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„Community composition of microbial biofilm and suspended stream water communities during biofilm succession“

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Abstract

Microbial biofilms in streams are formed by the attachment and subsequent growth of cells transported by the water column. In this study I compared the microbial community composition of stream biofilm growing on new substrate with its source community suspended in the stream water during the initial stages of biofilm succession using terminal restriction fragment length polymorphism of the 16S ribosomal RNA gene. The aim was to gain insight into how the biofilm community is shaped by the suspended source community during biofilm development. OTU richness and diversity were higher in the suspended than in the biofilm community. During succession richness and diversity of the biofilm community increased initially, but after a certain number of days richness decreased slightly again, whereas diversity stagnated. In the beginning the rank-abundance curves of the biofilm community showed a steep slope, which declined gradually later on. The microbial community composition of the biofilm differed considerably from the composition of the suspended community. The biofilm community composition changed during succession. Temporal variations of the source community suspended in the stream water showed no significant impact on biofilm community development. My findings indicate that stream biofilm community structure is shaped by a combination of environmental conditions through species sorting and autogenic successional processes within the biofilm.

Introduction

Bacteria are ubiquitous and abundant in huge numbers and therefore play a vital role in the functioning of ecosystems (Whitman et al. 1998). In streams, the majority of bacteria grow attached to surfaces as part of a biofilm (Costerton et al. 1995, Hall-Stoodley et al. 2004). Biofilms are microbial communities composed of bacteria, algae and fungi, which are enclosed by a matrix consisting of extracellular polymeric substances. Biofilms play a key role in ecosystem processes and trophic transfer due to the importance of heterotrophic microbes in the utilisation of dissolved organic carbon (DOC) (Meyer 1994, Battin et al. 2003, Singer et al. 2010).

New substrate in streams is colonised by bacteria transported by the water column. They attach to surfaces using extracellular polymers, and subsequently form colonies that develop into a mature biofilm (Davey and O'toole 2000, Hall-Stoodley et al. 2004). Conversely, bacteria in the biofilm are released back into the bulk fluid through sloughing, erosion and abrasion or as part of their normal life cycle (Leff et al. 1992). These cells can in turn colonise substrate further downstream. Biofilm communities of different habitat patches in stream networks are therefore linked by dispersal and can be regarded as metacommunities (Battin et al. 2007). According to metacommunity theory (Leibold et al. 2004, Holyoak et al. 2005), the interplay of local (abiotic environment, biotic interactions) and regional (dispersal) processes regulate the assembly of local communities. The advent of molecular technologies provides new possibilities to apply the concepts of metacommunity ecology on bacterial communities.

Streams collect and transport bacteria from multiple sources within the catchment, which suggests that the suspended microbial community in the stream water likely consists to a large extent of species from upstream habitats, such as riparian soil (Leibold et al. 2004, Crump et al. 2007). Not all of these organisms are necessarily adapted to the stream environment, but can be found in the community due to their high immigration rates. In the framework of metacommunity ecology, this is called the “mass-effects paradigm” (Shmida and Wilson 1985, Leibold et al. 2004). Notably, environmental factors appear to play an important role in determining community composition of stream biofilms (Besemer et al. 2007, Besemer et al.

2012). The increased residence time of microbial cells in biofilms seems to lower the impact of mass effects and raise the importance of "species sorting" (Leibold et al. 2004, Holyoak et al. 2005), i.e. OTUs capable of growing under certain environmental conditions are favoured. Accordingly, previous studies on stream biofilms found that biofilm community compositions differed considerably from the composition of the suspended community in the bulk fluid, demonstrating the importance of species sorting in shaping the biofilm communities (Araya et al. 2003, Beier et al. 2008). Additionally, autogenic successional changes within the biofilm have been shown to shape biofilm community structure, i.e., organisms interact and modify their surrounding environment, which facilitates or inhibits the presence of other populations (Besemer 2007).

Insight into community assembly and succession is crucial to better understand the functioning of biofilms. For a long time, most studies focused on the algal community of the biofilm (Stevensen et al. 1991, Johnson et al. 1997, Wellnitz and Rader 2003). Studies on succession in bacterial biofilm communities do exist, however (Jackson et al. 2001, Martiny et al. 2003, Lyautey et al. 2005, Besemer et al. 2007, Besemer et al. 2012, Wey et al. 2012). Using denaturing gel gradient electrophoresis Jackson et al. (2001) investigated succession of bacterial communities in biofilms that were grown on artificial substrates in mesocosms and a lake, which led to a conceptual model for bacterial biofilm succession (Jackson 2003). Few studies, however, have explicitly addressed the influence of the source community on the succession of biofilms in nature (Besemer et al. 2012).

The aim of my diploma thesis was therefore to compare the microbial community composition of stream biofilm with its source community suspended in the stream water during the initial stages of biofilm succession. Based on the previous findings that biofilms seem to be shaped by species sorting and autogenic successional processes, I hypothesized that biofilm communities would differ from stream water communities and that succession in biofilm communities would be largely independent from temporal variations in the suspended microbial community. Stream water has a very short residence time, which means that point samples taken of the suspended community inevitably neglect temporal dynamics of the

microbial stream water community, providing a limited picture of the diversity of this community. To overcome this problem, I sampled the stream water continuously and analysed time-integrated samples. This enabled me to get a full picture of the potential source community seeding the biofilm, a clear improvement compared to earlier studies. The method I used was terminal restriction fragment length polymorphism (T-RFLP) of the 16 S rRNA-gene. T-RFLP analysis is sensitive enough to be used for the characterization and comparison of complex bacterial communities (Moeseneder et al. 1999, Osborn et al. 2000).

Material and Methods

Experimental setup and sample processing

The experiment was conducted from March 22th, 2011 to April 6th, 2011. On the first day of the experiment, small sterile ceramic tiles (approx. 1.8 x 0.8 x 0.2 cm), which were glued onto larger ceramic tiles for stabilization using a silicon adhesive, were exposed in a small side stream of the Oberer Lunzer Seebach (Lower Austria) as substrate for biofilm growth. During the experiment no changes of discharge were observed.

Samples were taken each day in intervals of roughly 24 h. Two tiles were sampled for chlorophyll-*a* concentration measurements, two for bacterial cell abundance counts and three for community composition analysis. The tiles for the chlorophyll-*a* concentration measurements and for the community composition analysis were kept frozen at -20°C pending further analysis. The tiles for bacterial cell counts were fixed with a 1% formaldehyde solution and kept at 4°C.

Simultaneously, each day a water sample was taken from the small stream in which the tiles were exposed. In order to obtain a time-integrated sample of the full 24 h time interval between samplings and to avoid getting point measurements, stream water was slowly pumped into a large container, from which, after thorough mixing, a subsample was taken to the lab. Of each sample, 10 ml (in duplicates) were fixed for bacterial cell counts with formaldehyde (final concentration of 1% formaldehyde). For measurements of chlorophyll-a concentration, 2 l of the sample water were filtered (GF/F; Whatman) and frozen at -20°C. For community composition analysis approximately, 500 ml of the water sample were filtered through 0.2 µm pore-size filters (GSWP, Millipore) in duplicates and the filters were then frozen at -20°C.

Chlorophyll-*a*

Filters were cut into small pieces and mixed with 10 ml of acetone. Chlorophyll-*a* was then extracted at 4°C over night. The chlorophyll on the tiles was extracted in 2.5 ml of acetone, also over night at 4°C. The chlorophyll extract was then vortexed and filtered through a GF/F (Whatman) filter. Chlorophyll a concentrations were measured using fluorometry (EX435/EM675; Shimadzu RF-5301 PC). A standard curve was created for concentrations ranging from 0.1 to 2 µg Chl-α ml^{-1} using a spinach standard (Sigma).

Bacterial cell density

To detach the biofilms from the tiles, the tiles were shaken for 1 h in a 0.025 mmol l^{-1} pyrophosphate solution and subsequently sonicated (180 s, 40 W output; Branson). The formaldehyde-fixed water samples were also sonicated. The microbial cells were then stained with SYTOX Green nucleic acid stain and counted using flow cytometry (Cell Lab Quanta; Beckman Coulter).

PCR and T-RFLP

DNA was extracted using the PowerSoil® DNA Isolation Kit (MoBio). As a first step in the DNA extraction of the biofilm samples the PowerSoil® bead solution of the isolation kit was added to the tiles, vortexed and then transferred back to the bead tubes. This method effectively removed biofilm from the ceramic tiles and proved successful even for samples from the first sample days, when very low biomass was present on the tiles. For the water samples, the filters were cut into small pieces using ethanol-flamed scissors and tweezers and placed into the bead tubes. Afterwards DNA extraction was carried out according to the PowerSoil® DNA Isolation Kit manual.

The primers used for the polymerase chain reaction (PCR) were the hexachlorofluorescin-labelled bacteria-specific primer 27F-hex (5'-AGA GTT TGA TCM TGG CTC

AG-3') and the universal primer 519R (5'-GWA TTA CCG CGG CKG CTG-3') (Invitrogen). Each 50µl of PCR mixture contained both of the above mentioned primers at 0.4 µmol.L^{-1}, each deoxynucleoside triphosphate at 0.2 mmol.L^{-1}, $MgCl_2$ at 2.5 mmol.L^{-1}, 50 µg of bovine serum albumin (BSA), 1.25 U of *Taq* Polymerase, and the recommended PCR buffer (all MBI Fermentas). The PCR started with an initial 3 min long denaturation step at 94°C, followed by 30 cycles. Each cycle consisted of a 45 s denaturation step at 94°C, a 45 s long annealing step at 55°C and a 60 s long elongation step at 72°C. At the end a final elongation step at 72°C ran for 10 min. Each set of PCRs included a negative control. Integrity of PCR products was checked using agarose gel electrophoresis in combination with the Low DNA Mass Ladder (Fermentas) and cleaned using the QIAquick® PCR purification (Qiagen) kit according to the manufacturers recommendations.

The fluorescently labelled PCR products were digested separately with the restriction enzymes *Hha*I and *Hinf*I (Fermentas). 20 µl of the restriction digest mixture contained 10 µl cleaned PCR product, 10 U of restriction enzyme *Hha*I or *Hinf*I and the recommended buffer (Tango or R, respectively). The restriction digests were incubated for 16 hours at a temperature of 37°C, followed by a heat inactivation step at 65°C for 20 minutes. The sizing standard MapMarker 1000-ROX (0.5µl, BioVentures) was added to 1.5µl of the restriction digests. The samples were denatured in the presence of 10 µl deionized formamide (Applied Biosystems) at 94°C for 3 minutes and immediately placed on ice. Fluorescently labelled DNA fragments were separated and detected in an ABI 3130 XL capillary sequencer (Applied Biosystems). The electropherograms were analysed using the Peak Scanner software (Applied Biosystems). Restriction fragments smaller than 30 base pairs (bp) were excluded from further analysis to avoid detection of primers. Peaks that were higher than 25 fluorescence units were clearly distinguishable from background noise. The relative contribution of each operational taxonomic unit (OTU) was estimated as peak height divided by the cumulative peak height of the respective sample.

Data analysis

To analyse the T-RFLP data, the results of the *Hha*I and *Hinf*I restriction digests (as relative peak heights) were combined into one data matrix. The proportion of individual OTUs in the suspended and biofilm communities was illustrated in a heat map. A heat map is a graphical representation of the relative abundances of the OTU-matrix where the values are represented by colours. Data were cubic root-transformed prior to this analysis.

Diversities were estimated using richness *S*, i.e. the total number of OTUs in a given sample, and the Shannon index:

$$H' = -\sum_{i=1}^{S} p_i \ln p_1$$

where p_i is the relative proportion of OTU *i* in the sample. The richness and the Shannon index of biofilm and stream water communities were compared using Student's t-test.

Rank-abundance curves were created to investigate the distribution of OTUs abundances, richness and evenness (Magurran 2004). For this, the relative abundances of each OTU is plotted against its rank in a sample, i.e., the most abundant OTU has rank 1, the second most abundant rank 2 etc. After log transformation of the rank and abundance data linear regression models were fitted to each curve. The slopes of these curves were then compared using Student's t-test.

A similarity matrix of the community composition was calculated using the presence/absence-based Sørensen index:

$$QS_{ij} = \frac{2S}{(A_1 + A_{2)}}$$

where *S* is the number of OTUs shared between two communities and A_1 and A_2 are the total number of OTUs found in the respective communities. A similarity matrix based on the relative abundance of OTUs was calculated using the Bray-Curtis index:

$$BC_{ij} = \frac{\sum_{k=1}^{n} \left| x_{ik} - x_{jk} \right|}{\sum_{k=1}^{n} (x_{ik} - x_{jk})}$$

where x_{ik} and x_{jk} are the relative abundances of OTU *k* in samples *i* and *j*. In order to illustrate patterns of community composition nonmetric multidimensional scaling (nMDS) was performed on the similarity matrices. The biofilm and stream water community similarity matrices and a Euclidian distance matrix calculated for sample day were compared using the partial Mantel's Test with Pearson's correlation and 1000 permutations. The correlation between the biofilm and the stream water communities was calculated controlling for the effect of sample day, as well as the correlation between the biofilm communities and sample day, controlling for the stream water communities. The software packages used for data analysis were R 2.12.1, SPSS 18.0 and Sigma Plot 12.0.

Results

Microbial biomass

The concentration of chlorophyll *a* in the stream water samples ranged from 0.27 µg.L^{-1} to 1.33 µg.L^{-1} with an average concentration of 0.57 µg.L^{-1} (Figure 1). With the possible exception of the last three sample days, during which chlorophyll *a* concentration increased, no temporal trend in chlorophyll *a* concentration could be observed. Chlorophyll *a* concentration was extremely low in all biofilm samples, but increased over time from a mean concentration of 8.76 x 10^{-5} µg cm^{-2} on day 1 to 3.25 x 10^{-4} µg cm^{-2} on day 14.

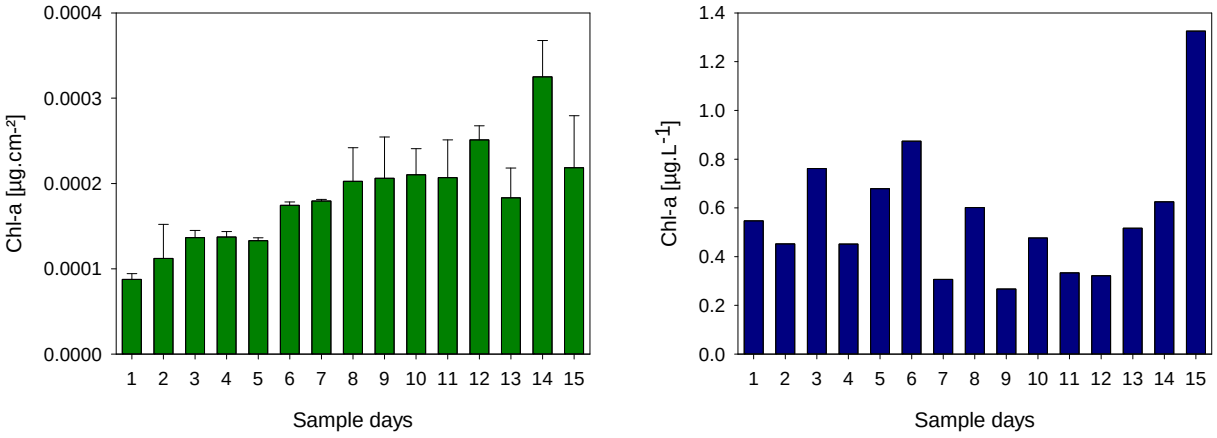

Figure 1: Amount of chlorophyll *a* in biofilm samples per cm² of substrate (left) and chlorophyll *a* concentration in stream water samples (right). Error bars in left graph indicate the standard deviation from the average (n=2 for each sample day).

The mean number of bacterial cells in the biofilm samples increased over the course of the experiment from 6.90 x 10^{5} cells.cm^{-2} on day 1 to 3.66 x 10^{7} cells.cm^{-2} on day 15 (Figure 2). The mean bacterial abundance in the suspended communities ranged from 1.27 x 10^{5} cells.ml^{-1} to 2.64 x 10^{5} cells.ml^{-1} with an overall mean of 1.63 cells.ml^{-1}. No trend over time could be observed.

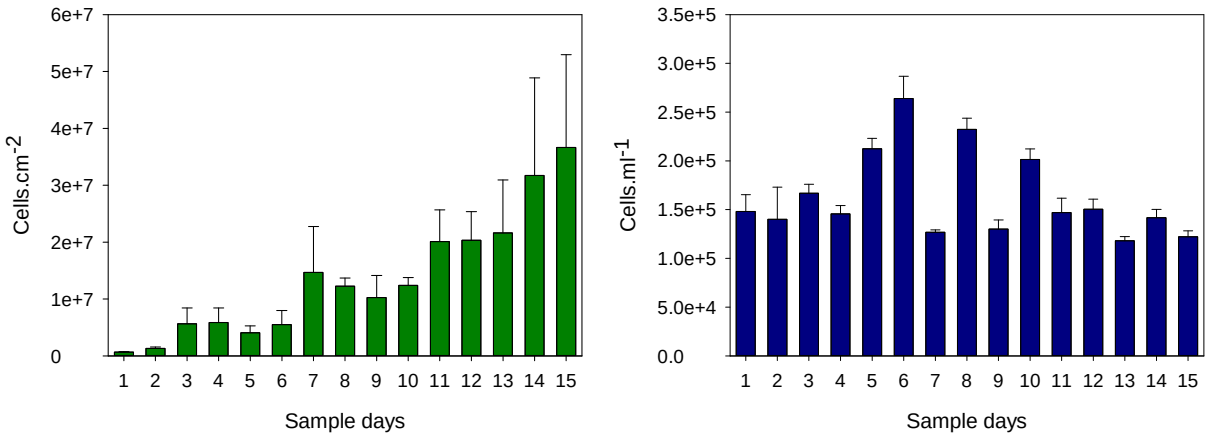

Figure 2: Number of bacterial cells in biofilm samples per cm² substrate (left) and per ml stream water in samples from the suspended community (right). Error bars indicate the standard deviation from the average (n=2 for each sample day).

Number and relative abundance of bacterial OTUs

With the results of both the *Hha*I and the *Hinf*I restriction digests combined, a total number of 202 OTUs were identified altogether (101 OTUs for each restriction enzyme). Of these, 90 OTUs occurred in both the biofilm and the suspended communities, 80 OTUs were exclusively found in the suspended community samples and 32 OTUs were exclusively found in biofilm samples.

The five most abundant biofilm OTUs from both the *Hha*I and the *Hinf*I restriction digest, respectively, were also present in the suspended communities (Table 1); likewise, the most abundant OTUs from the suspended community samples were also present in the biofilm (Table 2). OTUs corresponding to fragments 205 and 206 of the *Hha*I restriction digest and fragments 189, 299, 322, 323 and 324 of the *Hinf*I restriction digest had a high relative abundance in both the biofilm and the suspended microbial communities, whereas other OTUs were only prominent in either the biofilm or the suspended communities.

Table 1: Relative abundance of the five most abundant OTUS in biofilm samples for each restriction enzyme.

Fragment length [bp]	Relative abundance in biofilm communities [%]	Relative abundance in suspended communities [%]
*Hha*I		
479	19.412	2.719
530	12.455	0.416
205	11.139	5.740
206	9.418	6.094
480	4.692	0.489
*Hinf*I		
299	27.104	7.819
189	9.437	4.557
322	6.788	24.765
323	6.659	8.251
300	5.501	0.757

Table 2: Relative abundance of the five most abundant OTUs in the suspended communities for each restriction enzyme.

Fragment length [bp]	Relative abundance in suspended communities [%]	Relative abundance in biofilm communities [%]
*Hha*I		
86	17.849	2.551
331	6.680	0.073
206	6.094	9.418
205	5.740	11.139
84	5.495	1.236
*Hinf*I		
322	24.765	6.788
324	10.140	4.798
323	8.251	6.659
299	7.819	27.104
297	6.809	0.634

A heat map of the relative OTU-abundances showed a small number of OTUs that were dominant in the biofilm early in the experiment, but lost some of their relative importance later on (notably a479 and b 299), while others were important members of the biofilm community throughout the experiment (e.g. a205, a206, b189;Figure 3). A number of OTUs only occurred in the biofilm later in the experiment (e.g. a199, a228, b112, b315). Although the most abundant OTUs were present in both suspended and biofilm communities, some OTUs which constituted considerable proportions of the suspended community were never detected in the biofilm (e.g., a338, a504, a514 b188, b472).

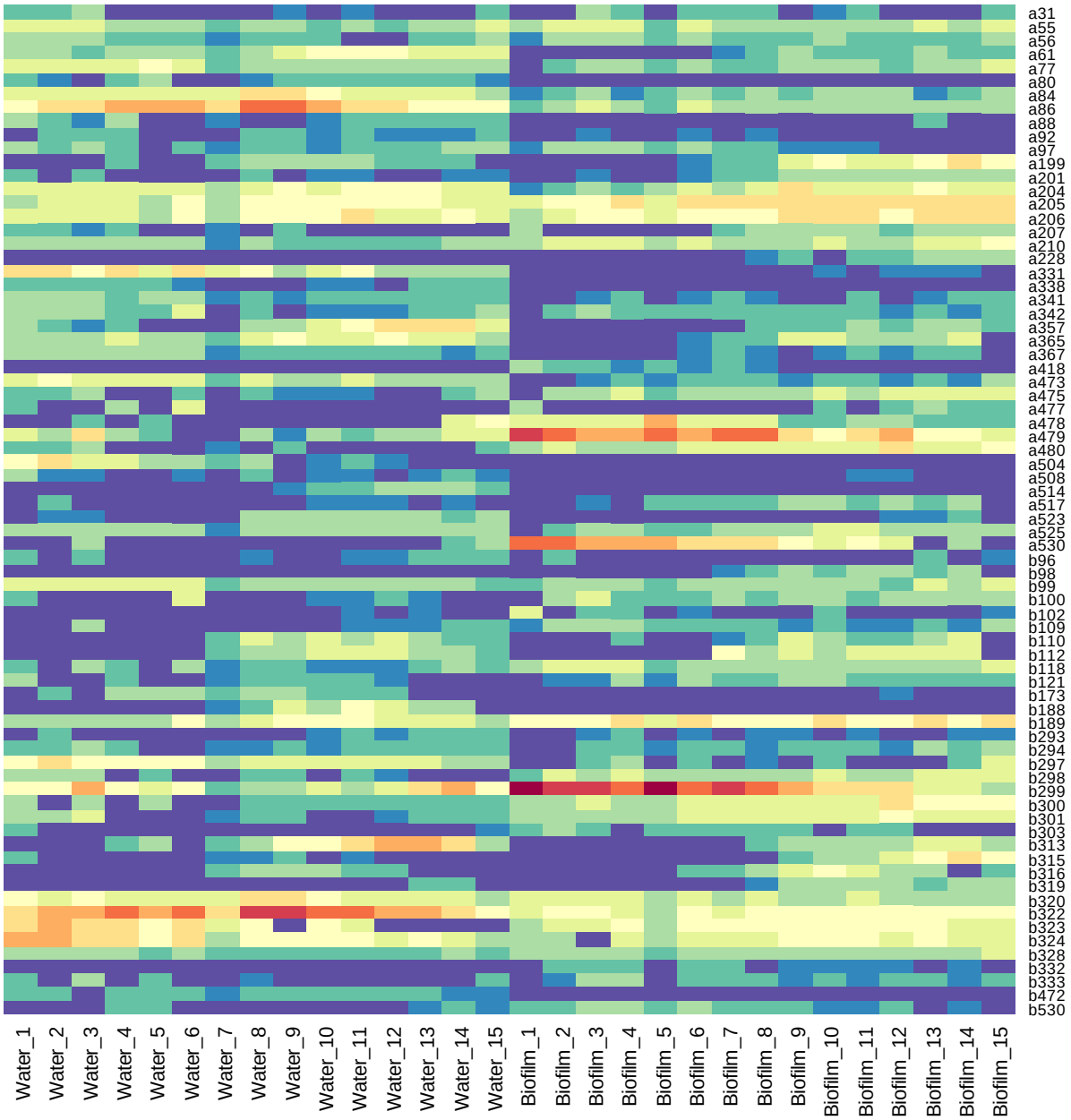

Figure 3: Heat map showing the cubic root-transformed mean relative abundances of the most important OTUs. The mean relative abundance is the mean of replicates from one day. OTUS which were always rare and only occurred at a small number of days were excluded from the heat map. Colours range from dark blue (low abundance) to dark red (high abundance). Labels on the x-axis give the names of the OTUs found in the T-RFLP analysis. OTU names starting with an "a" correspond to fragments found with the *Hha*I restriction enzyme, "b" indicates OTUs found with the *Hinf*I restriction enzyme. The number in the OTU name equals the length of the fragment in bp.

Microbial biodiversity

The results of the Shannon-index were found to be slightly but significantly higher in the suspended than in the biofilm communities ($t=2.009$, $d.f.=72$, $p=<0.05$), with a mean value of 5.41 for the suspended compared to 5.02 for the biofilm communities (Figure 4). With the exception of sample day 5 of the experiment, the Shannon-index of the biofilm communities increased over time starting from a Shannon index of 3.31 until it reached a plateau around a Shannon index of 5.7 on sample day 11. The results of the Shannon index for the suspended community fluctuated during the course of the experiment between a Shannon index of 4.12 and 6.21 but showed no clear temporal pattern.

Bacterial OTU richness was also significantly higher in the suspended communities than in the biofilm communities ($t=3.579$, $d.f.=72$, $p=<0.001$). The mean OTU richness of the biofilm communities was 45 OTUs per sample compared to a mean number of 55 OTUs per sample in the suspended communities. The OTU richness in the biofilm communities increased from a mean richness of 24 OTUs on sample day 1 to 58 OTUs on sample 12, after which it declined slightly again. An increase in OTU richness can also be observed in the suspended communities between sample day 6 and the end of the experiment on day 15.

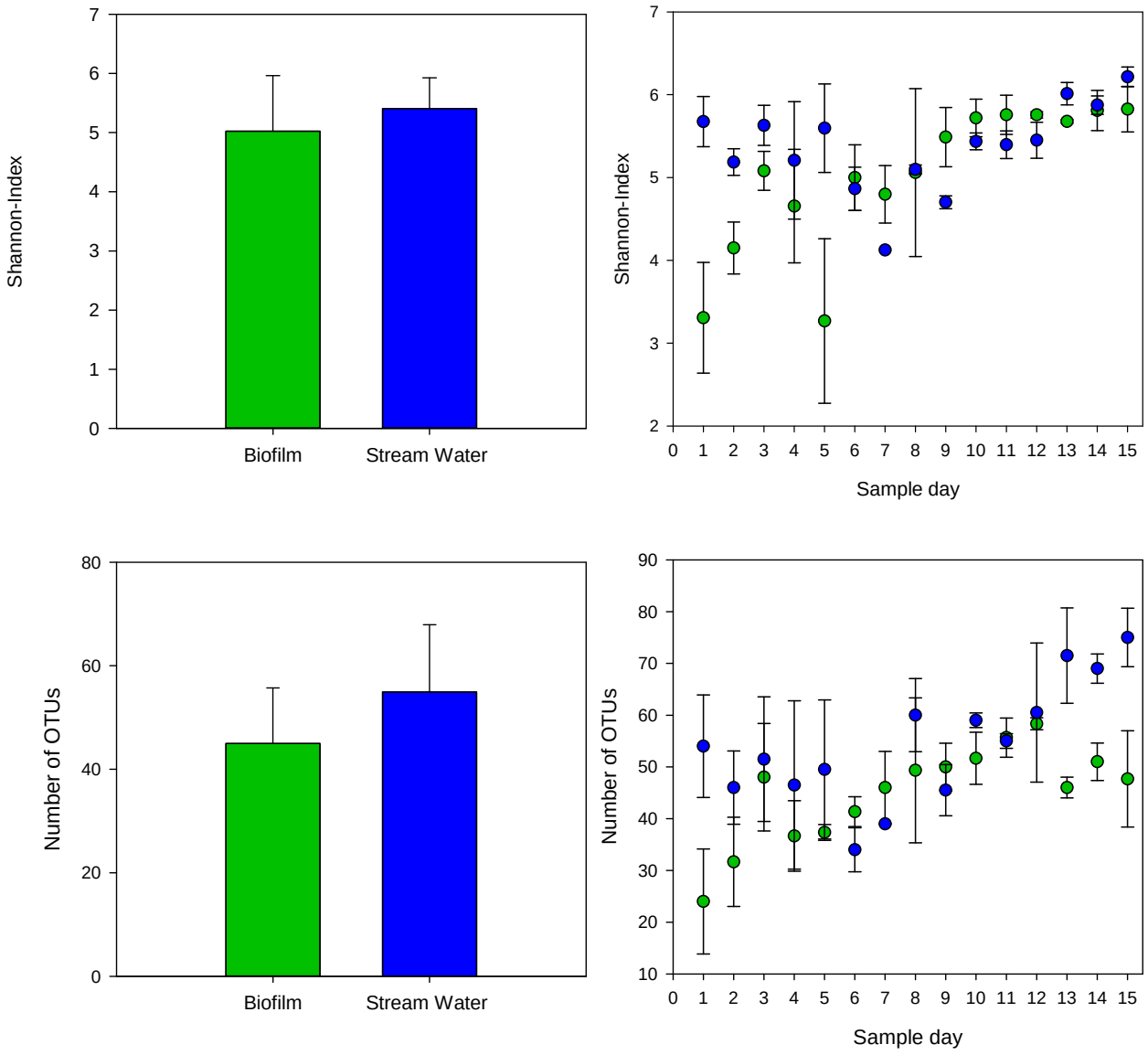

Figure 4: Shannon index (upper panel) and OTU richness (lower panel) of biofilm and the suspended communities as averages of all (left panel) and of daily replicates (right panel). The biofilm community is shown in green, the suspended community in blue. Error bars indicate the standard deviation from the average.

Rank-abundance curves

Rank abundance curves of the biofilm communities progressively changed from an early growth phase (sample day 1 to ca. sample day 5), characterized by a few very dominant OTUs and generally low richness, to a later growth phase (starting from approx. sample day 10), during which dominant OTUs accounted for a considerably smaller portion of the microbial community and the number of rare OTUs increased (Figure 5).

Initially, rank abundance curves of the biofilm and the suspended communities showed a clear difference, with less dominant OTUs and more rare ones in the suspended communities, but during the course of the experiment this difference disappeared. At the end of the experiment, dominant OTUs constituted a larger proportion of the suspended communities than of the biofilm communities. From day 6 of the experiment, the rank-abundance distributions of the biofilm communities had a lower slope than those of the suspended communities (Figure 6).Averaged over the whole experiment, the slopes of the regression models fitted to the rank-abundance curves were significantly ($t = -2.16$, d.f. = 72, $p < 0.05$) steeper in the suspended than in the biofilm communities.

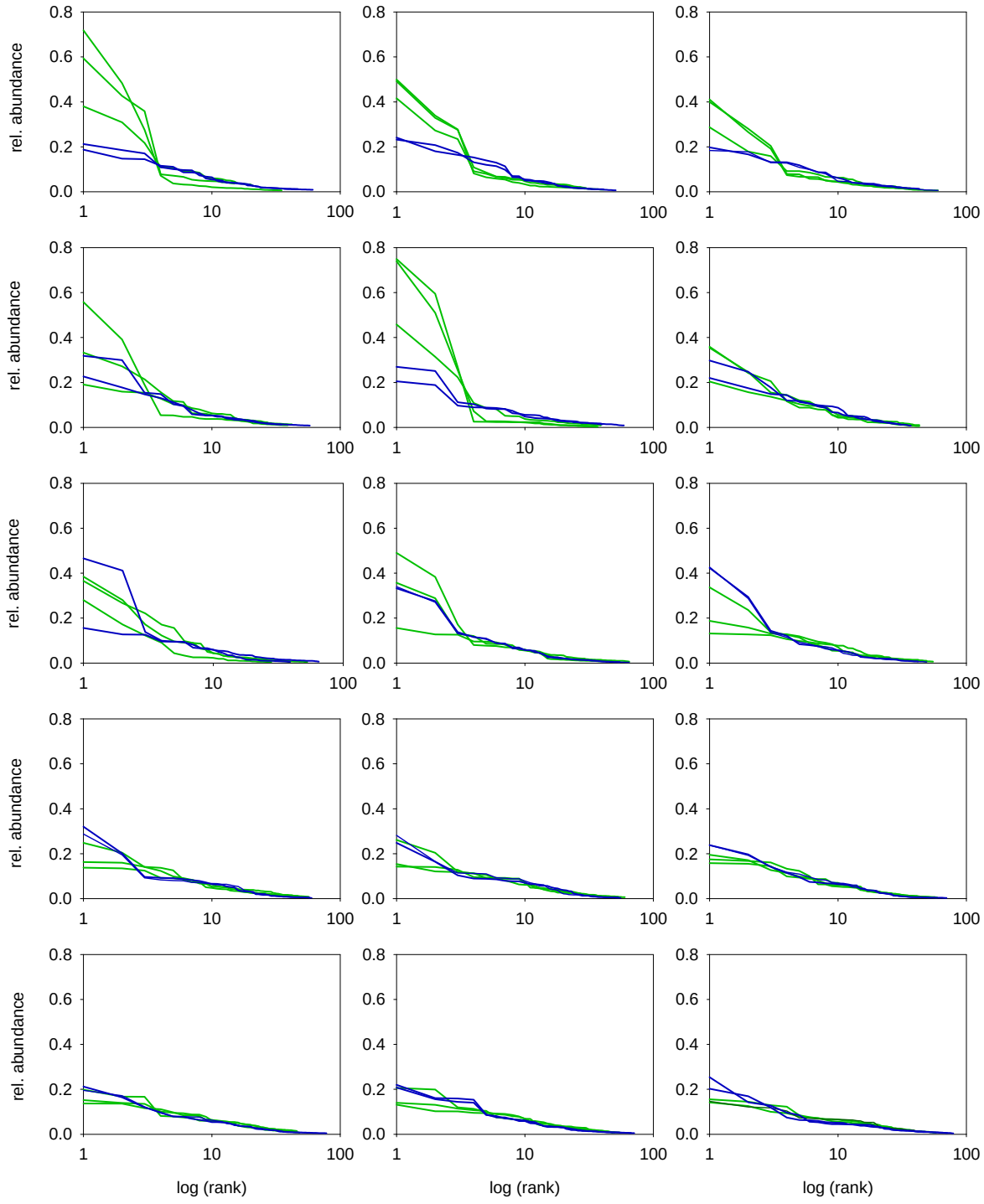

Figure 5: Rank abundance curves of biofilm (green) and suspended (blue) microbial communities from each sample day, starting from days 1-3 in the topmost row to days 13-15 in the bottommost row.

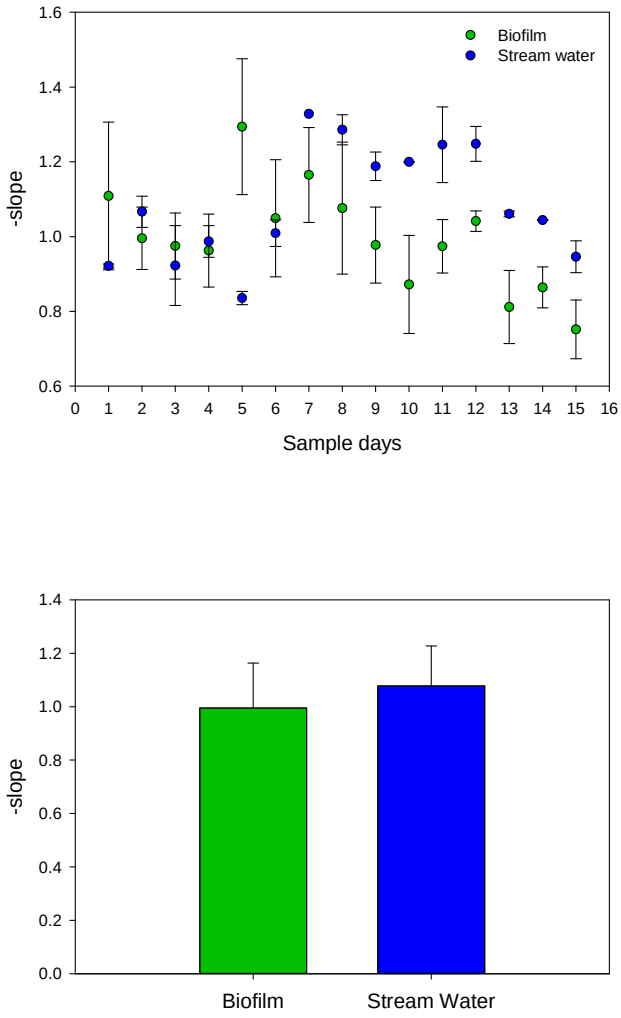

Figure 6: Average slope of biofilm and suspended communities for each sample day (upper panel) and of all rank abundance curves (lower panel). Error bars indicate the standard deviation from the average.

nMDS analyses

nMDS analyses were performed on both the Sørensen and the Bray-Curtis similarity matrices (Figure 7). Both nMDS plots revealed clear differences between biofilm and suspended communities, although these differences were less distinct in the nMDS analysis based on the presence/absence-based Sørensen similarity matrix. Both nMDS analyses showed a temporal progression in biofilm and suspended communities, with the exception of the suspended communities from day 14 and 15 in the nMDS analysis based on the Bray-Curtis similarity matrix. Partial Mantel statistics were performed to test for correlations between the suspended communities, the stream water communities and sampling day for both the Sørensen and the Bray-Curtis similarity matrices. The biofilm community composition was highly significantly correlated with sampling day (Sørensen index: $r=0.677$, $p=<0.001$; Bray-Curtis index: $r=0.715$, $p=<0.001$), but no significant correlation was found between the biofilm and the suspended communities (Sørensen index: $r=-0.001$, $p=0.47$; Bray-Curtis index: $r=0.108$, $p=0.23$).

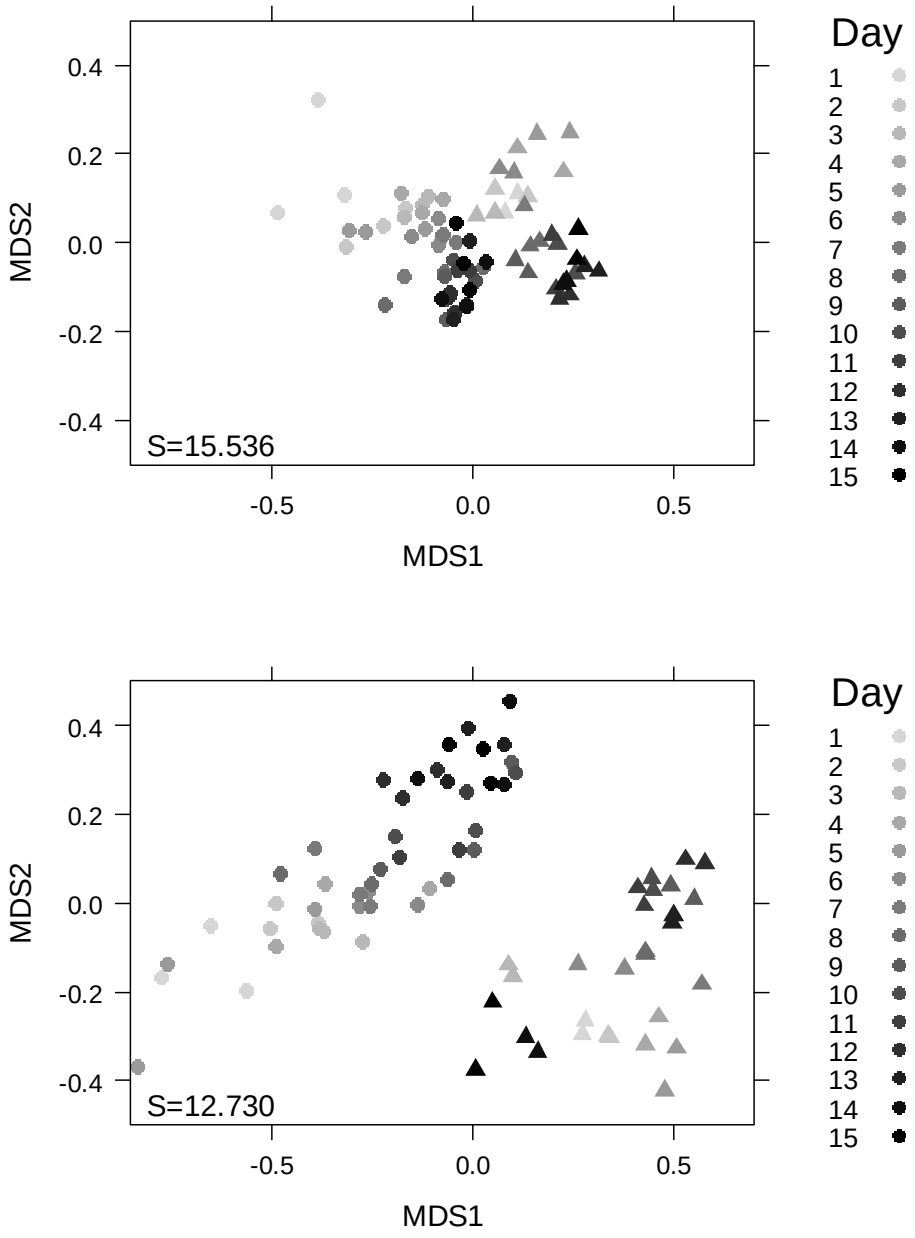

Figure 7: nMDS analysis of the composition of biofilm communities (circles) and suspended communities (triangles), calculated from the Sørensen index (above) and the Bray-Curtis index (below). Kruskal's standardized stress values (S) are given in per cent.

Discussion

Conceptual models predict that during biofilm development there is an initial phase of biomass accrual through colonization and growth, followed by a "loss phase" during which loss processes such as death, emigration, sloughing and grazing become dominant (Biggs 1996). Over the course of the experiment a steady increase in bacterial cell numbers as well as algal biomass in the biofilm could be observed. This increase in biomass indicates that an end of biomass accrual was not reached during the experiment. Hence the experiment only covers the initial stages of biofilm succession.

The T-RFLP analysis performed on the samples detected 202 OTUs with an average richness of 45 and 55 OTUs for biofilm and suspended stream water communities, respectively. In a comparable study (Besemer et al. 2012) found similar numbers of OTUs in biofilm and stream water microbial communities using T-RFLP. This same study found hundreds to thousands of OTUs per sample when using 454 pyrosequencing. This highlights the main problem of using T-RFLP data to measure diversity of microbial communities: since the vast majority of bacteria are rare and fall below the detection limit of T-RFLP (Blackwood et al. 2007, Bent and Forney 2008), diversity indices based on T-RFLP data do not reflect the real diversity of microbial communities. T-RFLP can, however, generate reliable patterns of community composition (Besemer et al. 2012) and provide insight into the diversity of the most abundant OTUs; in fact, T-RFLP is significantly more sensitive than, for instance, denaturing gradient gel electrophoresis (Moeseneder et al. 1999), which has been used to estimate successional changes in biofilm diversity (Jackson et al. 2001, Lyautey et al. 2005).

For plants and benthic algae a distinction between fast growing pioneer species, which colonize a site first and facilitate the establishment of new species, and slow growing, more competitive climax populations has been made (Swaine and Whitmore 1988, Biggs 1996). In this study, a small set of OTUs could be identified that make up a large proportion of the biofilm community during the early days of the experiment, after which their relative importance decreased over time, whereas another larger set of OTUs only appeared in the biofilm communities later on in the experiment (Figure 3). This is in accordance with Lyautey et al.

(2005), who could also identify pioneer and climax populations in bacterial biofilm communities. The most abundant OTUs occurred in communities of both habitats, albeit with different relative abundances (Table 1, Table 2). However, some of the OTUs were exclusive to either biofilm or stream water communities, notably a set of OTUs was identified, which accounted for considerable portions of the suspended community, but were never found in the biofilm community. Although the OTUs that were exclusively found in the biofilm must have originated from the stream water, many of these were probably present in the stream water at levels below the detection limit of T-RFLP analysis.

Richness and diversity were significantly higher in the suspended than in the biofilm community (Figure 4). Similar results with higher richness found in the suspended community were obtained by earlier studies (Martiny et al. 2003, Besemer et al. 2012, Wey et al. 2012) supporting the notion of stream water collecting microbes from multiple sources (Shmida and Wilson 1985, Leibold et al. 2004).

During biofilm succession an increase in OTU richness and diversity with time could be observed until day 12 (Figure 4), after which richness decreased slightly, while the Shannon index stagnated. Jackson et al. (2001) and Lyautey et al. (2005) also noted a reduction in OTU richness after the initial increase in richness. According to the conceptual model of biofilm succession developed by Jackson (2003), richness increases during the initial stage of biofilm development due to initial colonization by many bacterial species, followed by a decline as internal factors, such as competition begin to structure the community.

The rank-abundance curves of the biofilm community exhibited a steep slope between day 1 and day 5 of the experiment, after which the slope declined gradually (Figure 5). This suggests that at the beginning of the experiment, a few pioneer species dominated the community. As richness and diversity increased during succession, the slope of the rank-abundance distribution declined, due to a higher number of more evenly distributed OTUs. This is in accordance with an earlier biofilm study (Jackson et al. 2001). The slope of the biofilm rank-abundance curves became lower than that of the respective suspended communities after day 5, which indicates higher dominance of a few OTUs and a lower number of rare OTUs in the

suspended community (Figure 6). This is in contradiction to an earlier study based on 454 pyrosequencing, which found less dominant OTUs and a longer tail of very rare OTUs in stream water communities, compared to interstitial biofilm communities (Besemer et al. 2012). However, the limitations of the T-RFLP method should be kept in mind. It is reasonable to assume that this less sensitive method yielded a misleading picture of the structure of the microbial community (Blackwood et al. 2007, Bent and Forney 2008).

In the nMDS plots the suspended and the biofilm community are clearly separated (Figure 7). The fastest changes of biofilm community composition occurred during the first days of the experiment, as indicated by larger distances between samples in both the Sørensen-based and in the Bray-Curtis-based nMDS plots. However, not only the biofilm communities, but also the suspended communities exhibited a change in community composition over time, in roughly the same direction in the MDS analysis. This simultaneous change implies the question whether the change in the composition of the biofilm community was due to successional changes in the biofilm or caused by temporal dynamics of the suspended source community, i.e. allogenic changes. Partial Mantel tests revealed that while time significantly correlated with biofilm community composition, no significant correlation existed between the composition of the biofilm and the suspended microbial community. This indicates that biofilm community development does not simply reflect changes in the source community but exhibits autogenic successional changes. The finding that not all of the bacteria in the water column are able to grow in the biofilm suggests that the streambed habitat sorted OTUs able to invade biofilms and capable to cope with the local environmental conditions. The residence time of bacteria within the biofilm is higher than the residence time of bacteria suspended in the water column, increasing the importance of local environmental factors and lowering the impact of mass effects through immigration (Besemer et al. 2009, Besemer et al. 2012). My study therefore supports earlier findings that a combination of species sorting and autogenic successional processes plays a role in shaping the composition of biofilm communities (Besemer et al. 2009, Besemer et al. 2012).

In summary, my findings indicate that stream biofilm community structure is shaped by a combination of environmental conditions through species sorting and autogenic successional processes within the biofilm. Temporal variations of the source community suspended in the stream water showed no significant impact on biofilm community development. During biofilm succession, OTU-diversity and rank-abundance distribution patterns suggested an initial increase of richness and evenness, in accordance to conceptual models of biofilm succession (Jackson et al. 2001, Jackson 2003). This study therefore complements studies on biofilm succession (Jackson et al. 2001, Lyautey et al. 2005, Besemer et al. 2007) and metacommunity ecology in streams (Logue and Lindstrom 2008, Besemer et al. 2009).

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Zusammenfassung

Mikrobielle Biofilme in Fließgewässern entstehen durch die Anhaftung und das anschließende Wachstum von Zellen, die in der Wassersäule transportiert werden. In dieser Studie verglich ich die Zusammensetzung der mikrobiellen Gemeinschaft von Biofilm, der auf neuem Substrat wächst, mit jener der im Bachwasser suspendierten Gemeinschaft während der Anfangsstadien der Biofilmsukzession, um Informationen darüber zu gewinnen, wie die Biofilmgemeinschaft von der suspendierten Gemeinschaft während der Biofilmentwicklung geformt wird. Die dafür verwendete Methode war Terminal Restriction Length Polymorphism des 16S ribosmalen RNA-Gens. Die OTU-Richness und die Diversität waren in der suspendierten Gemeinschaft höher als in der Biofilmgemeinschaft. Im Verlauf der Sukzession nahmen die Richness und die Diversität der Biofilmgemeinschaft anfänglich zu, aber nach einigen Tagen nahm die Richness wieder leicht ab, während die Diversität stagnierte. Zu Beginn wiesen die Rang-Abundanz-Kurven der Biofilmgemeinschaft eine starke Steigung auf, die später allmählich abnahm. Die Zusammensetzung der mikrobiellen Gemeinschaft des Biofilms unterschied sich beträchtlich von der Zusammensetzung der suspendierten Gemeinschaft. Die Zusammensetzung der Biofilmgemeinschaft änderte sich im Laufe der Sukzession. Zeitliche Änderungen der im Bachwasser suspendierten Gemeinschaft zeigten keinen signifikanten Einfluss auf die Entwicklung der Biofilmgemeinschaft. Meine Ergebnisse legen den Schluss nahe, dass die Gemeinschaftsstruktur des Biofilms von einer Kombination aus Umweltfaktoren durch Species Sorting und autogene Sukzessionsprozesse innerhalb des Biofilms geformt wird.

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