodobacter	0.00	0.00	0.18	0.03	Ralstonia	0.00	0.00	0.02	0.06
Rhodococcus	0.00	0.40	0.31	0.03	Rhizobacter	0.22	0.91	0.48	1.20
Rhodoferax	0.14	1.50	0.29	0.05	Rhizobium	0.03	0.02	0.00	0.00
Roseomonas	0.00	0.00	0.00	0.02	Rhodobacter	0.14	0.11	0.05	0.06
Rubrivivax	0.00	0.00	0.06	0.17	Rhodococcus	0.00	0.00	0.03	0.01
Rugamonas	1.30	0.21	0.33	0.07	Rhodoferax	0.00	0.00	0.02	0.03
Salinibacterium	0.01	0.03	0.00	0.00	Rhodopirellula	0.00	0.00	0.02	0.00
Schlesneria	0.00	0.00	0.04	0.35	Rubrivivax	0.00	0.00	0.02	0.13
Sediminibacterium	0.08	0.00	0.00	0.00	Rugamonas	0.06	0.07	0.05	0.11
Segetibacter	0.00	0.00	0.20	0.02	Runella	0.02	0.00	0.00	0.00
Singulisphaera	0.00	0.00	0.00	0.02	Saccharofermentans	0.00	0.02	0.00	0.00
Solirubrobacter	0.00	0.00	0.02	0.00	Salinibacterium	0.02	0.00	0.00	0.00
Solirubrobacterales	0.00	0.00	0.31	0.02	Schlesneria	0.00	0.00	0.06	0.60
Sorangium	0.00	0.00	0.02	0.00	Sediminibacterium	0.00	0.02	0.00	0.00
Spartobacteria_genera_incertae_sedis	0.00	0.00	0.00	0.03	Segetibacter	0.00	0.04	0.02	0.01
Sphingobacterium	0.00	0.00	0.00	0.07	Singulisphaera	0.00	0.00	0.02	0.03
Sphingobium	0.09	0.56	0.02	0.19	Spartobacteria_genera incertae sedis	0.00	0.00	0.21	0.07

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
Sphingomonas	1.26	3.01	0.72	1.25	Sphingobium	0.09	0.13	0.02	0.10
Sphingopyxis	0.92	1.26	0.35	0.08	Sphingomonas	0.35	0.37	0.36	1.22
Sphingosinicella	0.00	0.00	0.06	0.00	Sphingopyxis	0.49	0.13	0.05	0.04
Staphylococcus	0.05	0.08	0.00	0.00	Sphingosinicella	0.00	0.00	0.08	0.00
Stenotrophomonas	0.00	0.00	0.00	0.08	Spirosoma	0.00	0.04	0.00	0.00
Streptophyta	0.00	0.03	0.00	0.00	Staphylococcus	0.02	0.00	0.00	0.01
Subdivision3_genera_incertae_sedis	0.00	0.00	0.23	0.17	Stenotrophomonas	0.02	0.00	0.00	0.06
Sulfuritalea	0.00	0.32	0.04	0.81	Steroidobacter	0.00	0.00	0.02	0.03
Terrimonas	0.00	0.21	0.68	0.49	Subdivision3_genera_incertae_sedis	0.02	0.20	0.17	0.14
Thermomonas	0.01	0.00	0.08	0.00	Sulfuricurvum	0.00	0.02	0.00	0.00
TM7_genera_incertae_sedis	0.00	0.00	0.06	0.02	Sulfuritalea	0.02	0.15	0.35	0.77
Turneriella	0.00	0.03	0.16	0.00	Terrimonas	0.00	0.54	0.48	0.50
unclassified_"Acidimicrobineae"	0.00	0.00	0.37	0.05	Tetrasphaera	0.00	0.02	0.00	0.00
unclassified_"Bacteroidetes"	0.11	0.51	2.38	0.44	Thermomonas	0.00	0.02	0.00	0.01
unclassified_"Chloroflexi"	0.00	0.00	0.02	0.00	TM7_genera_incertae_sedis	0.00	0.00	0.03	0.03
unclassified_"Flavobacteriales"	0.12	0.21	0.06	0.14	Turneriella	0.00	0.07	0.05	0.00
unclassified_"Proteobacteria"	28.96	8.80	6.28	2.62	unclassified_"Acidimicrobineae"	0.02	0.04	0.09	0.20
unclassified_"Saprospiraceae"	0.00	0.03	0.02	0.05	unclassified_"Bacteroidetes"	0.11	1.11	1.38	0.36
unclassified_"Sphingobacteriales"	0.00	0.19	0.53	0.34	unclassified_"Chloroflexaceae"	0.00	0.00	0.02	0.00
unclassified_"Verrucomicrobia"	0.00	0.00	0.04	0.00	unclassified_"Flavobacteriales"	0.02	0.24	0.11	0.10
unclassified_Acetobacteraceae	0.00	0.00	0.00	0.12	unclassified_"Proteobacteria"	3.33	6.35	5.13	3.09
unclassified_Acidimicrobiaceae	0.00	0.00	0.02	0.02	unclassified_"Saprospiraceae"	0.00	0.02	0.11	0.00
unclassified_Actinobacteria	0.00	0.05	0.41	0.37	unclassified_"Sphingobacteriales"	0.02	0.20	0.97	0.18
unclassified_Actinomycetales	0.00	0.00	0.10	0.08	unclassified_"Verrucomicrobia"	0.02	0.02	0.08	0.03
unclassified_Alcaligenaceae	0.00	0.00	0.00	0.05	unclassified_Acetobacteraceae	0.00	0.00	0.02	0.27
unclassified_Alphaproteobacteria	0.46	0.67	1.74	2.73	unclassified_Actinobacteria	0.00	1.37	0.42	0.31
unclassified_Anaerolineaceae	0.00	0.00	0.12	0.20	unclassified_Actinomycetales	0.02	0.07	0.09	0.08

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
unclassified_Bacillales	0.00	0.00	0.08	0.00	unclassified_Alcaligenaceae	0.00	0.02	0.00	0.03
unclassified_Bacilli	0.00	0.00	0.00	0.02	unclassified_Alphaproteobacteria	0.28	2.87	2.78	2.36
unclassified_Bacteria	0.29	2.44	12.19	5.27	unclassified_Anaerolineaceae	0.00	0.07	0.64	0.38
unclassified_Beijerinckiaceae	0.00	0.00	0.02	0.00	unclassified_Bacteria	0.58	4.70	6.76	4.71
unclassified_Betaproteobacteria	0.13	0.40	1.19	9.38	unclassified_Beijerinckiaceae	0.02	0.00	0.00	0.00
unclassified_Bradyrhizobiaceae	0.12	0.43	0.10	0.95	unclassified_Betaproteobacteria	0.35	1.44	1.78	9.16
unclassified_Burkholderiales	0.67	1.74	1.72	1.42	unclassified_Bradyrhizobiaceae	0.20	1.11	0.95	0.84
unclassified_Burkholderiales_incertae_sedis	0.65	3.25	1.66	9.03	unclassified_Burkholderiales	0.60	1.22	1.06	0.96
unclassified_Caulobacteraceae	0.28	0.19	0.37	0.00	unclassified_Burkholderiales_incertae_sedis	0.75	4.52	2.90	11.3
unclassified_Chitinophagaceae	0.00	0.16	2.61	0.69	unclassified_Caulobacteraceae	0.28	0.15	0.09	0.00
unclassified_Chloroplast	0.00	0.00	0.00	0.03	unclassified_Chitinophagaceae	0.02	0.67	0.70	0.56
unclassified_Chromatiales	0.00	0.00	0.00	0.02	unclassified_Comamonadaceae	1.18	1.50	0.67	0.42
unclassified_Clostridia	0.00	0.00	0.00	0.02	unclassified_Cryomorphaceae	0.00	0.00	0.02	0.03
unclassified_Clostridiales	0.00	0.00	0.02	0.00	unclassified_Cystobacteraceae	0.00	0.11	0.06	0.14
unclassified_Comamonadaceae	1.54	4.91	1.76	0.73	unclassified_Cystobacterineae	0.00	0.07	0.11	0.18
unclassified_Cryomorphaceae	0.01	0.05	0.00	0.10	unclassified_Cytophagaceae	0.05	0.00	0.00	0.00
unclassified_Cystobacteraceae	0.00	0.03	0.14	0.07	unclassified_Deltaproteobacteria	0.02	0.22	0.12	0.31
unclassified_Cystobacterineae	0.00	0.00	0.02	0.08	unclassified_Enterobacteriaceae	0.00	0.00	0.02	0.01
unclassified_Cytophagaceae	0.00	0.00	0.02	0.00	unclassified_Flavobacteriaceae	0.41	0.09	0.08	0.00
unclassified_Deltaproteobacteria	0.01	0.16	0.72	0.15	unclassified_Gammaproteobacteria	0.17	1.89	3.66	1.33
unclassified_Enterobacteriaceae	0.00	0.00	0.02	0.00	unclassified_Hyphomicrobiaceae	0.00	0.00	0.09	0.03
unclassified_Flavobacteriaceae	0.09	0.13	0.29	0.00	unclassified_Methylophilaceae	0.00	0.00	0.17	0.00
unclassified_Gammaproteobacteria	0.25	0.94	2.61	1.59	unclassified_Microbacteriaceae	0.00	0.11	0.05	0.11

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
unclassified_Hyphomicrobiaceae	0.00	0.00	0.10	0.00	unclassified_Micrococcaceae	0.08	0.07	0.11	0.01
unclassified_Intrasporangiaceae	0.01	0.00	0.02	0.00	unclassified_Micrococcineae	0.00	0.02	0.00	0.00
unclassified_Methylophilaceae	0.00	0.03	0.14	0.02	unclassified_Moraxellaceae	0.00	0.00	0.00	0.15
unclassified_Microbacteriaceae	0.00	0.00	0.10	0.14	unclassified_Myxococcales	0.02	0.30	0.18	0.13
unclassified_Micrococcaceae	0.01	0.00	0.08	0.02	unclassified_Nannocystaceae	0.00	0.07	0.00	0.00
unclassified_Moraxellaceae	0.01	0.00	0.27	0.15	unclassified_Nannocystineae	0.02	0.17	0.08	0.18
unclassified_Myxococcales	0.00	0.11	0.45	0.27	unclassified_Neisseriaceae	0.00	0.00	0.00	0.03
unclassified_Nakamurellaceae	0.00	0.00	0.02	0.00	unclassified_Nitrosomonadaceae	0.00	0.00	0.02	0.00
unclassified_Nannocystaceae	0.00	0.08	0.00	0.03	unclassified_Nocardiaceae	0.00	0.00	0.02	0.10
unclassified_Nannocystineae	0.00	0.00	0.08	0.20	unclassified_Nocardioidaceae	0.00	0.00	0.00	0.01
unclassified_Neisseriaceae	0.00	0.00	0.00	0.10	unclassified_Oxalobacteraceae	19.6	10.0	9.53	1.09
unclassified_Nitrosomonadaceae	0.00	0.00	0.00	0.02	unclassified_Phyllobacteriaceae	0.00	0.00	0.02	0.01
unclassified_Nocardiaceae	0.00	0.00	0.00	0.03	unclassified_Planctomycetaceae	0.00	0.13	0.70	0.77
unclassified_Nocardioidaceae	0.00	0.00	0.02	0.02	unclassified_Polyangiaceae	0.00	0.11	0.23	0.00
unclassified_Oxalobacteraceae	13.4	4.86	8.97	0.95	unclassified_Pseudomonadaceae	0.25	0.35	0.05	0.04
unclassified_Peptostreptococcaceae	0.00	0.00	0.02	0.00	unclassified_Pseudomonadales	0.08	0.61	0.82	0.29
unclassified_Phyllobacteriaceae	0.00	0.00	0.02	0.03	unclassified_Rhizobiaceae	0.00	0.02	0.00	0.00
unclassified_Planctomycetaceae	0.00	0.00	0.33	0.74	unclassified_Rhizobiales	0.60	0.37	1.00	2.81
unclassified_Polyangiaceae	0.03	0.03	0.12	0.00	unclassified_Rhodobacteraceae	0.88	1.54	1.71	1.52
unclassified_Propionibacteriaceae	0.00	0.00	0.00	0.03	unclassified_Rhodocyclaceae	0.14	0.13	0.33	0.89
unclassified_Pseudomonadaceae	0.05	0.03	0.16	0.02	unclassified_Rubrobacteridae	0.00	0.00	0.02	0.00
unclassified_Pseudomonadales	0.07	0.27	0.31	0.62	unclassified_Solirubrobacterales	0.00	0.02	0.08	0.04
unclassified_Rhizobiales	0.12	0.16	0.55	3.16	unclassified_Sorangiineae	0.00	0.09	0.11	0.00
unclassified_Rhodobacteraceae	0.16	0.54	0.92	1.57	unclassified_Spartobacteria	0.00	0.00	0.00	0.03

MIT039					MIT052				
genus	Day 49	Day 115	Day 263	Day 347	genus	Day 49	Day 115	Day 263	Day 347
unclassified_Rhodocyclaceae	0.00	0.38	0.18	1.01	unclassified_Sphingobacteriaceae	0.03	0.04	0.03	0.00
unclassified_Rubrobacteridae	0.00	0.00	0.08	0.00	unclassified_Sphingomonadaceae	0.57	0.78	0.47	1.03
unclassified_Sinobacteraceae	0.01	0.00	0.00	0.00	unclassified_Sphingomonadales	0.05	0.02	0.00	0.06
unclassified_Solirubrobacterales	0.00	0.00	0.29	0.02	unclassified_Spirochaetales	0.00	0.02	0.00	0.00
unclassified_Sorangiineae	0.03	0.03	0.06	0.02	unclassified_Verrucomicrobiaceae	0.00	0.07	0.05	0.00
unclassified_Sphingomonadaceae	1.04	4.75	5.62	0.93	unclassified_Xanthomonadaceae	0.14	0.30	0.41	0.84
unclassified_Sphingomonadales	0.14	0.13	0.06	0.05	unclassified_Xanthomonadales	0.00	0.02	0.00	0.00
unclassified_Veillonellaceae	0.00	0.00	0.00	0.03	Undibacterium	0.19	0.52	0.38	0.49
unclassified_Verrucomicrobiaceae	0.00	0.00	0.04	0.00	Vampirovibrio	0.00	0.00	0.03	0.00
unclassified_Xanthomonadaceae	0.13	0.16	3.69	1.00	Variovorax	0.41	0.26	0.12	0.00
Undibacterium	6.95	5.39	1.56	0.24	Vasilyevaea	0.00	0.00	0.00	0.01
Variovorax	0.04	0.03	0.27	0.00	Verrucomicrobium	0.00	0.02	0.05	0.00
WS3_genera_incertae_sedis	0.00	0.00	0.08	0.00	WS3_genera_incertae_sedis	0.00	0.00	0.08	0.03
Yersinia	0.00	0.00	0.00	0.07	Yersinia	0.00	0.00	0.00	0.11
Zavarzinella	0.00	0.00	0.04	0.02					

AUTHORSHIP CLARIFICATIONS

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

The idea and concept for the experiment was developed by the PhD candidate, Dr. Claudia Kellermann and Dr. Christian Griebler. The collection of samples was managed by the PhD candidate and Dr. Claudia Kellermann. The laboratory work including DNA extraction, cell counting, T-RFLP analysis, cloning and sequencing, the measurement of ATP, AOC and BCP as well as the statistic analysis were conducted by the PhD candidate. The concentration of ions in water samples were measured by colleagues from the central laboratory of the LfU. Together with Dr. Christian Griebler, results were discussed and evaluated. The PhD candidate wrote the entire manuscript which was improved and corrected by Dr. Christian Griebler. Dr. Christine Stummp contributed to the discussion of the hydrological part. The manuscript was published in FEMS Microbiol. Ecol. 2012 Jul; 81(1):230-242.

The colonization patterns of bacterial communities in a pristine aquifer revealed by 454 pyrosequencing

The concept and scientific design was developed by the PhD candidate and Dr. Christian Griebler. The sampling campaign was conducted by the PhD candidate and Dr. Claudia Kellermann. The PhD candidate performed DNA extraction and the preparation of clone libraries. The clone library was sequenced by Mrs. M. Schieweg in the Institute of Experimental Genetics. The statistic analysis and the pyrosequencing data were conducted by the PhD candidate. Dr. Giovanni Pilloni and Dr. Tillmann Lüders helped with an introduction to the statistical analysis. The evaluation of the results was done by the PhD candidate and Dr. Christian Griebler. The PhD candidate wrote the entire manuscript and Dr. Christian Griebler further improved and corrected it. The manuscript will be submitted to *ISME J*.

Microbial food web structure and carbon cycling in a pristine porous aquifer (Mittenwald, Germany)

The concept and research design was developed by the PhD candidate and Dr. Christian Griebler. The sampling was conducted by the PhD candidate and Dr. Claudia Kellermann. The PhD candidate performed modified FISH for eukaryotes counting. Dr. Michael Rothballer from the Research Unit Microbe-Plant Interactions provided an introduction to the method and Dr. Li Deng contributed to the modification of the FISH protocol. The virus counting was performed by the PhD candidate within the group of Dr. Markus G. Weinbauer from the Laboratoire d'Océanographie de Villefranche. Discussed with Dr. Christian Griebler, the calculation of carbon flux was done by the PhD candidate. The PhD candidate wrote the entire manuscript and Dr. Christian Griebler improved and corrected it. The manuscript will be submitted to *FEMS Microbiol. Ecol.*

ACKNOWLEDGMENTS

This PhD work was financially supported by the Helmholtz Zentrum München, in detail, the Institute of groundwater Ecology.

When I arrived in Munich in 2008, I did not know what exactly I am supposed to expect, neither working in microbiology nor living in Germany. Therefore, I especially like to thank my supervisor Dr. Christian Griebler who opened a door to the wonderful world of science for me. I learned a lot with regard to microbial ecology and scientific concepts. He is not only a great scientist but also a mentor. Whenever I was confused and stucked, he was always by my side and offered me help whenever I needed it. Many thanks for that!

I also like to thank Prof. Dr. Rainer Meckenstock, who enabled me to work at the Institute of Groundwater Ecology. I am indebted to him and Dr. Natuschka Lee (Technische Universität München), being part of my PhD thesis committee meeting and for valuable comments and discussion.

Many appreciations to Dr. Claudia Kellermann, who organized all the sampling campaigns and introduced me to laboratory work. Her scientific contribution greatly inspired my work.

I like to thank Dr. Tillmann Lüders and Dr. Giovanni Pilloni for introducing me to the molecular methods and related data analysis. Many thanks to (soon Dr.) Frederick von Netzer for helping me with the ARB software. I also want to thank Katrin Hörmann, who helped me a lot finding through protocols and technical guidelines when I started my molecular lab work.

Thanks to Dr. Anne Bayer introducing me to bacterial counting and ATP measurements. Also thanks go to (soon Dr.) Agnieszka Herzyk for her helpful scientific discussions and continuous support during my PhD work. I am obliged to Dr. Li Deng for her scientific suggestions on protozoa and viruses and introducing me to the BLAST analysis.

I like to thank Dr. Markus G. Weinbauer from the Laboratoire d'Océanographie de Villefranche for introducing me to the method of counting viruses and Dr. Michael Rothballer (Research Unit Microbe-Plant Interactions) for introducing me to the fluorescence in situ hybridization (FISH).

Micheal Stöckl kindly introduced me to DOC analysis and Harald Lowag to stable isotope measurements. Sigrid Kaschuba kindly provide technical support.

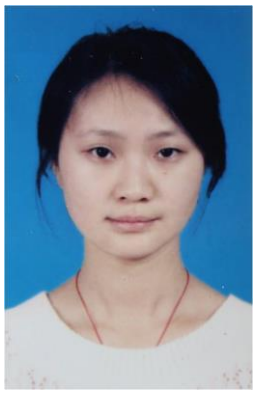
I want to thank my office roommates Agnieszka, Micheal and Gabriele for the nice and

relax working atmosphere. Same appreciation to Housna and Sviatlana. Their support really helped me out when I was upset.

Thanks the people from the KEW Karwendel Energie & Wasser GmbH in Mittenwald, especially M. Pöll and J. Gschwendtner. E. Eichenseher from the Wasserwirtschaftsamt Weilheim and M. Gierig from The Landesamt für Umwelt (LfU) Bayern in Wielenbach are acknowledged for support in organizing the Mittenwald project. Thanks go to M. Schmid and colleagues from the central laboratory of the LfU who helped with sample analysis.

Last not least, I would like to thank my family for their continuous support and encouragement all time long.

LEBENSLAUF



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Publikationen

1: **Y. Zhou**, C. Kellermann, C. Griebler. Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. FEMS Microbiol. Ecol. 2012 Jul; 81(1):230-242.

2: **Y. Zhou,** M.G. Weinbauer, L. Deng, C. Kellermann, C. Griebler. Microbial food web structure and carbon cycling in a pristine porous aquifer (Mittenwald, Germany). FEMS Microbiol. Ecol. In preparation.

3: **Y. Zhou,** T. Lueders, C. Kellermann, C. Griebler. The colonization patterns of bacterial communities in a pristine aquifer revealed by 454 pyrosequencing. *ISME J.* In preparation.

4: A. Herzyk, S. Qiu, **Y. Zhou**, M. Elsner, C. Griebler. Microbial indicators of system's response to organic contamination - resistance, resilience and evaluation. Submitted to *Ecological Indicators J*.

5: C. Lin, M.C. He, **Y. Zhou**, L.J Hu, W. Guo, X.C. Quan, Z.F. Yang. Mercury contamination and dynamics in the sediment of the Second Songhua River, China. Soil & Sediment Contamination. 2007, 16 (4): 397-411.