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Highlights

- Bacterial communities of cold drinking water showed a distinct seasonal cycle
- The hot water bacterial communities were formed within a few hours
- Hot water showed a highly different and less diverse bacterial community
- Rapid growth of thermophilic bacteria in hot water was indicated by a high RNA content
- Low abundant members of cold water functioned as a source for the hot water community

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**Seasonal dynamics of bacterial community structure and composition
in cold and hot drinking water derived from surface water reservoirs**

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Abstract

In temperate regions, seasonal variability of environmental factors affects the bacterial community in source water and finished drinking water. Therefore, the bacterial core community and its seasonal variability in cold and the respective hot drinking water was investigated. The bacterial core community was studied by 16S rRNA-based SSCP fingerprint analyses and band sequencing of DNA and RNA extracts of cold and the hot water (60°C). The bacterial communities of cold and hot drinking water showed a highly different structure and phylogenetic composition both for RNA and DNA extracts. For cold drinking water substantial seasonal dynamics of the bacterial community was observed related to environmental factors such as temperature and precipitation affecting source and drinking water. Phylogenetic analyses of the cold water community indicated that the majority of phylotypes were very closely affiliated with those detected in former studies of the same drinking water supply system (DWSS) in the preceeding 6 years, indicating a high stability over time. The hot water community was very stable over time and seasons and highly distinct from the cold water with respect to structure and composition. The hot water community displayed a lower diversity and its phylotypes were mostly affiliated with bacteria of high temperature habitats with high growth rates indicated by their high RNA content. The conversion of the cold to the hot water bacterial community is considered as occurring within a few hours by the following two processes, i) by decay of most of the cold water bacteria due to heating, and ii) rapid growth of the high temperature adapted bacteria present in the hot water (co-heated with the cold water in the same device) using the nutrients released from the decaying cold water bacteria. The high temperature adapted bacteria originated partially from low abundant but beforehand detected members of the cold water; additionally, the rare members ("seed bank") of the cold water are considered as a source.

Key words: bacterial community, SSCP fingerprints, 16S rRNA, community dynamics, seasonal variation, domestic hot water

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- The hot water bacterial communities were formed within a few hours
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1. Introduction

In temperate regions, reservoir based drinking water can be manifold impacted by seasonally changing environmental factors. The major impact can be considered as on the source water itself. Since it has been demonstrated previously that a fraction of the source water bacteria were transferred to drinking water despite water treatment, the seasonal changes of the bacterial community in the reservoir water can be expected to be partially reflected in the bacterial community of the finished drinking water (Eichler et al. 2006). Also, the seasonally variable availability of nutrients in the reservoir water may result in a different regrowth in the finished drinking water. In addition, temperature changes in the drinking water distribution system (DWDS) may influence directly the bacteria in the drinking water.

Here, we present a study investigating the seasonal dynamics of the bacterial core community in samples representative for the drinking water of the city of Braunschweig, Germany, supplied by two surface water reservoirs (Henne et al. 2012). The raw water is abstracted from the hypolimnion of the reservoirs and treated by sand filtration and chlorination as described elsewhere in more detail (Eichler et al., 2006). In addition to the cold drinking water, the seasonal variability of the respective hot drinking water, i.e. produced with the cold water, was investigated. A study comprising all seasons provides a more comprehensive picture of the bacterial communities, i.e. taking into account seasonal variabilities with respect to abundance and occurrence of bacterial species. Both cold and hot water bacterial communities are of relevance for health issues (Vital et al. 2007, Brettar and Höfle, 2008). Hot water can be of special relevance with respect to pathogenic species and it has been shown that its bacterial community has a strong impact on growth and survival of *Legionella pneumophila* (Wadowsky et al. 1985; Vervaeren et al.; 2006; Farhat et al., 2012).

Bacterial communities in drinking water have been studied, mostly with molecular methods to identify the bacteria at the phylum or class level (LeChevallier et al., 1980; Berry and Raskin, 2006; Burtscher et al. 2009). In these studies, the most abundant bacteria

1 belonged to gram-negative bacteria such as *Alpha*-, *Beta*- and *Gammaproteobacteria* and
2 *Bacteroidetes*, but also high numbers of gram-positive bacteria like *Actinobacteria* were
3 observed. Only few studies included not only DNA-based but also RNA-based techniques
4 (Eichler et al., 2006, Revetta et al., 2010). The use of RNA-based techniques enabled to detect
5 additional phyla such as *Planctomycetes*, *Cyanobacteria*, *Acidobacteria*, and *Nitrospira*
6 (Eichler et al., 2006). In addition, comparison of 16S rRNA-based and 16S rRNA gene-based
7 fingerprints allows gaining insights into the activity of single phylotypes (Brettar et al., 2012;
8 Brettar and Höfle, 2008, Höfle et al., 2008) and addressing aspects of regrowth in drinking
9 water, of special relevance also for hot water.

10 Although hot drinking water is a highly used consumer good, little is known about the its
11 bacterial community and comprehensive studies on bacterial community structure and
12 composition in hot drinking water supplies are still missing. Only few studies on domestic hot
13 water system were done, and most of them were concentrating on potential human pathogens
14 such as *Legionella* (Leoni et al., 2005; Kahlisch et al., 2010) . In the early 1970s Brock and
15 Boylen (1973) observed thermophilic bacteria with similarities to bacteria belonging to genus
16 *Thermus* in laundry and domestic hot-water heaters, confirmed by later studies (Pask-Hughes
17 and Williams, 1975). In a study based on heterotrophic plate counts (HPC) and total bacterial
18 counts, it was shown, that the number of bacteria in hot water may be even higher than in the
19 respective cold drinking water (Bagh et al., 2004).

20 Seasonal dynamics of bacterial communities in pelagic habitats of lakes and oceans have
21 been studied thoroughly and revealed a tight coupling of environmental parameters with the
22 bacterial community composition (Höfle et al., 1999; Giovannoni and Vergin, 2012). By
23 contrast, studies on the seasonality of drinking water communities are rare and restricted to
24 cultivation based techniques (Mazari-Hiriart et al., 2005) or on total bacterial biomass
25 (Amblard et al., 1996; Niquette et al., 2001). The study of seasonal dynamics of drinking
26 water bacterial communities is of high relevance because of the partial transfer of the bacterial

community of source water to the drinking water as demonstrated by Eichler et al. (2006). The seasonal dynamics of the bacterial community in the reservoir can be expected to vary as a response to season-wise changing environmental factors such as light, temperature, and precipitation (Hullar et al., 2006; Shade et al., 2008). Besides the seasonal impact on the reservoir bacteria, temperature and nutrients in drinking water distribution systems show seasonal oscillation impacting directly the bacterial community of the drinking water (Miettinen et al., 1997). Niquette et al. (2001) reported a major impact of water temperature above 15°C on the activity of suspended bacteria. But also a high impact of precipitation on the microbial community in drinking water reservoirs is described, especially after heavy rain events (Niquette et al., 2001; Kistemann et al., 2002). However, no comprehensive study of the seasonal dynamics of cold and hot drinking water community dynamics using molecular detection methods providing an assessment of the activity and regrowth potential of phylotypes is known to the authors until now.

Therefore, the present study focused on the seasonal variability of the bacterial community in hot and cold drinking water; this was achieved by analysing DNA and RNA extracts using 16S rRNA-based fingerprinting techniques followed by band sequencing and phylogenetic analyses. The study aims at contributing to the understanding of the following aspects: i) the influence of season-wise relevant environmental factors on the bacterial drinking water microflora, ii) the differences of the community structure, composition and activity between hot water and cold water bacteria, and (iii) the “transformation” of the cold water to the hot water bacterial community. Besides providing insight on seasonal dynamics and its driving environmental factors, a seasonal study provides also a more solid data basis for the study of the interrelationship between the bacterial communities of cold and hot water.

2. Materials and methods

2.1. Study sites and sampling

Cold drinking water was sampled monthly from May 2008 to October 2009. It was taken from the tap of laboratory D0.04 on the campus of the Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany, with five minute flushing to prevent stagnant water to be sampled. As shown in previous studies (Eichler et al. 2006, Henne et al. 2012), the drinking water sampled at the HZI is representative for the whole drinking water supply system of the city of Braunschweig with respect to the composition of the bacterial community. This drinking water originates from two surface water reservoirs (Grane reservoir, oligotrophic water, 51° 45' 44" N, 10° 22' 38" E), and Ecker reservoir, dystrophic water, 51° 50' 27" N, 10° 34' 45" E) situated in the Harz Mountains 40 km south of Braunschweig. Processing of the drinking water by the local supplier Harzwasserwerke GmbH included flocculation/coagulation, sand filtration and chlorination (0.2 - 0.7 mg l⁻¹). More details on the respective drinking water supply system are given elsewhere (Eichler et al., 2006). Hot drinking water was sampled in May 2008, and regular monthly sampling was done from September 2008 to October 2009. It was taken from a shower next to lab D0.04 also with several minutes flushing. The hot drinking water was made from the cold drinking water on the HZI campus by heating it up to 63°C (range 60-63°C) in two 600 liter heating vessels. The cold water supply for hot water generation is about 6 m³ per day, with the main turn over during working hours. The hot water at the HZI campus was transported in three circular supply systems pumped at a constant speed (approx. 2 m³/h for the cycling in the sampled building D, approx. 5 m³/hr for the total of 3 circular supply systems; after return from the circular systems the hot water had a decreased temperature of approx. 50°C) and repassed subsequently the two heating vessels. The cold drinking water was heated in the heating vessels together with the hot water of the circular systems at variable mixing ratios from approx.1:1 to 1:10 (cold :hot water) during work hours between 7 a.m. and 5 p.m. The

highest percentage of cold drinking water is added at noon time; very low cold water introduction was always recorded after work hours (< 10% of the daily use on work days).

Drinking water microorganisms from cold and hot drinking water were sampled by filtration according to Eichler et al., (2006). For each sampling date, three replicate samples were taken from hot and cold water, respectively. Per sample, 5 litres of drinking water were filtered through a filter sandwich consisting of a 0.2 µm pore size polycarbonate filter (90 mm diameter; Nucleopore; Whatman, Maidstone, United Kingdom) with a precombusted glass fiber filter on top (90 mm diameter; GF/F; Whatman). Biomass harvested on filter sandwiches was stored at -70°C until further analysis. for all samplings, total bacterial counts were determined as well as other relevant drinking water parameters, such as pH, conductivity, temperature, and chlorine concentration.

For total bacterial cell counts, formaldehyde-fixed samples (2% final concentration) of drinking water were stained with Sybr Green I dye (1:10000 final dilution; Molecular Probes, Invitrogen) and analysed directly with epifluorescence microscopy or stored frozen (-20°C) until examination as described in more detail by Henne et al. (2012).

2.2. Nucleic acid extraction and 16S rRNA and 16S rRNA gene based community fingerprints

DNA and RNA of the cold and hot water samples were extracted from the filter sandwiches. For extraction of DNA and RNA, a modified DNeasy/RNeasy protocol (Qiagen, Hilden, Germany) of the sandwich filters was used as described in more detail by Henne et al. 2012. The nucleic acids were quantified using Ribogreen (RNA or ssDNA quantification, Molecular Probes; Invitrogen) or Picogreen (dsDNA quantification, Molecular Probes; Invitrogen) according to the manufactures' protocol.

PCR amplification of 16S rRNA genes from the extracted nucleic acids was performed using previously described primers COM1F (5'-CAGCAGCCGCGGTAATAC-3') and

COM2R (5'-CCGTCAATTCCTTTGAGTTT-3'), amplifying positions 519 to 926 of the *Escherichia coli* numbering of the 16S rRNA gene (Schwieger and Tebbe, 1998). For the preparation of ssDNA and community fingerprints, the protocol described by Henne et al. (2012) was followed.

For evaluation of the SSCP fingerprint pattern, dried SSCP gels were digitized using an Epson Expression 1600 Pro scanner; bands with an intensity of >0.1% of the total lane were considered for further statistical analysis. Similarity coefficients were calculated using Dice algorithm. Dendrograms were constructed with the Neighbor-Joining algorithm using the GelCompare II software (Applied Maths, Kortrijk, Belgium). Community indices were calculated using the software Primer 6 (PRIMER-E Ltd, Ivybridge, UK). Replicate fingerprint analyses had a high comparability for the overall community as well as for the single phylotypes, also shown in an earlier comparison with real-time PCR (Brettar et al. 2006).

2.3. Sequencing and identification of 16S rRNA genes

Sequence information from the single bands of the SSCP fingerprints was obtained following the protocol of Eichler et al. (2006). Briefly, ssDNA bands were excised from the SSCP acrylamide gels, and boiled in extraction buffer (10 mM Tris-HCl, 5 mM MgCl₂, 5 mM KCl, 0.1% Triton X-100, pH 9). 7 µl of the extraction solution was used in a reamplification PCR with the unbiotinylated COM primers described above. These amplicons were purified (MinElute kit, Qiagen, Hilden, Germany) and subsequently sequenced by cycle sequencing (ABI Prism BigDye terminator cycle sequencing ready reaction kit; Applied Biosystems, Foster City, CA). Before analysis on an ABI Prism 3100 Genetic Analyzer, the products were purified using the BigDye Terminator purification kit (Qiagen). The phylogenetic identification of the sequences was done either by the NCBI Tool BLAST/blastn (Alschul et al., 1990) for comparison with the closest 16S rRNA gene sequence and for the identification of the closest described relative or the Ribosomal Data Base Project Seqmatch

and Classifier tool (Cole et al., 2009) for determination of corresponding taxonomic groups (NCBI Blast, 25 Sept 2012). The partial 16S rRNA gene sequences retrieved from the fingerprints are accessible at the GenBank/EMBL/DDBJ accession numbers and listed in Table 3 and Supplementary Table 1).

2.4. Meteorological Data

Meteorological data were obtained of the city of Clausthal-Zellerfeld from the Institute for Electrical Information Technology at the Clausthal University of Technology in Clausthal-Zellerfeld, Germany. Data of Clausthal-Zellerfeld in the West Harz are representative for the catchment area of the surface water reservoirs providing the studied drinking water, i. e. the Grane and the Ecker reservoirs (Eichler et al., 2006).

3. Results and Discussion

3.1. General characteristics of the drinking water

Cold drinking water was sampled monthly from May 2008 to October 2009. The mean temperature of the cold water was 12.5°C and varied, depending on the season, between 7.6°C in January and 16.9°C in September (Fig. 1 a). The pH of the cold drinking water varied between 8.2 and 8.8 with a mean of 8.5. During the sampling period no chlorine was detected (detection limit of 0.1 mg/l) and conductivity had a mean of 142.8 ± 7.5 µS/cm. The hot drinking water was heated to 60 - 63°C, entered the circular system with 60°C and the temperature fluctuated at the sampling point between 49.2°C and 57.9°C with an annual mean of 53.1°C; the pH varied between 7.9 and 8.5 (mean of 8.3); the mean conductivity was 146.4 ± 8.3 µS/cm.

The number of total bacterial counts of the cold drinking water varied between 0.78×10^8 and 4.81×10^8 cells/l with a mean of 2.41×10^8 cells/l showing no clear seasonal trend, i. e. no

maxima or minima with respect to seasons (Fig.1b). The dynamics of the hot water bacterial counts seemed to follow the cold water dynamics on a lower level until June 2009 with lower variability and a mean value of 1.93×10^8 bacterial cells/l. The number of bacteria measured in the hot drinking water was about 20% lower than in cold unheated drinking water (Fig.1b).

To shed light on the impact of seasonal environmental factors on the drinking water microflora, meteorological data (temperature, precipitation) of a meteorological station representative for the catchment of the reservoirs (Clausthal-Zellerfeld) and drinking water temperature were compared with microbiological observations in the drinking water. At first, a potential influence of the environmental parameters on the bacterial total cell counts was considered. Fig. 1 a shows the aerial temperature regime that can be considered as typical for the catchment area (maximal monthly mean aerial temperatures in August 2008 / 2009 with 16.0°C , minimum in January 2009 with -1.8°C). Also, the temperature of the drinking water showed a typical seasonal fluctuation. However, there was no recognizable relationship of these temperature regimes with the observed bacterial numbers in drinking water (Fig. 1b).

The precipitation record showed high monthly precipitation in June / August 2008, February / March 2009, June 2009 and October 2009 with volumes of 100 l/m^2 and above. Correlation analysis of the monthly mean of bacterial cell counts versus the monthly precipitation resulted in a rather low Pearson correlation coefficient of -0.724 (data not shown). Since the precipitation regime may cause changes of water parameters, the monthly precipitation was put into relation to the monthly change of bacterial counts in cold drinking water (equivalent to the first derivative with respect to time, $\Delta(\text{direct counts}) / \Delta(t)$) (Fig. 1 c). The curve of the monthly change in bacterial counts showed a clear inverse trend compared to the curve of the monthly precipitation in the catchment area of the reservoirs. A correlation analysis revealed a strong negative correlation between the precipitation and the change in bacterial counts in cold drinking water with a Pearson correlation coefficient of -0.801 (Fig. 1 d, line 1). In this correlation, all four outliers in the change of bacterial counts were in the

months with the highest drinking water temperature (around 15°C in August and September in both years). Exclusion of these four outliers improved the correlation coefficient (r_2 -0.918) (Fig. 1d, line 2). Niquette et al. (2001) reported a higher bacterial activity and growth, when the drinking water temperature exceeded 15°C. Therefore, the drinking water temperature is considered also for this study as an environmental factor influencing the bacterial density in addition to the precipitation regime.

The strong negative correlation between the change in bacterial counts of cold drinking water and the precipitation regime is interpreted as a dilution effect, i.e. due to increased precipitation the water in the reservoir was diluted and the number of bacteria decreased what was presumably reflected in the reduced bacterial numbers of cold drinking water. This dilution effect seems to be in contrast to other studies, where bacterial blooms in the reservoir were observed after heavy rain events (Kistemann et al., 2002). The increased amount of bacteria after heavy rain events was explained by run off causing an inflow of organic and inorganic nutrients into the reservoir system and a subsequent growth of bacteria due to higher concentration of nutrients. In our study we did not correlate the change of bacterial numbers with short term heavy rain events, but with the total volume of precipitation per month. In most cases, the precipitation was spread over the month without high precipitation events; thus, we assume a more steady inflow of water into the reservoirs leading to a dilution effect that is finally transferred to the resulting drinking water.

3.2. Seasonal dynamics of the community structure in cold drinking water

16S rRNA (based on extracted RNA) and 16S rRNA gene-based (based on extracted DNA) SSCP fingerprints of samples taken in monthly intervals were used to analyse the community dynamics of cold drinking water. According to culture based and environmental observations, the DNA-based analyses is considered as representing the present bacterial community; the RNA-based analyses represents bacteria with a high RNA content that

usually can be considered as “active”, i.e. actively growing and/or actively metabolising (Brettar et al. 2012).

DNA-based fingerprints showed some constant bands and some with changing intensity over time, and only a few suddenly appearing or vanishing bands (Fig. 2 a). Comparative cluster analysis of the DNA-based fingerprints reflected different subclusters for different seasons, i. e. a large cluster for the warmer part of the year, comprising summer and autumn (May to October) 2008 and 2009, and a large cluster for the colder part of the year divided in two subcluster for winter (November to January) and spring (February to May) (Fig. 2 b). The 16S rRNA-based fingerprints showed some constant bands and many with changing intensity over time, and several suddenly appearing or vanishing bands (Fig. 2 c). Again, two large subclusters were obtained with one large subcluster comprising subclusters for summer/autumn 2008 and winter 2008/2009, and a second large subcluster comprising subclusters for summer/autumn 2008/2009, spring 2008, and spring/summer 2009 (Fig. 2 d). Transition phases were observed between subclusters in June/July 2008, September/October 2008, January/February 2009, and June/July 2009 (indicated by arrows in Fig. 2 c). Thus, clusters obtained for the RNA-based fingerprint pattern was by far less apparent than for DNA-based analyses.

DNA- and RNA-based fingerprints of the cold drinking water were substantially different. We concluded that those bacteria which had a high abundance were not necessarily the “active” ones. In summary, the fingerprint patterns were more stable over the year for DNA than for RNA extracts. Changes of the present bacteria were moderate and slightly, while changes of the “active” bacteria could be abrupt and intense. In both, DNA-based as well as in RNA-based fingerprints, the summer clusters of 2008 and 2009 showed similarities to each other indicating seasonal reoccurrence for present and active bacteria. While the DNA-based cluster analysis distinguishes the cold and the warm season and could be explained by seasonally variable environmental parameters such as temperature, the pattern of RNA-based

analyses was more complex. Interestingly, the transition phases of the RNA-based fingerprints occurred simultaneously to local minima in the curve of the monthly change of direct counts coinciding with months with high precipitation (above 100 l/m²) (Fig. 1 c). Time periods spanned by subclusters of the RNA-based fingerprints were consistent with time periods between months with high precipitation: e.g. between two periods of strong precipitation in February/March 2009 and in August 2009 we found a subcluster (Fig. 1a, 2 c, transition phases are indicated by small arrows in both figures). A month of high precipitation and thereby changed environmental parameters may have had a strong impact on the activity of bacteria in the deep water of the reservoir and consecutively in the drinking water; while the DNA-based community structure was not affected (Fig. 2 a).

Compared with former studies of the drinking water of the Harzwasserwerke, a good similarity of both DNA- and RNA-based fingerprints over the studied six years period was observed (Eichler et al., 2006, Henne et al., 2012, Kahlisch et al., 2012). We conclude that, though a seasonal variation could be observed that is more pronounced on the RNA level, the similarity of the fingerprint pattern indicates a high stability of the cold drinking water community structure over the preceeding six years.

3.3. Seasonal dynamics of the community structure in hot drinking water

For hot drinking water, the DNA-based fingerprints showed many constant bands, a few with changing intensity over time, and only very few suddenly appearing or vanishing bands (Fig. 3 a). Comparative cluster analysis revealed three seasonal subclusters (winter/spring 2009, summer 2009, and autumn 2008) with similarities to each other varying between 25% and 52% (Fig. 3 b). RNA-based fingerprints showed many constant bands and some with a changing intensity over time, and few suddenly appearing or vanishing bands (Fig. 3 c). As in DNA-based fingerprints, the same three seasonal subclusters were detected (Fig. 3d) with similarities between 22% and 62%. In hot water, RNA-based fingerprints showed very similar

banding pattern to those of DNA-based fingerprints. In both types of fingerprints, five major constant bands were observed (Fig. 3 a/c, indicated by arrows). These bands occurred in RNA and DNA-based fingerprints and were most pronounced in samples taken after a pressure loss event in the hot water supply system (Fig. 3a,c). For all samples, RNA-based fingerprints showed higher intensities of these five bands.

Seasonal variability of the bacterial communities in hot drinking water was rather low because the banding pattern in DNA- and RNA-based fingerprints did not vary much over time. Nevertheless, for both kinds of fingerprints the same seasonal cluster were observed indicating a weak seasonal variation. More important was the rather similar banding patterns between DNA- and RNA-based fingerprints presumably indicating that the present bacteria were also active, an issue addressed below in more detail in section 3.6.

3.4. Comparison of community structure of cold and hot drinking water

The community structure of cold and hot drinking water differed substantially in many respects. A major difference is the similarity of the fingerprint pattern for RNA and DNA-based analyses for hot water compared to cold water (Fig. 2 a, c and 3 a, c) indicating a higher similarity for “active” and present bacteria in hot water.

Differences of the DNA-based community structures in cold and hot water were well reflected by rank abundance plots of the respective communities (Fig.4). We used relative band intensities from the fingerprints to assess the relative abundance of bands or phylotypes. To obtain comparable datasets we applied a cut-off of 0.1% relative abundance, as this is the detection limit for reliable estimates of relative abundances for SSCP fingerprints. Regression analysis of these abundance data resulted in different rank-abundance curves for cold and hot drinking water. For hot water, the slope of the exponential regression was steeper and the detection limit was reached at lower ranks than in cold water (mean rank representing the average richness of the community: 55 for cold, 40 for hot water) indicating that the mean

richness was markedly higher for cold than for hot water. The abundance of the most abundant phylotype varied from 7.4% to 16.2% (mean 10.0%) in cold water, to 20.9% to 38.3% (mean 28.6%) in hot water, respectively. In general, these differences in diversity measures were strong indicators for a markedly different bacterial community structure in cold and hot drinking water. Substantial differences in the community structure of cold and hot drinking water were already indicated by earlier studies on domestic water that showed a high discrepancy (by a factor of 20) for the ratio of HPC vs. total counts (Bagh et al., 2004).

Seasonal dynamics was more pronounced for cold water than for hot water. Though seasonal clusters were detected for both, a higher seasonal impact on cold water was reflected by much more pronounced changes of the fingerprints especially for the RNA-based analyses.

3.5. Taxonomic composition of the cold drinking water community

Excising and sequencing of the major SSCP-bands (approximately all bands above 0.1% relative band intensity) of cold drinking water fingerprints and subsequent alignment of the obtained sequences resulted in a set of 40 distinct phylotypes (Table SM1). 22 phylotypes were obtained from DNA-based, and 25 phylotypes from RNA-based fingerprints, with 7 identical phylotypes among DNA-based and RNA-based sequences. With the about 400nt long sequences we could resolve up to the species level. The phylogenetic identification of obtained phylotypes is summarized in Table 1. Most of the detected phylotypes were very closely affiliated with phylotypes of former studies of this DWDS (Eichler et al. 2006, Kahlisch et al. 2012, Henne et al. 2012). Only 6 of the 40 phylotypes retrieved in this study can be considered as “new” for this DWSS, with all of them being most closely affiliated with environmental bacterial sequences of aquatic origin.

The most numerous phylotypes belonged to the *Actinobacteria* phylum (9 phylotypes (PTs)) with 4 phylotypes in each DNA and RNA-based fingerprints, and one in both types of fingerprints. *Bacteroidetes* had 7 phylotypes with four found in both, DNA and RNA-based

fingerprints. Only *Actinobacteria*, *Alphaproteobacteria* (6 PTs) and *Betaproteobacteria* (7 PTs, 16%) had at least one phylotype in common between DNA-based and RNA-based fingerprints. Other observed phylotypes were *Gammaproteobacteria* (4 PTs), *Cyanobacteria* (3 PTs), *Planctomycetes* (2 PTs) and *Firmicutes* (1 PT, 2%). According to the former studies this phylotype composition can be regarded as typical for this DWSS.

To obtain an overview on the seasonal dynamics of the phyla and phylotypes, the relative abundances of the single phylotypes were plotted against the sampling period. The relative abundance of the phylotypes was estimated by their relative band intensity compared to all bacteria of the respective sample. For the DNA-based fingerprints (Fig. 5 a) two main phases of the bacterial community composition could be distinguished, i.e. winter and summer (Fig. 2 b, Fig. 5 a). The winter phase (October 2008 to May 2009) was characterised by an increased abundance of *Alphaproteobacteria* (25.4%) and *Bacteroidetes* (27.2%). The summer phase (ended in September 2008, begin in June 2009) was characterized by an increased abundance of *Actinobacteria* (24.5%) and *Gammaproteobacteria* (9.6%). During the whole sampling period, the abundances of *Betaproteobacteria* (12.0%), *Cyanobacteria* (0.6%) and *Planctomycetes* (1.0%) remained rather constant.

The 16S rRNA abundance plot of the RNA-based fingerprints showed an entirely different picture for the seasonal dynamics (Fig. 5 b). At least three clearly separated main phases could be identified with elevated rRNA abundances of only a single phylotype. The beginning and ending of each phase coincided with the transition phases between the seasonal subclusters (Fig. 2 d, Fig. 5 b). All three phases started with periods of heavy rain coinciding with a decrease of the bacterial numbers. The first phase in July to October 2008 was characterized by an increased abundance of the gammaproteobacterium C11R (42.3%). The second phase (November 2008 - February 2009) was characterized by an increased abundance of the cyanobacterium C13R (42.3%). The third phase (February/March 2009 - June 2009) was characterized by an increased abundance of *Betaproteobacteria* (27.8%, phylotypes

1 C01R, C08R) and *Actinobacteria* (2.3%). The ending of the third phase again coincided with
2 a high monthly precipitation in July 2009. During the whole sampling period, the rRNA
3 abundances of *Planctomycetes* (1.7%), *Bacteroidetes* (1.5%) and *Alphaproteobacteria*
4 (15.8%) remained rather constant.

5 Based on the hypothesis, that DNA-based fingerprints represent the “present” bacteria,
6 while the RNA-based ones represent the “active” bacteria, we thus had observed a different
7 seasonal pattern for active and present bacteria (Prosser et al., 2010, Brettar et al., 2012).
8 Summer and winter communities were observed for the “present” bacterial community whose
9 change coincided with mixing periods in the dimictic reservoirs. The changes of
10 environmental conditions such as temperature, nutrients and carbon availability through
11 mixing induce a remarkable shift in the bacterial community of the reservoir which were
12 considered as have been detected in the drinking water community. Another well known
13 effect occurring in freshwater ecosystems in summer was also observed in the cold drinking
14 water: the amount of *Actinobacteria* doubled in the summer phase compared to the winter
15 phase. This effect was explained by the small, grazing resistant size of *Actinobacteria*, which
16 allows for better carbon competition during low primary production (Hahn and Höfle, 2001;
17 Jezbera et al., 2006). For the “active” bacterial community, the community shifts correlated
18 with periods of heavy rains. Possibly, due to environmental impacts related to the high
19 precipitation periods, environmental conditions changed to a major extent and led to a change
20 or partial decay of the bacterial community coinciding with a decrease of the total bacterial
21 abundance. Subsequently, the community may have regrown to higher levels until the next
22 month of heavy rainfall. Interestingly, only single phylotypes dominated in these regrowth
23 periods after heavy rain months in the drinking water; presumably these were best adapted to
24 the actual reservoir conditions or the respectively changed conditions in the drinking water.

25 An unexpected result was the high relative amount of 16S rRNA of a *Synechococcus*
26 species (PT C13R) in the RNA-based fingerprints during winter. This could be explained by

1 cyanobacterial blooms commonly observed in autumn which then sink down to the
2 hypolimnion. In addition, high levels of rRNA have been observed in *Cyanobacteria* and
3 have been explained by a storage function of rRNA for phosphorus and nitrogen (Asato,
4 2003; Cuhel and Waterbury, 1984; Lami et al., 2009).

5 Comparing abundances of the DNA-based fingerprints with those of the RNA-based
6 fingerprints, it can be inferred that the most active bacterial phylotypes were those that are not
7 detectable in DNA-based fingerprints. Only 7 out of 40 phylotypes were found in both types
8 of fingerprints. In addition, some taxonomic groups tended to possess low rRNA-levels
9 despite their high abundances on DNA-based fingerprints (e.g. *Actinobacteria* and
10 *Bacteroidetes*). Especially for *Actinobacteria* in drinking water reservoirs the low content of
11 ribosomes has been described as a result of their small size, resulting in a lower detection rate
12 also by other molecular detection methods like FISH (Nielsen et al., 2006, Lee et al., 2009).

14 **3.6. Taxonomic composition of the hot drinking water community**

15 We detected a total of 19 bacterial phylotypes in the hot drinking water community. 12
16 phylotypes were found in DNA-based analyses and 13 phylotypes in RNA-based analyses,
17 with six of these phylotypes occurring in both types of analyses (Table 1). The common six
18 phylotypes were those with the highest abundances (> 5% DNA or > 3% RNA, Table 2).
19 Although the comparative cluster analysis of the hot drinking water fingerprints assigned a
20 community pattern according to seasons, no definite seasonal phases were observed in the
21 dynamics of the abundances of phylotypes (Fig. 6). The hot water community was
22 characterized by five dominating taxonomic groups, i.e. *Betaproteobacteria*, *Acidobacteria*,
23 *Alphaproteobacteria*, *Bacteroidetes* and *Planctomycetes* (Table 2). Each taxonomic group
24 was dominated by a single phylotype which did not show significant seasonal variations in its
25 abundance. Similar trends were observed for DNA- and RNA-based fingerprints showing no
26 clear seasonal variation in the abundances of phylotypes with the same five dominating

1 phylotypes, i.e. H06DR, H09DR, H10DR, H15DR and H12DR summing up to a total
2 abundance of 65.5% (DNA) and 80.9% (RNA), respectively (Table 2, arrows in Fig. 3).

3 The source of the hot water bacteria is an interesting question with respect to an
4 understanding of their ecology, but also with respect to pathogenic or noxious bacteria, e.g.
5 those promoting corrosion. To estimate the conceivable source habitat of the phylotypes, the
6 habitat or ecology of phylogenetically closely related bacteria was analysed and summarized
7 in Table 3. The phylogenetic environment together with their “behaviour” and occurrence in
8 hot and cold water, can give an estimate on the potential of the phylotype in the hot water.

9 Five phylotypes detected in the hot water were identical with phylotypes detected in the
10 cold water of this or previous studies. The three phylotypes present in the cold water of this
11 study had a decreased abundance compared to the cold water with two occurring only in the
12 DNA-based analyses (alphaproteobacterium C34D=H19D, betaproteobacterium C03D=H01D)
13 and gammaproteobacterium C11R (=H08R) only detected in RNA-based analyses.

14 This contrasts with two phylotypes detected in an earlier study: betaproteobacterium
15 H06DR (=PT(E)10) and bacteroidetes H15DR (=PT(E)29) had a comparable and high
16 abundance in DNA (32 and 5%) and RNA (30 and 5%)-based analyses of hot water. In the
17 former study of this DWSS sampled in June 2003 they had a low abundance and were
18 detected only in the DNA (<2%) of the cold water (Eichler et al., 2006). According to their
19 phylogenetic neighbourhood, the three phylotypes detected in the cold drinking water of this
20 study could be regarded as non-thermophilic, while those detected in the former study are
21 affiliated with thermophilic bacteria. This could explain our observations in a way that the
22 non-thermophilic phylotypes of high abundance in the cold water decayed and the low
23 abundant thermophilic ones grew in the hot water as indicated by an increased abundance in
24 the DNA and especially the RNA-based analyses.

25 The most highly abundant betaproteobacterium H06DR is phylogenetically affiliated with
26 the thermophilic *Thiobacillus aquaesulis* (Table 3) and has an identical sequence with

bacteria from shower head biofilms (EU633732, EU633468), and a 95% similarity with a bacterial sequence from a hot spring (JX125567). The bacteroidetes H15DR is more difficult to be assigned to high temperature adaptation because there are no closely related cultured neighbours (16SrRNA similarity of less than 86%); however, a 16S rRNA sequence similarity of 95% with bacteria from geothermal springs (EF429643) could indicate its high temperature adaptation. These phylogenetic affiliations make an adaptation to high temperatures for these two phylotypes likely. Despite their assumed thermophilic nature, the reservoirs are not unlikely to be the source of these phylotypes; related 16S rRNA sequences were also retrieved from lake sediments (95% sim. of H06DR to HM243969 and JN825456; 94% sim. of H15DR to JN825414) and subsurface environments (99% sim. of H06DR to FR846917), i.e. environments in close neighbourhood to the deep water of lakes and reservoirs.

In total, 68% of all phylotypes in hot drinking water were affiliated with bacteria of “hot habitat” mostly of geothermal nature (Table 3). All of them, except the actinobacterium, were detected in the RNA-based analysis. Their high RNA content can be interpreted as active growth of these phylotypes in the hot water. Only the low abundant actinobacterial phylotype can be considered as high temperature adapted and not detected on the RNA side; as discussed earlier this is presumably due to their specific low RNA content.

For the hot water phylotypes that have not yet been detected in the cold water, the source could also be the cold water among other possibilities. The cold water could have been a source in a way that these phylotypes were present at very low abundances. According to the model of “abundant and rare members” describing the bacterial community composition in pelagic environments (Höfle et al., 2008; Huber et al., 2007; Pedros-Alio, 2006) the community consists of a core community with few taxa that are highly abundant and a huge “seed bank” with nearly infinite numbers of very low abundant phylotypes. A technique analysing the low abundant members such as Next Generation Sequencing could allow to finally assess the cold water and the reservoirs as a source.

3.7. Transformation from cold to hot water bacterial consortium

From our observations the following three different mechanisms for the transformation of the cold water to the hot water bacterial community were derived: i) survival of cold water bacteria (bacterial “group A”), ii) growth of high temperature adapted bacteria previously detected in the DWSS at low abundance (“group B”), iii) growth of high temperature adapted bacteria not yet observed in the DWSS (“group C”).

Survival was observed for three phylotypes of the cold water that were detected either only in DNA- or only in the RNA-based analyses (Group A: C34D, C03D, C11R). They all showed a strong decrease compared to the cold water. For these phylotypes growth is not likely, and the viability of the bacteria still remains to be assessed, means they could have been detected in a decaying or dead state. Due to their low abundance (5% of the DNA, 1% of the RNA) and presumably low growth their role for the hot water community is low.

Two phylotypes (H06DR, H15DR) form group B, i.e. bacteria that show growth in the hot water and occurred in the cold water as shown previously at a low abundance but were not detected in the present study. These bacteria can be considered as high temperature adapted, accounted for more than a third of the hot water bacteria, and had an almost identical abundance in RNA (35%) and DNA (37%).

The majority of the phylotypes (group C: 55% RNA, 30% DNA) has not been detected beforehand in the investigated DWSS and are considered as adapted to high temperatures. Among these were the most dominating acidobacterium H10DR and the alphaproteobacterium H09DR that account together for 43% of the RNA- and 24% of the DNA-based abundances, respectively. A higher abundance in the RNA- compared to DNA-based analyses was observed for most phylotypes of this group. The source of these bacteria might be the cold water, means low abundant and so far undetected members of the “seed bank” of the cold water (see above). Due to their high growth and abundance groups B and C

are considered as most relevant for the hot water, while the fraction of “just surviving” bacteria introduced with the cold water is small.

This severe change of the bacterial community of the cold to the hot water can be assumed to occur within a rather short period of time. During work hours, i.e. the sampling period, the turn over of the cold water to hot water was high, on average 1.2 m^3 within 2-3 hours. The cold water was mixed with the returning hot water for heating in the vessels. We assume that the cold water bacteria were mostly destroyed during the heating process. The released nutrients were re-used for rapid regrowth of the bacterial community of the returned hot water because the high RNA content of the hot water bacteria indicated their intense growth. During work hours we can consider the heating vessels as a continuous reactor for re-growth of hot water bacteria based on the released nutrients of the cold water microflora. This procedure promotes a rapid and substantial change of the bacterial community within a few hours.

3.8. Overall comparison of bacterial communities in cold and hot water

The hot and cold water bacterial community showed pronounced differences with respect to taxonomic composition, activity and seasonal dynamics. The strong discrepancy with respect to the composition is evidenced by the fact that there were only three common phylotypes for hot and cold water of the present study. This is more striking as this was the case even for a monthly study for more than year. The diversity of the cold water community was by far higher than the hot water as best shown by the different rank abundance curves. While the hot water was to a major extent consisting out of five dominating phylotypes over the whole year, there was a far lower dominance of single phylotypes in the cold water and those were even fluctuating during the year.

The cold drinking water showed the typical strong discrepancy between DNA- and RNA-based analyses consistent with all previous observations. Only a minor fraction of the phylotypes was detected in both fingerprints. A strong discrepancy between active and

present bacterial species is considered as the major reason for this observation. This contrasts with the analyses of the hot drinking water. A rapid growth of most bacteria in hot water can be assumed as reason for a high RNA content of most hot water bacteria leading to a more similar picture for RNA and DNA-based analyses.

Seasonal variation was not well pronounced for the hot water consistent with the low variability of environmental factors. By contrast, seasonal variation could be observed for the cold water with distinct changes for the cold and the warm part of the year; additionally, conceivable environmental influences on the source water bacterial communities (such as precipitation) are considered to have been transferred to the cold drinking water. The latter observation was supported by the coincidence of the variation of the bacterial numbers and the “active” bacterial communities with the precipitation regime.

The transformation of the cold drinking water community to the hot one was considered a rapid process that can happen within a few hours occurring during co-heating of cold and returning hot water. Our observations indicate a rapid decay of most cold water bacteria (> 95%) and a rapid growth of the bacteria of the hot water based on the nutrients released due the cold water bacterial decay reaching a final level of approx. 20% below the cold water bacterial numbers.

The origin of phylotypes in the hot water is considered as partially derived from the cold drinking water: three “low temperature adapted” phylotypes were observed in the cold water of this study; two phylotypes had been observed in this DWSS in a former study at low abundance and are considered high temperature adapted. For the remaining hot water phylotypes, it is hypothesized that they are low abundant members of the cold water. In a former study the transfer of source water bacteria to the drinking water along the DWSS has been demonstrated (Eichler et al. 2006). With the transfer of five phylotypes from the cold to the hot water as shown in this study, we suggest that the bacterial community of the reservoirs even influenced the hot drinking water community, i.e. via the cold drinking water.

4. Conclusions

- Bacterial communities in cold drinking water showed a distinct seasonal pattern that was more pronounced in RNA-based than in DNA-based fingerprints.
- Seasonal environmental factors such as temperature and precipitation are considered to influence the bacterial community in the drinking water via transfer of bacteria of the reservoir bacterial community; the observed variation of bacterial numbers in correspondence to the precipitation regime exemplified such an environmental influence.
- Bacterial communities of hot drinking water did not show a pronounced seasonal variability and DNA- and RNA-based fingerprints provided a similar picture of the bacterial community structure and composition.
- Bacterial communities of hot drinking water communities were substantially less diverse than those from the corresponding cold drinking water and had a community composition dominated by five thermophilic and thermotolerant phylotypes.
- Several mechanisms of community species sorting were used to explain the transition in community composition from cold to hot drinking water.
- The rapid conversion of the cold water to the hot water bacterial community can be understood as a fast decay of the cold water bacteria and a rapid and substantial regrowth of thermophilic bacteria as indicated by their high RNA content.

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19

1 **Tables**

2

3 **Table 1** - Number of retrieved phylotypes from cold water and hot water (presence/absence
 4 data). DNA: Number of phylotypes found in DNA-based fingerprints. RNA: Number of
 5 phylotypes found in RNA-based fingerprints. DNA+RNA: Number of phylotypes found in
 6 both types of fingerprints.

Phylum / Class	Cold water				Hot water			
	DNA	RNA	DNA+RNA	Total	DNA	RNA	DNA+RNA	Total
<i>Alphaproteobacteria</i>	3	4	1	6	2	1	1	2
<i>Betaproteobacteria</i>	4	4	1	7	3	5	2	6
<i>Gammaproteobacteria</i>	1	3	0	4	0	2	0	2
<i>Acidobacteria</i>					1	3	1	3
<i>Actinobacteria</i>	5	5	1	9	1	0	0	1
<i>Bacteroidetes</i>	6	5	4	7	4	1	1	4
<i>Cyanobacteria</i>	1	2	0	3				
<i>Firmicutes</i>	0	1	0	1				
<i>Planctomycetes</i>	1	1	0	2	1	1	1	1
unknown	1	0	0	1				
total	22	25	7	40	12	13	6	19

Table 2 – Major taxonomic groups and their dominating phylotypes in hot drinking water revealed by DNA- and RNA-based fingerprints. Relative abundance values are average values across all samples. Phylotypes marked with D only occurred in DNA-based fingerprints, marked with R only in RNA-based fingerprints, and phylotypes marked with DR occurred in both. Phylotypes in bold were also occurring in the cold water of this or a former study (“Cxx” and “Hxx” indicates the phylotype signature for the cold (Cxx) and hot (Hxx) water of this study, “PT(E)xx” indicates a phylotype detected in 2003 by Eichler et al. 2006). Phylotypes marked with an asterics are considered as thermophilic based on their phylogenetic affiliation (for details see Table 3).

DNA				RNA			
Phylum / class	Rel. abundance (%)	Phylotype	Rel. abund. (%)	Phylum / class	Rel. abund. (%)	Phylotype	Rel. abund. (%)
<i>Acidobacteria</i>	14.7	H10DR*	14.7	<i>Acidobacteria</i>	26.3	H10DR*	23.0
						H11R*	1.5
						H14R*	1.7
<i>Actinobacteria</i>	0.1	H13D*	0.1				
<i>Alphaproteobacteria</i>	13.7	H09DR*	9.0	<i>Alphaproteobacteria</i>	20.6	H09DR*	20.6
		H19D (=C34D; PT(E)22)	4.7				
<i>Bacteroidetes</i>	6.70	H15DR* (=PT(E)29)	5.1	<i>Bacteroidetes</i>	5.1	H15DR*	5.1
		H17D	0.5				
		H18D	0.9				
		H20D	0.2				
<i>Betaproteobacteria</i>	32.4	H06DR* (=PT(E)10)	31.8	<i>Betaproteobacteria</i>	36.8	H06DR*	30.2
		H03DR*	0.6			H03DR*	3.1
		H01D (=C03D; PT(E)13)	0.1			H02R*	2.4
						H04R*	1.0
				<i>Gammaproteobacteria</i>	1.2	H08R (=C11R; PT(E)49)	1.2
<i>Planctomycetes</i>	5.4	H12DR*	5.4	<i>Planctomycetes</i>	2.0	H12DR*	2.0

- 1 **Table 3.** Taxonomic identification of single phylotypes (PT) found in hot drinking water fingerprints shown in Fig. 3.
- 2 Legend: D/R = presence in DNA, RNA-based analyses: D, only present in DNA-based fingerprints; R, only present in RNA-based fingerprints; DR,
- 3 present in DNA and RNA-based fingerprints. ^a Phylotype already observed in previous studies: PT (E) = Eichler et al. 2006, PT (N) = new
- 4 phylotype, * 1-3nt different from original phylotype; ^b % similarity and accession number of “new” phylotypes; DW = Drinking water, NA = not
- 5 applicable.

Designation of SSCP band	D/R	Taxonomic group	PT ^a signature	Accession No. of PT	Closest neighbour sequence of new PT(N) ^b	Habitat	Closest described species (% similarity, accession number, species name)
H01D	D	<i>Betaproteobacteria</i>	PT(E) 13*	DQ077603		source water (GR1,GR2)	99% NR_024935 <i>Acidovorax facilis</i>
H02R	R	<i>Betaproteobacteria</i>	PT(N) H02R	HF567849	100% AY324139	thermophilic isolate from a hot water tank	100% AY324139 <i>Tepidimonas aquatica</i>
H03DR	D R	<i>Betaproteobacteria</i>	PT(N) H03DR	HF567850	100% DQ417336	thermophilic bacterium isolated from a hot spring	100% DQ417336 <i>Schlegelella aquatica</i>
H04R	R	<i>Betaproteobacteria</i>	PT(N) H04R	HF567851	99% HM991576.1	thermophilic sediments at Hot Springs National Park, AR	94% NR_025839 <i>Rhodocyclus tenuis</i>
H05R	R	<i>Betaproteobacteria</i>	PT(N) H05R	HF567852	99% HM186667	subsurface microbial community	91% NR_026438 <i>Azoarcus anaerobius</i>
H06DR	D R	<i>Betaproteobacteria</i>	PT(E) 10*	DQ077622		DW	93% NR_044793 <i>Thiobacillus aquaesulis</i>
H07R	R	<i>Gammaproteobacteria</i>	PT(N) H07R	HF567853	100% HQ899376	Initial microbial coloniser of Eyjafjallajökull lava flows, Iceland	97% NR_025441 <i>Thermomonas haemolytica</i>
H08R	R	<i>Gammaproteobacteria</i>	PT(E) 49*	DQ077602		DW	93% NR_026063.1 <i>Methylocaldum gracile</i>

H09DR	D R	<i>Alphaproteobacteria</i>	PT(N) H09DR	HF567854	97% FQ740415	soil sample ferralsol, Madagascar	95% NR_029125 <i>Rhodoplanes elegans</i>
H10DR	D R	<i>Acidobacteria</i>	PT(N) H10DR	HF567855	99% DQ791443	geothermal soil from Hawaiian volcano park	84% NR_025345.1 <i>Chondromyces lanuginosus</i>
H11R	R	<i>Acidobacteria</i>	PT(N) H11R	HF567856	98% HQ755778	bowel diseases, human ileum	81% NR_042719.1 <i>Thermovenabulum ferriorganovorum</i>
H12DR	D R	<i>Planctomycetes</i>	PT(N) H12DR	HF567857	99% EU376432	hot spring mats from Western Thailand	91% NR_037010 <i>Gemmata obscuriglobus</i> 2246
H13D	D	<i>Actinobacteria</i>	PT(N) H13D	HF567858	93% JF717667	volcanic CO2 vent	92% NR_036979 <i>Oxalophagus oxalicus</i>
H14R	R	<i>Acidobacteria</i>	PT(N) H14R	HF567859	97% HM991545	Thermophilic Sediments Hot Springs National Park, AR	84% NR_025815.1 <i>Silanimonas lenta</i>
H15DR	D R	<i>Bacteroidetes</i>	PT(E)29*	DQ077604		DW, water processing (GT)	85% NR_025743 <i>Algoriphagus mannitolivorans</i>
H17D	D	<i>Bacteroidetes</i>	PT(N) H17D	HF567860	90% EU626714	sea urchin symbiont	88% NR_042135.1 <i>Sphingobacterium thalpophilum</i>
H18D	D	<i>Bacteroidetes</i>	PT(N) H18D	HF567861	96% NR_042135	human clinical specimen, blood, wound	96% NR_042135 <i>Sphingobacterium thalpophilum</i>
H19D	D	<i>Alphaproteobacteria</i>	PT(E)22*	DQ077623		DW, source water (GR1)	84% NR_025835 <i>Rhodothalassium salexigens</i>
H20D	D	<i>Bacteroidetes</i>	PT(N) H20D	HF567862	92% FQ740678.1	soil sample (ferralsol, Madagascar)	88% NR_040920 <i>Marinoscillum furvescens</i>

Figures legends

Fig. 1 - (a) Climate data of Clausthal-Zellerfeld representing climatic parameters for the catchment area of the Grane reservoir and the Ecker reservoir and the monthly temperature of the drinking water. (b) Total bacterial cell counts in cold and hot drinking water during 2008 and 2009. Continuous line with diamonds: cold water; dashed line with triangles: hot water. (c) Monthly precipitation and monthly change in total abundances of bacteria in cold drinking water. Arrows at the x-axis indicate a strong decrease in bacterial numbers and transition phases in the cluster analysis of corresponding RNA-based fingerprints (see Fig. 2 d) (d) Correlation analysis (Pearson) between monthly precipitation and monthly change in total abundance of bacteria in cold drinking water. r_1 : correlation coefficient including all data points. r_2 : correlation coefficient excluding four outliers (open diamonds) belonging to months with high precipitation.

Fig. 2 - (a) 16S rRNA gene based SSCP fingerprints of cold water. (b) Comparative cluster analysis of DNA-based SSCP fingerprints of cold water. (c) 16S rRNA-based SSCP fingerprints of cold water. (d) Comparative cluster analysis of RNA-based SSCP fingerprints of cold water.

Fig. 3 - (a) 16S rRNA gene based SSCP fingerprints of hot water (b) Comparative cluster analysis of DNA-based SSCP fingerprints of hot water (c) 16S rRNA-based SSCP fingerprints of hot water (d) Comparative cluster analysis of RNA-based SSCP fingerprints of hot water. Arrows indicate major phylotypes, 1 = H06DR, 2 = H15DR, 3 = H10DR, 4 = H12DR, 5 = H09DR. Pressure loss event was on November 21, 2008.

Fig. 4 - Rank abundance plot of cold water (blue) and hot water (red) using relative band intensities from DNA-based fingerprints as a measure for relative abundance. Lines show the regression analysis of plotted rank abundance curves: blue line, cold water; red line, hot water.

Fig. 5 - Seasonal variation of relative abundances of the phlotypes obtained from cold water. The colors are corresponding to the major phylogenetic groups. Phlotypes are separated by solid lines. Arrows indicate phase transitions of the cluster analysis of the respective fingerprints. Blue bars at the x-axis indicate months with high precipitation. (a) Phlotypes from the DNA-based SSCP fingerprints. (b) Phlotypes from the RNA-based SSCP fingerprints.

Fig. 6 - Seasonal variation of relative abundances of the phlotypes obtained from hot water. The colors are corresponding to the major phylogenetic groups. Phlotypes are separated by solid lines. (a) Phlotypes from the DNA-based SSCP fingerprints. (b) Phlotypes from the RNA-based SSCP fingerprints.

Fig. 1

Fig. 1

(c)

d)

Fig. 2

Fig. 3 - (a)

(b)

(c)

(d)

Fig. 4

Fig. 5

Fig. 6

(a)

(b)

Supplementary Material - Fig. 1

Supplementary **Fig. 1** - Comparison of DNA- and RNA-based relative abundances of the major taxonomic groups in hot drinking water. Relative abundances are average values across all samples as given in detail in Table 2. *Actinobacteria* have a DNA-based abundance of 0.1%.

Supplementary Table S1. Taxonomic identification of phylotypes (PT) found in cold drinking water fingerprints shown in Fig. 2.

Legend: D/R. = presence in DNA,RNA-based analyses: D, only in DNA-based fingerprints; R, only in RNA-based fingerprints; DR, present in DNA and RNA-based fingerprints. ^a Phylotype already observed in previous studies: PT(E) = Eichler et al. 2006; PT(K) = Kahlisch et al. 2012, PT(H) = Henne et al. 2012, PT(N) = New phylotype, * 1-3nt different from original phylotype, ** 4-7nt different from original phylotype; ^b % similarity and accession number, only given for new phylotypes; ^c similarity below 85%; DW = Drinking water, NA = not applicable.

Designation of SSCP band	D/R	Taxonomic group	PT ^a signature	Accession No. of PT	Closest neighbour sequence of new PT(N) ^b	Habitat	Closest described species (% similarity, accession number, species name)
C12R	R	<i>Actinobacteria</i>	PT(N) C12R	HF567863	95% HM486292	wetland	94% NR_024742 <i>Actinoplanes digitatis</i>
C17R	R	<i>Actinobacteria</i>	PT(N) C17R	HF567864	96% HE616254	boreal lake plankton	91% NR_043495 <i>Streptomyces regensis</i>
C18D	D	<i>Actinobacteria</i>	PT(H) T018*	FR796672		DW	88% NR_043717 <i>Humicoccus flavidus</i>
C19D	D	<i>Actinobacteria</i>	PT(H) T019**	FR796673		DW	88% NR_043461 <i>Tetrasphaera jenkinsii</i>
C20R	R	<i>Actinobacteria</i>	PT(E) 19**	DQ077626		source water (ER1,ET)	90% NR_043460 <i>Tetrasphaera vanveenii</i>
C21D	D	<i>Actinobacteria</i>	PT(E) 19**	DQ077626		source water (ER1,ET)	90% NR_043460 <i>Tetrasphaera vanveenii</i>
C22R	R	<i>Actinobacteria</i>	PT(E) 18**	DQ077609		DW, source water (GR1,GR2, GT)	91% NR_041419 <i>Streptacidiphilus jiangxiensis</i>
C23DR	D R	<i>Actinobacteria</i>	PT(E) 16*	DQ077613		DW, source water (GR1,GR2, GT)	91% NR_043465 <i>Demequina aestuarii</i>
C33D	D	<i>Actinobacteria</i>	PT(E) 21**	DQ077610		DW, source water (GR2)	90% NR_041633 <i>Ilumatobacter fluminis</i>
C25R	R	<i>Alphaproteobacteria</i>	PT(H) B082**	FR796624		DW-biofilm, HWW (H)	89% NR_026428 <i>Hyphomicrobium hollandicum</i>
C26D	D	<i>Alphaproteobacteria</i>	PT(N) C26D	HF567866	94% FR796624	DW-biofilm, HWW (H)	94% NR_037121 <i>Blastochloris sulfoviridis</i>

C27R	R	<i>Alphaproteobacteria</i>	PT(H)T027	FR796681		DW	99% NR_028799 <i>Bosea vestrisii</i>
C29R	R	<i>Alphaproteobacteria</i>	PT(E) 55*	DQ077572		DW	98% NR_024685 <i>Sphingomonas oligophenolica</i>
C34D	D	<i>Alphaproteobacteria</i>	PT(E)22*	DQ077623		DW, source water (GR1)	84% NR_025835 <i>Rhodothalassium salexigens</i>
C35DR	D R	<i>Alphaproteobacteria</i>	PT(N) C35DR	HF567867	97% DQ077623	DW, source water (GR1)	83% NR_044315 <i>Insolitospirillum peregrinum</i>
C36DR	D R	<i>Bacteroidetes</i>	PT(N) C36DR	HF567868	90% HE616317	boreal lake plankton	89% NR_044197 <i>Sediminibacterium salmoneum</i>
C37D	D	<i>Bacteroidetes</i>	PT(E)73*	DQ077593		source water (GR1, GR2)	85% NR_044568 <i>Solitalea koreensis</i>
C38D	D	<i>Bacteroidetes</i>	PT(H)T035*	FR796689		DW	87% NR_040906 <i>Solitalea canadensis</i>
C39R	R	<i>Bacteroidetes</i>	PT(E)82*	DQ077584		DW, source water (GR1)	91% NR_044568 <i>Solitalea koreensis</i>
C41DR	D R	<i>Bacteroidetes</i>	PT(E)35*	DQ077625		DW, source water (GR2,GT)	98% NR_044197 <i>Sediminibacterium salmoneum</i>
C42DR	D R	<i>Bacteroidetes</i>	PT(E)35*	DQ077625		DW, source water (GR2,GT)	98% NR_044197 <i>Sediminibacterium salmoneum</i>
C43DR	D R	<i>Bacteroidetes</i>	PT(K)37*	GQ917153		DW	96% NR_044197 <i>Sediminibacterium salmoneum</i>
C01R	R	<i>Betaproteobacteria</i>	PT(E) 77*	DQ077559		DW	99% NR_028714 <i>Acidovorax delafieldii</i>
C02D	D	<i>Betaproteobacteria</i>	PT(E) 77*	DQ077559		DW	99% NR_028714 <i>Acidovorax delafieldii</i>
C03D	D	<i>Betaproteobacteria</i>	PT(E) 13*	DQ077603		source water (GR1,GR2)	99% NR_024935 <i>Acidovorax facilis</i>
C04R	R	<i>Betaproteobacteria</i>	PT(E) 77**	DQ077559		DW	99% NR_028714 <i>Acidovorax delafieldii</i>
C05D	D	<i>Betaproteobacteria</i>	PT(E) 8*	DQ077605		source water (ER1,ER2, ET)	99% NR_042382 <i>Polynucleobacter necessarius</i>
C06DR	D R	<i>Betaproteobacteria</i>	PT(E) 1*	DQ077612		DW, source water (GR1,GT)	96% NR_041257 <i>Methylophilus methylotrophus</i>
C08R	R	<i>Betaproteobacteria</i>	tentatively identified ^c	NA		NA	NA
C13R	R	<i>Cyanobacteria</i>	PT(K) 46*	GQ917161		DW	94% NR_028762 <i>Prochlorococcus marinus</i>
C14R	R	<i>Cyanobacteria</i>	PT(N) C14R	HF567864	98% JQ927353	coastal water	96% NR_028762 <i>Prochlorococcus marinus</i>
C15D	D	<i>Cyanobacteria</i>	PT(K) 46**	GQ917161		DW	94% NR_028762 <i>Prochlorococcus marinus</i>
C16R	R	<i>Firmicutes</i>	tentatively identified ^c	NA		NA	NA
C09D	D	<i>Gammaproteobacteria</i>	PT(E) 85*	DQ077579		DW	99% NR_027549 <i>Escherichia fergusonii</i>

C10R	R	<i>Gammaproteobacteria</i>	PT(H) T032*	FR796686		DW	100% NR_042107 <i>Methylobacter tundripaludum</i>
C11R	R	<i>Gammaproteobacteria</i>	PT(E) 49*	DQ077602		DW	93% NR_026063 <i>Methylocaldum gracile</i>
C24R	R	<i>Gammaproteobacteria</i>	tentatively identified ^c	NA		NA	NA
C30R	R	<i>Planctomycetes</i>	tentatively identified ^c	NA		NA	NA
C31D	D	<i>Planctomycetes</i>	tentatively identified ^c	NA		NA	NA
C32D	D	<i>Planctomycetes</i>	PT(H) T005D*	FR796659		DW	NA