Impact of the Flow Velocity and Sedimentation on Microbial Biofilms in the Stream Ilm (Thuringia / Germany)

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General Introduction

Introduction into stream ecosystems

Streams are linear definite one dimensional structures and several theoretical concepts aiming the stream ecology are existing. The River Continuum Concept (RCC) for instance describes streams as a continuous series of physical gradients and associated biotic adjustments indicating that the stream organisms interact with the longitudinal changing environment (Vannote et al., 1980). The products of streams are highly appropriate to be transported downstream than reaching the river bottom (Schönborn, 2003). Most of the organic matter available in streams is of allochthonious origin, as leaves from the riparian zones (99%). After entering the stream, allochtonious material undergoes three phases of processing: leaching, microbial colonization (conditioning) and fragmentation by physical forces and invertebrate feeding (Fisher and Likens, 1973). The amount of terrestric organic carbon transported or transformed by stream and river ecosystems world wide is about 2 Pg per year whereas the metabolic capacity in these fluvial systems may result from microbial attachements as biofilms (Battin et al., 2008).

Occurance of microbial biofilms and the economic impact

Biofilms are structured communities of bacteria, algae, cyanobacteria, fungi, and protozoa embedded in a polymeric (EPS) matrix (Lock et al., 1993). The first recorded observation of biofilms comes from Henrici (1933) who found that it is quite evident that for the most part water bacteria are not free floating organisms, but grow upon submerged surfaces. These cells develop structures that are morphologically and physiologically different from free living bacteria (Davies et al., 1998; Sauer and Camper, 2001).

Prokaryotes benefit from the association with surfaces by using cellular division of labor, accessing resources that cannot effectively be utilized by single cells, collectively defending against antagonists, and optimizing population survival by differentiating into distinct cell types (Beveridge et al., 1997; Shapiro, 1998; Ghigo, 2001).

The economic role of biofilms is significant. They foul the surfaces of ships, oil rigs, heat exchangers, water reticulation and hydro-electric systems etc. (Marshall and Blainey, 1991). Microbial biofilms are known for their resistance against antibiotics (Nickel et al., 1985;

Costerton et al., 1999; Purevdorj et al., 2002) and the protection against UV-radiation (Hodoki, 2005a). Harmful biofilms are abundant reaching from dental plaque (Kolenbrander and London, 1993; Kaplan et al., 2003) to *Pseudomonas aeruginosa* biofilms in the alveoli of fibrosis patients. On the other hand microbial biofilms can play a beneficial role in certain wastewater treatment operations (e.g. rotating biological contractors), natural stream purification, and specific fermentation processes (Bryers and Characklis, 1981).

Formation and structural appearances of microbial biofilms in different environments

Biofilm formation begins with the interaction of planktonic cells with the surface which become sessil as a result of phenotypic changes in the bacteria. Cell-cell communication, a response to microbial population density (Quorum Sensing, QS) may play a role in this initial attachment process (Sauer and Camper, 2001). QS has been described for single bacterial species biofilms (Fuqua et al., 2001) and between different bacterial species (Whitely, 2001).

Development of biofilm can be recorded in 5 steps, (1) initial attachment with the surface, (2) production of the exopolymeric matrix (EPS) resulting in a more firmly adhered “irreversible” attachment, (3) early development of biofilm architecture, (4) biofilm maturation, (5) dispersion of single cells from the biofilm (Stoodley et al., 2002). Behind the initial attachment different mechanisms can occur. For *P. aerugenosa* typ IV pili-mediated twitching motility plays a key role (O'Toole and Kolter, 1998). A second mechanism is the binary division of attached cells, daughter cells spread outward and upward from the attachment surface and build cluster (Heydorn et al., 2000). A third mechanism of aggregation is the recruitment of cells from the bulk fluid to the developing biofilm (Tolker-Nielsen and Molin, 2000). The main hallmark of an “irreversible” attached biofilm is the EPS matrix which surrounds the bacteria and attaches them to a substratum. Microbial EPS are biosynthetic polymers that can be highly diverse in chemical composition and may include substituted and unsubstituted polysaccharides and proteins, nucleic acids, and phospholipids (Wingender et al., 1999). The ability of bacteria to secrete polymeric substances is impressive. It has been calculated that a single cell of *Azotobacter sp.* can produce enough EPS to coat more than 500 particles (0.4 µm diam) per day (Underwood and Paterson, 1995). During the maturation phase a complex architecture with pores, channels and a redistribution of bacteria away from the substratum occurs (Davies et al., 1998). Finally cells and entire biofilms can detach from the surface in response to starvation which allows bacteria to search for nutrient-rich habitats

(O'Toole et al., 2000). Biofilms are not rigid fixed to the surface. Single cells, cell cluster and entire biofilms can flow over porous media with velocities up to 1 mm per hour (Hall-Stoodley et al., 2004).

Three conceptual models of biofilm architecture exist (i) the water-channel model, with a proliferation of mushroom forms attached by stalks of EPS and microorganisms, water channels penetrate almost to the base of the film (Costerton et al., 1995), (ii) the heterogeneous mosaic biofilm model, an extreme form of the water-channel model, were microorganisms form stacks attached to the substratum but well separated to the neighbors, and (iii) the dense biofilm models used for dental plaque and in-dwelling medical catheters reviewed in Wimpenny and Colasanti (1997). These models are based on bacterial biofilms whereas natural biofilms grown in aquatic environments with more “trophic levels” form a more complex building with hexagonal structures and ripples dependent on the environmental conditions (Okabe et al., 1998; Battin et al., 2003).

The biofilm matrix exhibits great microheterogeneity and is a dynamic environment in which numerous microenvironments exist. The component microbial cells appear to reach homeostasis and are optimally organized to use all available nutrients (Sutherland, 2001) (Fig 1).

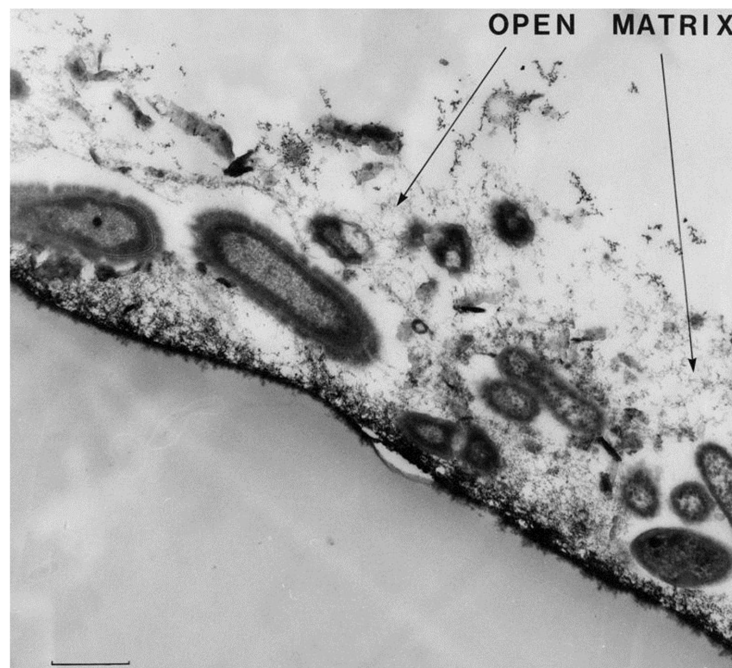

Fig.1. **Transmission electronic microscope images of a biofilm on coarse sediment in the Stream Breitenbach (Germany, Hesse). Shown is a loose matrix of fibrillar polysaccarides. Photo by Ditner and Lock (University of Wales, Bangor).**

Colonization patterns of bacteria and algae in aquatic environments

Colonization of surfaces in natural aquatic systems displays a successional pattern. Initial biofilm colonizers are bacteria and algae. Within the bacterial community early colonization is dominated by *Betaproteobacteria* (BETA) followed by *Alphaproteobacteria* (ALPHA). Bacteria from the *Cytophaga-Flovobacterium* (CF) group appear at later stages (Manz et al., 1999). The proportion of *Actinobacteria* (HGC) usually account for less than 1% (Araya et al., 2003) of the community.

Diatoms represent the main compound in algal assemblages in riverine biofilms (Roberts et al., 2004; Patil and Anil, 2005). Coccal green algae *Chlorococcus* are highly competitive in early biofilm development and appear as primar colonizers, in later developmental state diatoms are more dominant (Sekar et al., 2002; Sekar et al., 2004), whereas low profile forms appear first followed by long-stalked and large-rosette diatoms. Finally filamentous greenalge and cyanobacteria appear (Hoagland et al., 1982; Sekar et al., 2004).

The relationship of bacteria and algae in biofilms is positive. Both organism groups encourage the immigration of the other (Lock, 1993; Rier and Stevenson, 2001; Hodoki, 2005b) and a direct competition for nutrients could not be detected so far (Currie, 1990; Carr et al., 2005). Algae and bacteria have specific abilities for nutrients and other organic and inorganic compounds, but biofilm thickness may affect these abilities, both through a decrease in diffusion and by enhancing recycling within biofilms (Sabater et al., 2002).

In streams grazing interferes with the relative efficiency of biofilms, by simplifying the composition of the biofilm community and by decreasing the amount of sorption and uptake of the biofilm (Sabater et al., 2002). In some cases biofilm bacteria are successfully adapted to situations of protozoan grazing. In early biofilm formations of *Pseudomonas aerugenosa* the bacteria are resistant against protozoan (*Rhynchomonas nasuta*) grazing due to QS whereas organinms in mature biofilms produce toxicants against protozoans (Matz et al., 2005).

Metabolic activity of biofilm organisms and the capacity of the EPS matrix

In addition to numeric dominance attached bacteria in natural environments have been found to be metabolically more active than planktonic bacteria (Van Loosdrecht et al., 1990).

Under functional aspects, CF degenerates biopolymeres as cellulose, chitin, and pectine, ALPHA are often found in environments with low sediment organic matter content, low dissolved organic carbon (DOC) content, and low nitrite/nitrate concentrations. BETA and *Gammaproteobacteria* (GAMMA) are involved in nitrogen cycling and be found in

environments with high DOC and nitrite/nitrate concentrations (Teske et al., 1994; Kirchman, 2002; Zwisler et al., 2003; Gao et al., 2005).
The productivity within biofilms is dependent on the availability of external carbon supplies and the productivity of algae which release extracellular organic carbon that can be rapidly utilized by bacteria and so periphyton plays a key role in carbon and nutrient dynamics of stream ecosystems (Sundh and Bell, 1992; Romani and Sabater, 1999; Rier and Stevenson, 2002).
EPS (extracellular polymeric substances) released by bacteria and algae perform a wide range of important functions in aquatic and terrestrial systems (Hirst et al., 2003). It acts as a site of entrapment for soluble and particulate matter and the accumulation of extracellular enzymes (Sinsabaugh et al., 1991; Lock, 1993). In streams and rivers biofilms are seen as the major sites of carbon cycling (Romani et al., 2004). Biofilms react slowly to dissolved organic matter (DOM) reductions in the river water, suggesting the presence of a substantial organic matter reservoir within the biofilm. So the matrix provides a buffer against changing organic nutrients (Lock, 1993; Freeman and Lock, 1995; Sutherland, 2001).
Cellulose and hemicellulose are the main high molecular weight carbon sources in a stream (Atlas and Bartha, 1987) and the extracellular enzymes beta-glucosidase and beta-xylosidase are involved in the degradation of both (Chrost, 1990). Beta-glycosidase is predominantly produced by bacteria and fungi in aquatic environments (Hoppe, 1983). Phosphate is one of the limiting nutrient in rivers (Mohamed et al., 1998). Alkaline phosphatase catalyses the hydrolysis of a variety of phosphate esters (Barman, 1969). It has been demonstrated in filtrates of algal cultures, in phytoplankton, bacteria, protozoans, and zooplankton as well as dissolved (extracellular) in aquatic environments. As well as for carbon attached bacteria have disproportionately higher protein-degrading activities than planktonic bacteria. Leucine aminopeptidase is associated with heterotrophic bacteria. It has been shown that a high concentration of algal proteins after blooms induces a release of this enzyme (Chrost, 1989).
Biofilm organisms itself serve as an important nutrient source for higher organisms as protists (Hunt and Parry, 1998; Lawrence, 2002) and macroinvertebrates (Burns and Walker, 2000).

Flow velocity: The main force for biofilm formation and function in streams

The surfaces of bacteria are highly interactive with their environment (Beveridge et al., 1997). In streams and rivers the biofilm structure is related to the current velocity. Initial biofilms under flow conditions in flumes transform into ripple-like structures and

quasihexagonal networks due to the prominence of pennate diatoms orientated along their axes to form the hexagons. Biofilms that develop under slow velocities are thicker and have larger surface sinuosity and higher areal densities than those grown under higher velocities (Battin et al., 2003). A honeycomb structure of biofilm makes it more resistance to water flow (Okabe et al., 1998). Water velocity influences the uptake of nutrients. Under increasing flow velocity the spiral length of single nutrients decreases (Lock, 1993). The DOM spiralling model suggests that the uptake lengths for DOM molecules increase with decreasing flow velocity (Kaplan and Newbold, 2004). In biofilms, problems of slow diffusive transport under low flow velocity conditions, and high biofilm densities can reduce nutrient availability as phosphor. Therefore, cellular excretion of algae may be an important phosphorous source for biofilm bacteria, which may lack the same capacity algae have for sequestering external sources of phosphorous. Since bacteria have phosphate uptake kinetics superior to those of algae, bacterial cells within biofilms may benefit from the phosphatase activity of nearby algal cells (Espeland and Wetzel, 2001). But the current also has a direct impact on the organisms. Bentic algal biomass is tightly related to fluctuations of the discharge (Romani and Sabater, 1999). Diatom immigration rates are greater in areas protected from the direct stream flow. Occasional diatom densities are more than three times as dense in slow current (15 cm per s) than under fast flow (40 cm per s) (Lock, 1993). Finally high flow velocities and shear stress can lead to biofilm detachement and sediment relocation leaving empty spaces for colonization (Blenkinsopp and Lock, 1994).
In the RCC a close interactive relation of the biota to the longitudinal changing environment is suggested. The habitats offer different and changing resources and the communities may reflect the instable requirements. For invertebrate communities a specialization of organism groups to the different organic matter sources offered and so an adaptation to special locations in the stream systems are already demonstrated (Vannote et al., 1980). However whether microbial biofilms reflect the ecological environment in a stream is in question so far.

Regulation of the flow velocity in stream by small dams

World-wide more than half of the large river systems are affected by fragmentation by dams (Nilsson et al., 2005). Generally dams alter the down-stream flux of water and sediment, which modifies biogeochemical cycles, change water temperature, influences organismal bioenergetics and hinders biotic exchanges. Very distinct is the impact of dams as sedimentation traps (Poff and Hart, 2002; Stanley and Doyle, 2002). The fact, that many streams are disrupted by more than one weir the river continuum as proposed by Vannote

(1980) becomes highly fragmented which possibly leads to a serial pattern of small continuums between the weirs.

An important theoretical concept describing modern riverine systems altered by fragmentation, the Serial Discontinuity Concept (Ward and Stanford, 1983) predicts that dams can shift stream ecosystem structure upstream or downstream depending on dam size. Low head dams often do not completely act as barriers (Benstead et al., 1999) and effects on water characteristics often are not predictable (Santucci et al., 2005; Nichols et al., 2006). Fish and the macroinvertebrate community seem to be more affected by impoundments (Growns and Growns, 2001; Grubbs and Taylor, 2004; Santucci et al., 2005) than periphytic diatoms (Growns and Growns, 2001; Nichols et al., 2006). How fragmentation and an increase of sedimentation affect the microbial community as biofilms which are highly effective in organic matter conditioning is unclear so far.

Small dams provide sites with predictable flow conditions with slow flow velocities in front and fast flow velocities behind the barrier. Because of the close proximity of the sites the nutrient conditions are expected to be equally. Thus small dams are excellent habitates for investigations of the impact of the flow velocity and sedimentation on microbial biofilms in a natural stream. The Stream Ilm (Thuringia, Germany) is highly fragmentated by more than 50 small dams and was the study object of a graduated school founded by the DFG (266/3 at the Friedrich-Schiller-University Jena).

In earlier investigations of the stream the impact of a low-head dam on the macroinvertebrate community and their resources was only local and the occurrence of more than 50 dams along the stream did not alter the zonation of invertebrates along the stream (Arle, 2005).

Thesis structure

In the present work microbial biofilms grown on glass slides exposed in the 3rd order Stream Ilm has been investigated. In contrast to flumes and rotation reactors which are often used for biofilm investigations, a natural stream provides a permanent change in large (floods) and small (drought) scale fluids as well as laminar and turbulent flow. The permanent changes of the flow motions as well as the temperature and nutrition may affect the microbial community structure and function.

The colonization pattern and community structure of bacteria and algae and the extracellular enzyme activity of the community have been investigated. In a second experiment the focus was on the impact of the flow velocity on the structure and function of the biofilm

community. Biofilm thickness was used to prove the matrix stability at different flow velocities in the stream and organic and inorganic matter content which may provide important resources for the community were measured. In a flow channel experiment under laboratory conditions the impact of different amounts of inorganic sediment particles on the bacterial community structure, algal biomass, biofilm thickness, and the turnover of an added carbon source have been investigated. Finally the abundance of bacteria at three different weirs in close approximaty was investigated to prove the impact of fragmentation in the stream.

Hypotheses:

1. Colonization of empty spaces occurs rapidly by bacteria and algae.
2. Slow flow velocities enhance sedimentation which changes the community structure and metabolic activities of biofilm organisms.
3. Stability of biofilms is dependent on the magnitude of the current and the amount of inorganic sediment incorporated.
4. Smal weirs provide sites of nutrient accumulation in a stream entailed by sedimentation which in turn enhance biofilm turnover.

The present work is based on the following questions:

1. Which time interval is needed for early colonizers to contribute to the degradation of organic matter in streams?
2. Does sedimentation entailed by slow flow velocities change the biofilm community structure and function in a stream?
3. Does the incorporation of increasing amounts of sediment particles modify the biofilm structure and the carbon turnover of microbial biofilms?
4. Do weirs affect the microbial community in biofilms and which are the consequences?

The first chapter “**Pioneering Bacterial and Algal Communities and Potential Extracellular Enzyme Activities of Stream Biofilms”** gives an insight into the initial biofilm formation at a natural sampling site in the Stream Ilm (Thuringia, Germany). Glass slides where exposed and the bacterial and algal community has been investigated. Measurements of the extracellular enzymatic activity represent the functional aspect of the community (Qustion 1).

In the second chapter “**Distinct Flow Velocities and Sedimentation Affect Microbial Biofilms in a Stream”** the main focus was on the impact of the flow velocity and sedimentation of inorganic matter on the structure and function of the biofilm community. Besides the microbial community structure and the enzymatic activities the biofilm thickness as a parameter for biofilm stability has been measured. Glass slides for biofilm sampling were exposed in front and behind a weir to ensure that the flow conditions at the natural stream remained predictable during the sampling season (Question 2).

The chapter three, “**Influence of Incorporated Inorganic Sediment on Stream Biofilms**” gives an insight into the impact of sedimentation on the structure and function of biofilms in flow channels. Besides the bacterial community structure the consumption rate of an added carbon sorce as well as the stability of the biofilm matrix at high flow velocities has been proved (Question 3).

In chapter four “**Enhanced Abundances of Biofilm Bacteria at Small Weirs in the Stream Ilm (Thuringia, Germany)**” the impact of three different weirs in a close proximity on the abundance of the biofilm bacteria has been investigated (Question 4).

The chapter five **“Querying the Obvious: Lessons from a Degraded Stream”** gives an outline for restoration ecology purposes. The question whether weirs provide a disturbance or a benefit for a stream ecosystem has been discussed (Question 4).

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Pioneering bacterial and algal communities and potential extracellular enzyme activities of stream biofilms

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Abstract

Microbial biofilms are important for the turnover of organic matter in small streams. A rapid colonization of epilithic surface will become more important given the predicted increase of flood events. Here, we elucidated the pioneering community structure and activity of stream biofilms. Colonization of glass slides exposed in a small stream for 1, 4, 8, 12, and 24 hours were compared with those exposed for 7 days or 5 months. 40,000 bacterial cells and 10 algae per cm^2 attached to the glass slides within 1 hour of exposure. Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) demonstrated that the pioneer community which settled within 12 hours was dominated by *Cytophaga-Flavobacteria*. Later stages were characterized by an enrichment of *Gammaproteobacteria* and *Betaproteobacteria* especially after 24 hours. Green algae dominated the pioneering algal groups but were outnumbered by filamentous algae after the attachment period. Potential activity of alkaline phosphatase was already detected after 4 hours, β-glucosidase after 8 hours, and β-xylosidase only after 7 days of biofilm formation. Thus, biofilm formation occurred rapidly and the functionality of the assemblages was given within few hours. However, the potential activity ratios of β-xylosidase:β-glucosidase suggested that initial biofilms relied more on autochthonous than on allochthonous carbon sources in contrast to mature biofilms.

1 **Introduction**

2 Microbial biofilms, i.e. assemblages of bacteria, archaea, fungi, algae, and protozoans, are
3 formed at any submerged surface in rivers, such as stones, plants, and roots (Zubkov &
4 Sleigh, 1999). Structure and function of biofilms are dependent on the environmental
5 conditions given (Beyenal & Lewandowski, 2002; Crump *et al.*, 2003; Stoodley *et al.*, 2005).
6 In streams, the flow velocity is the primary physical force shaping biofilm colonization
7 dynamics and biofilm structure-function coupling (Battin *et al.*, 2003). Under laminar flow
8 cell transport to the surface and mass transfer of nutrients are the limiting steps. Thus, the
9 extent of biofilm growth increases with increasing flow velocity (Costerton *et al.*, 1995;
10 Characklis, 2009). However, high flow velocities under flood events can cause a significant
11 biofilm removal (Marxsen, 2001). Sediment fractions which are relocated during floods
12 provide new epilithic surfaces for colonization. Grazing by invertebrates like snails will also
13 yield empty surfaces (Sheldon & Walker, 1997). Thus, stream biofilms are subjected to
14 frequent disturbances and stable late growth phases like in controlled microcosm experiments
15 (Besemer *et al.*, 2007) will seldom reached by the biofilm community.

16 Colonization of surfaces displays a successional pattern. Initial biofilm colonizers in
17 streams are bacteria, which reach abundances up to 0.2 x 10^9 cells cm^{-2} after 2 days (Battin *et*
18 *al.*, 2003). The community structure of bacteria in aquatic environments is mainly represented
19 by *Alpha*-, *Beta*-, and *Gammaproteobacteria, Cytophaga-Flavobacteria* and *Actinobacteria*
20 (Glöckner *et al.*, 2000; Kirchman *et al.*, 2004). The proportion of these bacterial groups can
21 change over time according to environmental factors. In stream biofilms *Betaproteobacteria*
22 are early colonizers whereas in mature states *Alphaproteobacteria* and *Cytophaga-*
23 *Flavobacteria* dominate (Manz *et al.*, 1999).

24 The dominant algae in riverine biofilms are diatoms (Roberts *et al.*, 2004; Patil &
25 Anil, 2005). Pennate diatoms are a major component in mature biofilms and filamentous
26 algae oscillating in the water flow can develop in these biofilms (Battin *et al.*, 2003). Initial
27 colonizers on plexiglas surfaces in a fresh water reservoir are small green algae followed by
28 colonial green algae and diatoms, whereas filamentous green algae predominate after 10 days
29 (Sekar *et al.*, 2004). Only few studies have investigated the combined succession of bacteria
30 and algae during the initial colonization phase in streams (Sobczak & Burton, 1996; Battin *et*
31 *al.*, 2003; Besemer *et al.*, 2007). However, in these studies sampling intervals started after
32 more than one day which excluded the initial colonization phase. Studies focusing on marine
33 biofilm formation demonstrate a change in the bacterial community within the first 9 hours of

colonization indicating that pioneering organisms have to be identified with much finer temporal resolution (Lee *et al.*, 2008).

Biofilms are hot spots for the turnover of organic matter in small streams (Geesey *et al.*, 1978). The biofilm matrix permits the storage of nutrients and extracellular enzymes (Lock, 1994). These extracellular enzymes that can be free or bound to the cell (Wetzel, 1991) hydrolyze high-molecular-weight (HMW) organic compounds into smaller molecules, which than are available for microbial uptake. Their activity is regulated by catabolic processes (Chrost, 1989) and can be correlated with the relative abundance of different bacteria groups (Kirchman *et al.*, 2004). Indeed no single species can express all extracellular enzymes with high activity, and these results in variation of extracellular enzyme activities among bacterial species (Castillo, 2000). Particularly bacteria from the *Cytophaga-Flavobacteria*-cluster may have a specialized role in dissolved organic matter (DOM) uptake though the degradation of biopolymers such as cellulose and chitin (Kirchman, 2002). The degradation of organic matter is further influenced by the bacteria to algae biomass ratio in biofilms, because the extracellular enzyme activity is higher in autotrophic biofilms than in heterotrophic ones (Romani & Sabater, 2000).

Since floods and drying events will occur more frequently in the future (Labat *et al.*, 2004), abrasions and colonization of epilithic surfaces will become more important in streams. This poses the question, which time interval is needed for early colonizers to contribute to the degradation of organic matter in streams. Thus, the goal of this study was to investigate the structure of the pioneering community of stream biofilms and to link the succession of bacterial and algal communities during the first 24 hours of biofilm formation with biofilm extracellular enzyme activities. This initial colonization phase was compared with the community structure and function of a seven days and a five months old biofilm.

Material and methods

Sampling

Biofilm samples were taken in the third order stream Ilm at Manebach (50°44′58′′N, 11°02′14′′E; Thuringia, Germany). The Ilm is a typical mountain stream which arises about 800 m above sea level in the northern part of the Thuringian forest. The reach is situated at the head water region with a mean discharge of 2.45 $m^3 s^{-1}$. Sampling cylinders of perforated (perforation diameter 0.8 cm) stainless steal (cylinder diameter 8 cm, length 16 cm) for 6 glass slides (Fig. 1 A) (modified after Marxsen, 1982) were exposed horizontally on tubes

1 near the streambed (Fig. 1 B). The cylinders had to be used to avoid destruction of the glass
2 slides by moving gravel. The flow velocity inside the cylinders is reduced to approximately
3 50% due to the flow resistance of the cylinders (Risse-Buhl & Küsel, 2009). Exposure of the
4 cylinders to the stream water 5 months (March 2005), 7 days and 1 day, as well as 12, 8, 4,
5 and 1 hours before sampling on August 17th 2005. Six sampling cylinders containing 36 glass
6 slides were sampled per time point. The period of March-August was selected to minimize the
7 likelihood of flood events. The main sampling period was done in the summer to ensure the
8 presence of algae.

9

10 **Water sample analyses**

11 Triplicate samples from the water column were filtered (Whatman, polycarbonate, 0.45 µm)
12 during the period of February 2003 to August 2005 for measurements of dissolved organic
13 carbon (DOC), ammonia (NH^{4+}), nitrate (NO_3^-) and orthophosphate (PO_4^{3-}). Concentrations
14 of PO_4^{3-} were determined with the ascorbic acid method (Clesceri, 1998), NO^{3-} concentrations
15 with the ultraviolet spectrophotometric screening method (Clesceri *et al.*, 1999) (Uvikon 931,
16 Kontron Instruments, Italy). Ammonium was determined spectrophotometrically (Gadkari,
17 1984) (Uvikon 931, Kontron Instruments, Italy). DOC was measured using a DIMATOC 100
18 infrared spectrometer (Dimatec Analysentechnik GmbH, Germany). Oxygen content, pH,
19 salinity, conductivity and turbidity were measured in the water column (Horiba U 10,
20 Multiparameter, Water Quality Meter, Kyoto, Japan). Flow velocity was measured near the
21 tubes after removing the cylinders in 5 cm steps beginning from the river bottom to the top of
22 the water column (Flowmate, MARSH McBirney, USA).

23

24 **Biofilm sample analyses**

25 Biofilms from triplicate glass slides were scraped off and weighed after drying for 48 h at
26 60°C and a second time following combustion for 4 h at 550 °C. For Chl *a* content
27 measurements, biofilms were also scraped off and frozen in liquid nitrogen for
28 transportatation. Chl a content was determined spectrophotometrically (Uvikon 931, Kontron
29 Instruments, Germany) following an acetone extraction (Clesceri, 1998) and incubation at
30 6°C in the dark for 12 h.

Bacterial community structure

To estimate the abundance of biofilm prokaryotes, biofilms (5-6 replicates) were scraped from the glass slides using sterile glass slides and kept in 50 ml Greiner tubes containing 35 ml prefiltered stream water (Whatman, polycarbonate, 0.2 µm pore size) and paraformaldehyde at a final concentration of 4%. To estimate the abundance of water column prokaryotes, three replicates of 50 ml stream water were fixed with paraformaldehyde (4% final concentration). Abundance of bacteria belonging to different groups were determined by catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) (Pernthaler et al., 2004) after sonification of the biofilm samples for 1 min (Sonopuls HD2200, Bandelin, Korea), because determinations with fluorescence in situ hybridization (FISH) showed very low efficiency and poor reproducibility (data not shown). Samples were filtered onto polycarbonate filters (GTTP, Millipore, Eschborn, Germany, pore size 0.2 µm) and attached with low gelling agarose 0.1%. Cells were permeabilisized with lysozyme (10 mg ml^{-1}) and achromopeptidase (60 U ml^{-1}). Horseradish peroxidase (HRP) labeled oligonucleotide sequences (Biomers, Ulm) specific for the domain Bacteria (Amann et al., 1990), *Alpha,*- *Beta*- and *Gammaproteobacteria* (Manz et al., 1992), *Actinobacteria* (Roller et al., 1994; Berchtold et al., 1999), and *Cytophago-Flavobacteria* (Manz et al., 1994) (Table S1) were linked with $Alexa_{488}$ as fluorochromic dye. Finally filters were embedded into a glycerol-PBS-DAPI (1 µg $µl^{-1}$) mixture and cells were counted at 1000x magnification [Zeiss Axioplan, 450-490 FT 510 LP 515 ($Alexa_{488}$) and G 365 FT 395 LP 420 (DAPI)].

Algal community

Algal morphotypes (pennate, central and filamentous diatoms, coccale and filamentous green algae) were quantified microscopally (Ettl, 1983; Komarék & Fott, 1983; Ettl & Gärtner, 1988; Kramer, 2000) after staining with Lugol's solution at 100x magnification (Zeiss-Axiovert) (Utermöhl, 1958). Cells were differentiated as living and dead according to chlorophyll content, which was visible under the light microscope. Those cells completely filled with the chloroplast were counted as live, whereas those cells with a shrunken or no visible chloroplast were counted as dead.

Extracellular enzyme activities

Potential activities of β-glucosidase, β-xylosidase and alkaline phosphatase were measured with artificial 4-methylumbelliferyl (MUF) substrates (MUF-ß-D-glycoside, MUF-ß-D-

xyloside, MUF-phosphate (Sigma)). To 100 mg of substrate 2 ml of hydroximethylether (Sigma) were added in sterile Greiner-tubes to facilitate its dissolution. Fifty ml of autoclaved distilled water was added and the solution was kept in the freezer (-20°C) until use. Three replicates of biofilm on the glass slides at each time step were transferred within minutes after sampling into 50 ml Greiner tubes with filtered (Whatman glass fiber, 0.2 µm pore size) and autoclaved river water containing the artificial substrate (0.3 mmol l^{-1}) and incubated for two hours. Fluorescence was determined after adding 1 mL of 0.05 M glycine buffer (pH 10.4) (for 100 mL 80.35 mL NaOH 0.2 M and 19.64 mL Glycine 0.2 M) to stop the reaction and enhance fluorescence that was measured with a fluorescence-spectrophotometer (Perkin Elmer, LS 50 B; em 450 nm, ex 365 nm, slit 2.5). Quantification was achieved by calibration with standard MUF solutions (1-10 µmol L^{-1}) (Hoppe, 1983).

Statistical analyses

Concentration of nutrients and environmental variables were analyzed using one way ANOVA (SigmaStat). Data of bacterial and algal abundance were analyzed using pairwise analysis (SigmaStat).

Results

Environmental parameters

Flow velocity was much lower in August compared to March 2005 (Table 1). In addition, the discharge with 0.5 m^3 s^{-1} in August was below the mean annual discharge (2.44 m^3 s^{-1}, gauge Gräfinau-Angstädt; data provided by the Thüringer Landesanstalt für Umwelt und Geologie, Jena). No flood or extreme drying event occurred during March and August 2005. The concentrations of nutrients in the stream water were low as expected for a mountain stream. Ammonia and orthophosphate concentrations decreased during the exposure period of five months. In contrast, DOC concentrations were elevated in August. In general, all parameters measured during the time of exposure were in the normal range of those obtained during a 2.5 year period. Seven flood events with discharges ranging from 10 up to 46 m^3 s^{-1} and one extreme drying event with a discharge of only 0.1 m^3 s^{-1} occurred during this 2.5 year period.

1 **Initial substratum colonization**
2 After 1 hour of exposure in August 2005, approximately 40,000 DAPI stained cells and 10
3 live algae per cm² were detected on the glass slides indicating a rapid colonization of new
4 substrata. After 12 hours of exposure, approximately 88,000 DAPI stained cells and 82 live
5 algae per cm² were detected. DAPI counts and abundance of algae increased 16- and 10-fold,
6 respectively, between 24 hours and 7 days (168 hours) of exposure (Fig. 2 A, B). After 5
7 months (3672 hours) of exposure the abundances increased further 8- and 10–fold,
8 respectively. The abundance of both groups was significantly correlated ($P<0.001$). In
9 general, Chl *a* contents (Fig. 2 C), organic matter (Fig. 2 D) and biofilm dry mass (data not
10 shown) increased over the exposure time. Chl *a* contents were stable during the first 12 hours
11 of exposure, although the abundance of algae steadily increased. The increase of biofilm dry
12 mass between 7 days (168 hours) and 5 months (3672 hours) of exposure was mainly due to
13 the increase of organic matter, because the amount of seston was stable.

14

15 **Bacterial community structure**
16 The proportion of bacterial cells that hybridized with the probe specific for Eubacteria relative
17 to the DAPI counts varied between 35 and 75%. Variations did not correlate with increasing
18 time of substratum exposure. This discrepancy might be caused by the low CARD-FISH
19 efficiency and /or the presence of archarea picoeucaryote cells not accounted in this study.
20 With the exception of *Gammaproteobacteria*, all specific groups addressed with probes were
21 detected after 1 hour of exposure (Table 2). Cells that hybridized with the *Cytophaga-*
22 *Flavobacteria* probe were the dominant pioneering group after 1, 4, and 8 hours of exposure.
23 Only in mature biofilms (5 months) this group was outnumbered by *Actinobacteria* and
24 *Betaproteobacteria*. The abundances of all groups did not show an increasing trend during the
25 first 24 hours of exposure. Between 24 hours and 7 days of exposure, cell abundances
26 increased 8 to 130 fold. The mean relative proportion of other cells which could be not
27 identified with specific probes in this study approximated 71%. Again, variations did not
28 correlate with increasing time of substratum exposure.

29

30 **Algal community structure**
31 A high proportion of dead algal cells were always detected. After 1 hour and 7 days of
32 exposure this proportion approximated 72 and 44%, respectively (Table 3). *Melosira*- and
33 *Fragilaria*-like species and pennate diatoms dominated the dead algal community. Within the

1 live algal morphotypes the proportion of green algae dominated the early colonization periods
2 of 1 to 4 hours with 78 and 68% respectively. No further increase was observed between 24
3 hours and 7 days of exposure, whereas the abundance of pennate diatoms increased. The main
4 representatives of the green algae community colonizing the substratum within the first day
5 were taxa from the orders of Chlorococcales (spherical and cylindrical morphotypes),
6 Chlamydomonadales (e.g. *Chlamydomonas*) and Desmidiales (e.g. *Closterium*). After 7 days
7 and 5 months of exposure, the green algae community contained mainly taxa from
8 Klebsormidiales (e.g. *Klebsormidium*), Chlorococcales (e. g. *Scenedesmus*),
9 Chlamydomonadales (e.g. *Chlamydomonas*), and Desmidiales (e.g. *Closterium*). Pennate
10 (31%) and filamentous diatoms (58%) dominated after 7 days of exposure. Pennate diatoms
11 were represented by *Navicula*, *Pinnularia*, *Cymbella*, *Fragilaria*, *Stauroneis, Gomphonema,*
12 *Tabellaria, Cocconeis, Synedra, Meridion,* and *Diatoma*. Central diatoms played a minor role
13 at all time steps. Representatives of the centric diatoms were *Melosira* and *Cyclotella* (Table
14 3).

15

16 **Extracellular enzyme activity**

17 There was no extracellular enzyme activity detectable after 1 hour of substratum exposure.
18 Activity of alkaline phosphatase was detectable after 4 hours of exposure followed by the
19 detection of β-glucosidase after 8 hours (Table 4). β-xylosidase was first detected after 7 days
20 of exposition. Between 7 days and 5 months of exposure, the activity of alkaline phosphatase,
21 β-glucosidase, and β-xylosidase increased 7-, 11, and 46-fold, respectively.

22

23 ## Discussion

24 **Pioneering bacterial community structure**

25 Although biofilm formation is initiated by attachment of specific groups of free-living
26 bacteria present in fresh- or seawater, the composition of bacterial communities in biofilms
27 differs from that of the water phase (Besemer *et al.*, 2007; Lee *et al.*, 2008). The community
28 difference can be explained by a rapid growth of a subpopulation of pioneer species that are
29 present in the water at low abundances or by a continuous attachment of these pioneer species
30 to the substratum. Surface properties of the submerged material are not so crucial, because
31 only slight differences in the bacterial community are detected for acryl, glass, and steel
32 substrata (Lee *et al.*, 2008). In this study, 40,000 DAPI stained cells per cm^2 attached to glass

slides within 1 hour of exposure suggesting that these cells present the pioneer population of this stream. The high number of cells was surprising as was the fact that the identified bacterial cells were dominated by *Cytophago-Flavobacteria* and not by *Betaproteobacteria*. It is proposed that *Betaproteobacteria* attach more easily to surfaces during initial biofilm formation than other groups of bacteria and, thus, dominate biofilm succession (Araya *et al.*, 2003). Similarly, fine temporal resolution of the formation of marine biofilms show the predominance of *Gammaproteobacteria* and not of *Alphaproteobacteria* in the pioneering population (Lee *et al.*, 2008). *Gammaproteobacteria* did not belong to the early pioneering population in this stream biofilm, because the first cells were detected after 4 hours on the glass surfaces.

Bacterial community succession

In this study, bacterial development occurred in several phases beginning with attachment apparently without growth during the first 12 hours resulting in an increasing density of bacterial cells between 1 and 7 days. Comparative community structure analyses indicated that the pioneer community in stream biofilms settled within 12 hours and developed into later stages by an enrichment of *Gammaproteobacteria* and *Betaproteobacteria* especially after 24 hours. Mechanisms of the succession of bacterial communities in biofilms are characterized as the sequence of pioneer-driven accumulation of biomass followed by an enrichment of other groups (Jones *et al.*, 2007). However, the exact time frame for settlement of pioneer groups and subsequent recruitment of other groups have not been known, because previous studies on succession in stream biofilms were performed at >1-day intervals. The community structure of the 7 days old biofilm was dominated by *Cytophaga-Flavobacteria*, *Gamma*- and *Betaproteobacteria* (Fig. 3) which are typical for stream water environments (Hullar *et al.*, 2006; Beier *et al.*, 2008). Settlement of pioneer groups and subsequent recruitment of other groups should not be dependent on the flow velocity, since it was recently shown that flow velocity does not affect the initial bacterial community of stream biofilms (i.e. the first 10 days) (Besemer *et al.*, 2007). The flow velocity measured at this site varied between laminar and turbulent flows, but transitional to turbulent flows dominated during the time of exposure. No flood events with very high flow velocities occurred during the period March-August. The main sampling period was done in August to ensure the presence of algae, because developmental patterns appear to be driven by algae which modulate their microenvironment (Besemer *et al.*, 2007). After 5 months of exposition, the

1 abundances of the investigated groups were similar to each other, representing a typical
2 stream bacterial community (Glöckner *et al.*, 2000; Hullar *et al.*, 2006; Beier *et al.*, 2008).
3 The abundance of DAPI stained cells approximated 2 x 10^6 cells cm^{-2} after 7 days and 16 x
4 10^6 cells cm^{-2} after 5 months, which is in the range of cell numbers (5 to 80 x 10^6 cells cm^{-2})
5 reported from stream biofilms (Geesey *et al.,* 1978; Freeman *et al.*, 1990; Romani &
6 Marxsen, 2002).
7 Although probes that hybridize with specific groups typical for freshwater
8 environments were used, the amount of “other cells” varied between 16 and 94% indicating
9 high abundances of unknown groups. However, other molecular techniques like phylogenetic
10 analysis of dominant DGGE bands from stream biofilms yield sequences related to the known
11 typical groups (Besemer *et al.*, 2007). Cells that hybridized with the Eubacterial probe
12 accounted only for 35 to 75% of the DAPI cell numbers similar to studies done with the
13 benthos of nine streams (Gao *et al.*, 2005). The low recovery during the first samplings was
14 surprising, because the EPS matrix which could act as a barrier for the labeled probes should
15 not be so pronounced in the very early phase of biofilm formation. Other reasons than matrix
16 effects have to be responsible, because FISH analyses of lake bacterioplankton reveal also a
17 low recovery of 10 to 59% of the DAPI counts (Zwisler *et al.*, 2003).

18

19 **Algal community succession**

20 Algae colonized the surfaces very rapidly within 1 hour (Fig. 2 B) and algal abundance
21 increased continously over the time of exposure. Algae can settle passively per sedimentation
22 or actively by motile species (Sekar *et al.*, 2002; Sekar *et al.*, 2004). Within the algal
23 community the dominance of small green algae represented by *Chlorella spec*.
24 (*Chlorophyceae*) within the first 4 hours is possibly based on the ability to fast attachment of
25 this group. With increasing exposure time algae abundance increased, but their overall
26 propotion was low, which might be due to a low competive ability, a specialized herbivore or
27 a decreased ability in settling on pre-occupied aerea. Filamentous algae outnumbered other
28 groups similar to other studies (Besemer *et al.*, 2007), after the attachement phase, whereas
29 pennate diatoms dominated the 5 months old biofilm. Although centric diatoms are dominant
30 in the water phase (Patil & Anil, 2005), this group was always of less importance in this
31 study. The high percentage of dead cells might be caused by an increased number of dead
32 cells in the water phase in late summer caused by nutrient limitations (e.g. ammonia, Table 1).
33 This phenomenon is known to explain the viability of marine algae which show a significant

reduction in late summer (Hayakawa, 2008). The association within the biofilm matrix may allow algae and bacteria to persist during periods of nutrient limitation, because autotrophics and heterotrophics in the biofilm use nitrogen and phosphorus from the stream water leading to an increased downstream nutrient decline (Sabater *et al.*, 2002).

Algal biomass is a good predictor of epilitic bacterial density. However, for a quantitative relationship between algae and bacteria, a minimum algal biomass (> 5 µg Chlorophyll a per cm^2) appears to be necessary (Rier & Stevenson, 2001), which was given in the biofilms after 7 days. Filamentous green algae can form structural templates on which bacterial communities develop (Besemer *et al.*, 2007). Similarly to stalked diatoms that can be important as secondary substrata for bacteria forming a loosely attached biofilm fraction (Tuji, 2000; Rier & Stevenson, 2002).

Extracellular enzyme activity

River biofilms are critical for organic matter processing (Sabater *et al.*, 2002). Extracellular enzymes accumulate in the biofilm and are both adhered to the bacteria (up to 81% in young and up to 37% in old biofilms) or are interspersed within the matrix (Romani *et al*., 2008). The appearance of phosphatase activity after 4 hours of exposure might be due to the *Betaproteobacteria* and the early settlement of algae (Bruckmeier *et al*., 2005; Wilczek *et al.*, 2005). *Betaproteobacteria* have been found to be highly correlated with phosphatase activity in surface waters (Kirchman *et al.*, 2004). Potential activities of the three enzymes measured after 7 days were in the upper range of activities reported from other streams (Romani & Marxsen, 2002) suggesting a highly active population despite their small cell number. The increased extracellular enzyme activities in 5 months old biofilms are comparable with results from stomatolitic algal patches from La Solana River (Spain) (Romani & Sabater, 1998). Conspicuous is the switch from a very low ratio of β-xylosidase:β-glucosidase to a higher ratio of 0.4 after 7 days and 5 months, respectively, indicating a change in organic matter resources which were utilized by the biofilm community. High ratios indicate the importance of hemicellulose which is mainly of allochthonous origin (Romani & Sabater, 2000). Low ratios show that microorganisms preferentially hydrolyze cellobiose which originates from a great variety of autochthonous and allochthonous sources. Thus, our data suggest a switch from more autochthonous carbon sources delivered directly by attached algae and used by the bacterial community in the initial stage of development to increased importance of allochthonous compounds in biofilms that reach a mature state.

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26
27

Table 1. Environmental parameters measured in triplicates on March and August 2005 at the sampling site. In addition, the range of these parameters measured during February 2003 and August 2005 is presented.

Parameter	Unit	March 2005	August 2005	Range
Flow velocity	cm s^{-1}	78 ± 39	18 ± 9	0 - 120
Temperature	°C	1.5± 0.0	12.8 ± 0.0	1.5 - 16.3
Conductivity	µS cm^{-1}	165 ± 0.0	137 ± 0.0	105 - 172
pH		7.81 ± 0.0	7.22 ± 0.0	6.2 - 7.6
Ammonium	µmol l^{-1}	12.2 ± 0.0	<1	<1 - 20
Nitrate	µmol l^{-1}	129 ± 0.0	119 ± 50.2	12 – 159
Phosphate	µmol l^{-1}	1.9 ± 0.3	0.51 ± 0.03	1.6 - 4.9
DOC	mg l^{-1}	1.3 ± 0.8	1.7 ± 0.0	0.2 - 1.6

Table 2. Mean abundance (± standard deviations) of bacterial cells (* 10^4 cm^{-2}) (n between 3 and 6) that hybridized with probes specific for Eubacteria, *Alpha,*- *Beta*-, and *Gammaproteobacteria*, *Cytophaga-Flavobacteria*, and *Actinobacteria* on glass slides exposed for 1, 4, 8, 12, 24 hours, 7 days, and 5 months in the stream Ilm. The group "others" represents the percentage of cells which hybridized with the Eubacterial probe but not with the more specific ones.

Targeted groups	Mean abundance (± standard deviations) of cells (* 10^4 cm^{-2}) and percentage of others on glass slides exposed for						
	1 h	4 h	8 h	12 h	24 h	7 days	5 months
Alphaproteobacteria	0.04 (± 0.03)	0.12 (±0.15)	0.09 (±0.08)	0.13 (±0.03)	0.06 (±0.04)	0.06 (±0.04)	21.4 (±10.4)
Betaproteobacteria	0.05 (±0.02)	0.16 (±0.11)	0.06 (±0.06)	0.21 (±0.00)	0.06 (±0.04)	2.25 (±0.45)	39.2 (±18.7)
Gammaproteobacteria	0	0.05 (±0.04)	0.24 (±0.19)	0.06 (±0.06)	0.03 (±0.17)	3.89 (±1.99)	11.9 (±11.9)
Cytophaga-Flavobacteria	0.40 (±0.40)	0.20 (±0.05)	1.10 (±0.91)	0.27 (±0.13)	0.31 (±0.18)	3.89 (±1.99)	30.9 (±34.7)
Actinobacteria	0.10 (±0.10)	0.09 (±0.10)	0.09 (±0.10)	0.13 (±0.04)	0.03 (±0.05)	1.20 (±0.68)	39.2 (±28.9)
Eubacteria	1.69 (±0.54)	6.65 (±5.23)	1.87 (±2.27)	2.75 (±1.68)	7.99 (±1.46)	66.5 (±31.9)	702.0 (±480)
Others	65.1	90.7	15.5	70.9	93.9	79.9	79.9

Table 3. Mean abundance (± standard deviations) of live algal cells (cm^{-2}) (n=3) and percentage of dead cells on glass slides exposed for 1, 4, 8, 12, 24 hours, 7 days, and 5 months in the stream Ilm.

Algal groups	Mean abundance (± standard deviations) of cells (cm^{-2}) and percentage of dead cells (%) on glass slides exposed for						
	1 h	4 h	8 h	12 h	24 h	7 days	5 months
Centrales	0.2 (± 0.4)	0.4 (±0.7)	1.0 (±1.0)	0	0	120 (±30)	179 (±161)
Pennales	1.0 (±1)	2.0 (±2.0)	18 (±4.0)	13 (±3.0)	74 (±97)	468 (±145)	13538 (±18889)
Filamentous diatoms	1.0 (±1)	6.0 (±8)	11 (±8)	29 (±22)	20 (±4)	882 (±202)	1472 (±1544)
Green algae	8 (±13)	18 (±31)	8 (±8)	40 (±52)	65 (±91)	40 (±46)	441 (±686)
Dead cells	72	48	51	45	56	34	54

Table 4. Mean activity rates (± standard deviations) of extracellular enzymes of the community on glass slides (n=3) exposed for 1, 4, 8, 12, 24 hours, 7 days, and 5 months in the stream Ilm.

Extracellular enzymes	Mean activity rates (± standard deviations) of the community (nmol cm^{-2} h^{-1}) on glass slides exposed for					
	1 h	4 h	8 h	12 h	24 h	7 days	5 months
Alkaline phosphatase	n.d.*	0.3 (±0.7)	0.8 (±1.0)	10.4 (±22.6)	9.1 (±13.5)	91 (±71)	611 (±151)
β-glucosidase	n.d.	n.d.	1.4 (±2.5)	n.d.	1.6 (±2.8)	60 (±28)	630 (±237)
β-xylosidase	n.d.	n.d.	n.d.	n.d.	n.d.	5.3 (±8.3)	242 (±97)

* n.d.: not detected

Figure legends

Fig. 1. (A) Sampling basket for 6 glass slides. (B) Exposition of the basket horizontally to the stream bed.

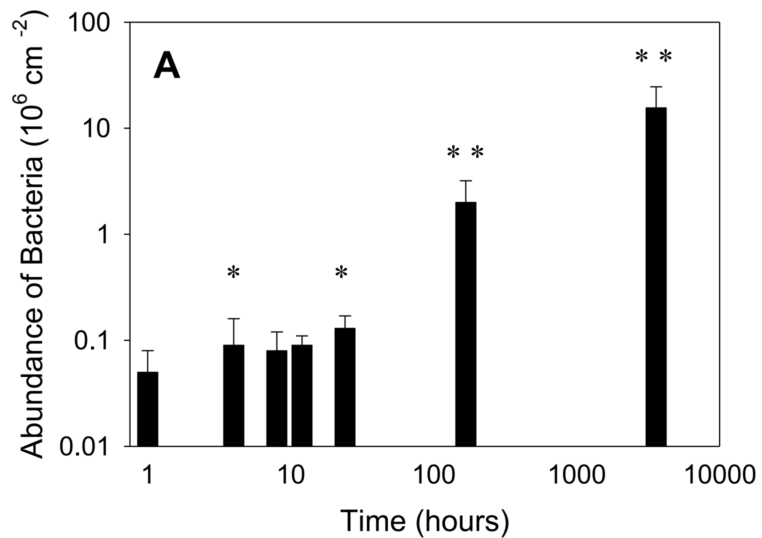
Fig. 2. Abundances (mean ± standard deviations) of bacteria (A) and algae (B), as well as Chl *a* (C) and organic matter (D) content on glass slides exposed for 1, 4, 8, 12, 24 hours, 7 days, and 5 months in the stream Ilm. Asterisks above the columns indicate significant differences (* $P<0.05$, ** $P<0.001$).

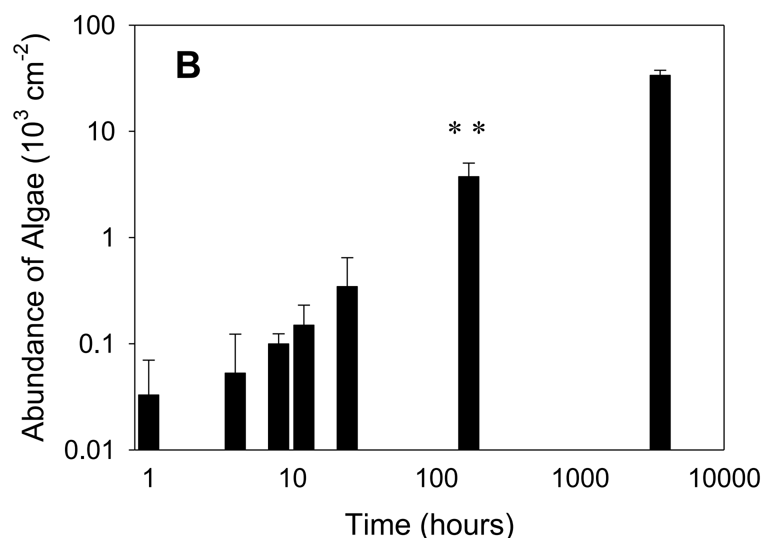
Fig. 3. Frequencies of bacterial cells that hybridized with probes specific for *Alpha*-, *Beta*-, and *Gammaproteobacteria*, *Cytophaga-Flavobacteria*, and *Actinobacteria* relative to the total number of cells affiliated to these groups on glass slides exposed for 1 h, 12 h, 7 days, and 5 months in the Stream Ilm.

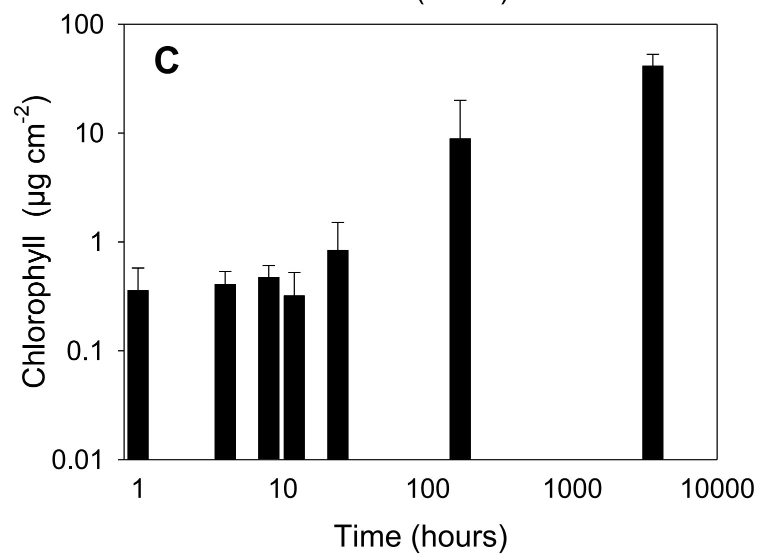
Figure 1

A **B**

Figure 2







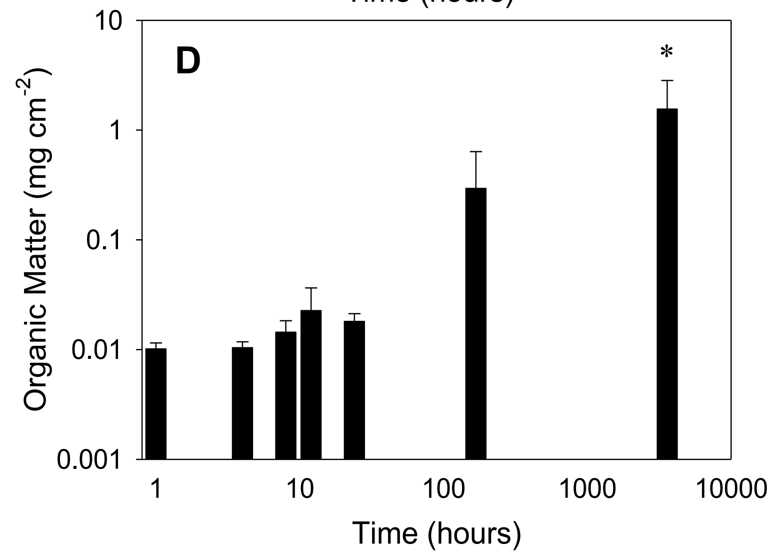
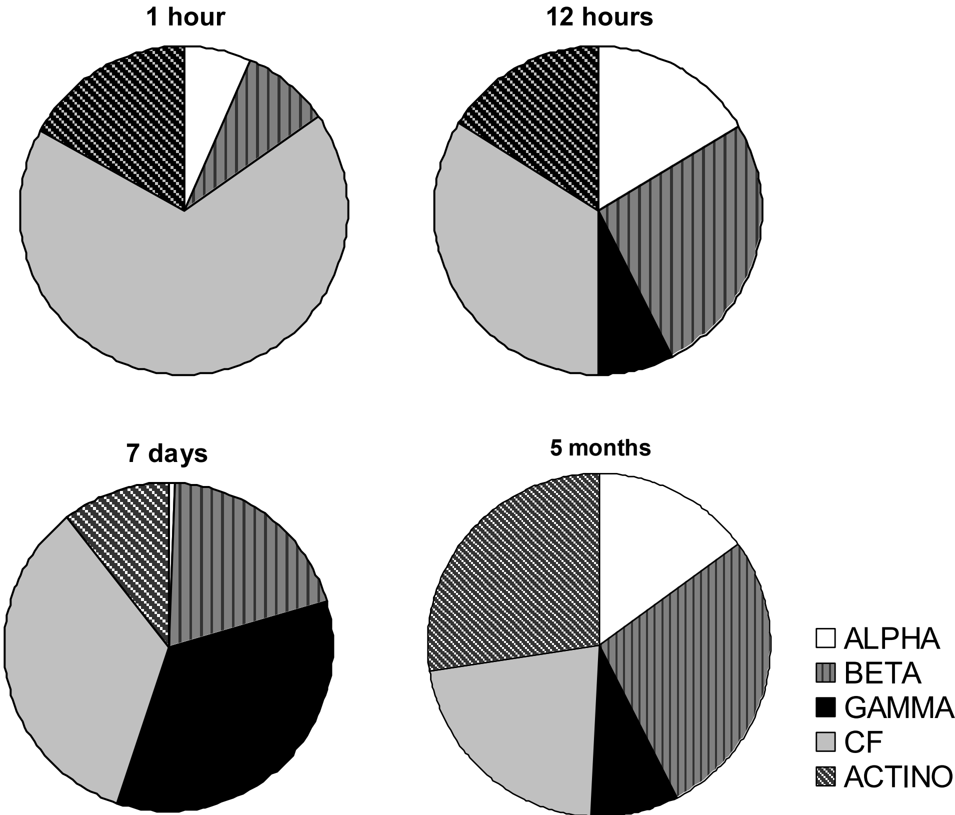


Figure 3



Supplement 1. Sequences of oligonucleotide probes and references used for CARD-FISH

Specificy	Common Name	Sequence (5´- 3´)	Reference
Bacteria	S-D-Bact-0338-a-A-18	GCTGCCTCCCGTAGGAGT	(Amann et al. 1990)
Alpha-Proteobacteria	S-Sc-aProt-0019-a-A-17	CGTTCGYTCTGAGCCAG	(Manz et al. 1992)
Beta-Proteobacteria	L-Sc-bProt-1027-a-A-17	GCCTTCCCACTTCGTTT	(Manz et al. 1992)
Gamma-Proteobacteria	L-Sc-gProt-1027-a-A-17	GCCTTCCCACATCGTTT	(Manz et al. 1992)
Cytophaga-Flavobacteria	S-P-CyFla-0319-a-A-18	TGGTCCGTGTVAGTAG	(Manz et al. 1994)
Actinobacteria	HCG69a	TATAGTTACCACCGCCGT	(Roller et al. 1994)
			(Berchtold et al. 1999)

Distinct Flow Velocities and Sedimentation Affect Microbial Biofilms in Streams

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Running Titel: FLOW VELOCITIES AFFECT MICROBIAL BIOFILMS

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Biofilm, bacteria, algae, community structure, flow velocity, inorganic sediment, stream

Abstract

We examined the impact of the flow velocity and sedimentation on the structure and function of stream microbial biofilms. Glass slides were exposed in a stream at slow flow velocities (0.17 m s^{-1}) in front and high velocities (0.4 m s^{-1}) behind a barrier as well as at a natural site (0.4 m s^{-1}). In front and behind the barrier high fractions of inorganic matter were incorporated. At this sites the total bacterial abundance, biofilm thickness, and *Betaproteobacteria* in 2 weeks old biofilms were enhanced. Extracellular enzyme activities at the natural site per bacterial biomass were significantly higher indicating the demand for low molecular weight nutrients at this site. The biofilm thickness in biofilms grown at slow flow velocities was significantly reduced after 7 weeks obviously caused by small flood events. To evaluate the impact of inorganic sediments on the structure and function of the biofilm community 0, 1, 5 and 15 mg cm^{-2} of inorganic sediment particles were added to biofilms in flow channels. The abundance of bacteria, algal biomass, biofilm thickness, organic matter content, and the carbon turnover was enhanced after the addition of sediment at a flow velocity of 0.7 cm s^{-1}. With addition of 5 mg cm^{-2} sediment *Beta*- and *Gammaproteobacteria* were enhanced and *Alphaproteobacteria* reduced. After exposing to higher flow velocities (20 and 60 cm s^{-1}) for 1 h the biofilms with inorganic sediment of 5 and 15 mg cm^{-2} sustained loss in thickness. These results show that inorganic sediment fractions incorporated in the matrix enhance the bacterial abundance and turnover capacity but in higher concentrations lead to instability.

INTRODUCTION

Biofilms are ensembles of autotrophic and heterotrophic organisms formed at any submerged surface in streams, such as stones, plants, and roots (Fuchs et al. 2000). The biofilm matrix formed by extracellular polymeric substances (EPS) provides a refuge for the microbial community against shear stress and desiccation and capture extracellular enzymes and products from hydrolysis (Freeman and Lock 1995, Ramasamy and Zang 2005). Activity of extracellular enzymes in biofilms is linked to representatives of the bacterial community (Kirchman 2002), the nutrient condition in the stream (Artigas et al. 2008), and the presence of algae (Rier et al. 2007). Most abundant heterotrophic bacterial groups in aquatic environments include 3 subdivisions of *Proteobacteria* (*Alpha-, Beta-, Gammaproteobacteria*), *Cytophaga-Flavobacteria*, and *Actinobacteria* (Glöckner et al. 2000, Kirchman 2004). Diatoms represent the main part of algae in stream biofilms (Roberts et al. 2004; Patil and Anil 2005).

The architecture and dynamics of biofilms in natural streams depends on the current velocity. Biofilms grown under slow flow velocities (6 cm s^{-1}) in flumes are thicker with higher abundances of bacteria and differ in structure compared with the counterparts from fast flow velocities (23 cm s^{-1}) resulting in dissimilar turnover DOC conditions. Biofilms grown under fast velocities are more dependent on the DOC load from the water column (Battin et al. 2003). Under slow flow velocities biofilms exhibit low densities in internal architecture and high effective diffusivity but cannot resist higher shear stress, whereas biofilm grown at higher flow velocities are denser and can resist higher shear stress but have a lower affective diffusivity (Beyenal and Lewandowski 2002). Honeycomb structures of biofilms for instance result in a resistance to water flow (Okabe et al. 1998). High flow velocities (15.5 cm s^{-1}) can intensify shear stress and frictional forces both acting on the amount of surface attached biomass (Cheng et al. 1997).

Disturbed freshwater communities can recover their numerical and species composition by succession within 14 days. The recovery of the microbial community in biofilms is dependent on age and taxonomic composition of the community, nutrient condition, shading, and the flow velocity (Stevenson 1990; Peterson and Stevenson 1992; Peterson 1994; Railkin 1998). In a stream 40,000 bacteria and 10 algae (8 green algae) per cm^2 colonized the surface within 1 h (Pohlon et al. submitted), whereas marine biofilms in a laboratory experiment consisted of 460,000 bacteria and 16,300 diatoms per cm^2 after 1 h (Railkin 1998). Initial colonization of surfaces by ciliates occurs faster at slow flow velocities (9 cm s^{-1}) in a stream and in flow channels with a flow velocity of 5 cm s^{-1} (Risse-Buhl and Küsel 2009).

Sediment fractions play a key role in bacterial community and production in rivers. Bacterial production in sediments is higher than in the pelagic zone (Fischer and Pusch 2001). But bacterial carbon production (BCP) is dependent on sediment conditions in a stream. In sandy (0.5 mm) sediments the BCP is higher (7.8 µg per mL sediment and day) as in coarse (>0.5 mm) sediments (3.6 µg per mL sediment and day) (Marxsen 2001). Furthermore bacteria prefer colonization of fine fractions (<0.063 mm) of sediments whereas the coarse sediments (0.063-1 mm) contribute the major proportion of particulate organic matter (Koutny and Rulik 2007).

In the current study the structure and function of microbial biofilms grown under different flow velocities in a natural stream has been investigated. For this porpose a small weir with predictable flow velocities in the Stream Ilm (Thuringia; Germany) was chosen. In front of the barrier slow flow velocities were predominant and significantly higher behind (Table 1). Furthermore small weirs are known as sedimentation traps (Stanley et al. 2002; Magilligan et al. 2003) and so the impact of sedimentation on the bacterial and algal community structure, the enzymatic activity, and the biofilm thickness in 2 and 7 weeks old biofilms has been investigated. Additionally colonization of glass slides by bacteria between 4 hours – 14 days at different flow velocities were mesured. Glass slides were exposed in front and behind the barrier as well as at a natural site with high flow velocities. In a flow channel experiment the impact of the amount of inorganic sediment particles on the microbial community structure and the carbon turnover has been evaluated. After adding 0, 1, 5 and 15 mg cm^{-2} of inorganic sediment particles to biofilms the bacterial community structure, algal biomass, biofilm thickness and sucrose degradation was measured at a flow velocity of 0.7 cm s^{-1}. Finally these biofilms were exposed to flow velocities of 20 or 60 cm s^{-1} respectively for one hour to evaluate biofilm stability.

MATERIAL AND METHODS

Sampling. Biofilm samples were taken in the third order stream Ilm (50°44´58´´N, 11°02´14´´E; Thuringia, Germany) at Manebach (natural site, river km 120) and at Griesheim (weir, river km 100). The reach Manebach is situated at the head water region with a mean discharge of 2.45 m^3 per s^{1}. The pool is situated in front of a small weir and the outlet behind of the same weir.

Sampling baskets of punched (diameter of the holes 5 mm) stainless steal (diameter 8 cm, length 16 cm) for 6 glass slides were exposed on tubes above the river ground so that the

slides were aligned horizontally to the surface water (Pohlon et al. submitted). The initial colonization (1, 3, 5, 7, and 14 days) of biofilm bacteria (DAPI-counts) was estimated in November 2003 and in May 2004 (4 and 8 hours and 3 days of exposition). In February 2005 samples for the examination of 2 and 7 weeks old biofilms were taken. The community structure of biofilm bacteria and algae, biofilm tickness, and organic- and inorganic matter content was estimated. Extracellular enzyme activity was measured at March 3rd 2005 in 3 weeks old biofilms grown on clay tiles (Sabater and Romani 1996).

Laboratory biofilms were grown in flow channels (50 x 16 x 4 cm; length x width x height) containing 24 glass slides in a climate chamber at 13 C in a 12 h light-dark cycle. Photosynthetic active radiation approximated 100 µmol m^{-2} s^{-1} above the water surface during the light period. Flow velocity in all channels was adjusted to 0.7 cm s^{-1}, all channels were connected to one pump reservoir. Five liters of stream water per channel were circulated using one rotary pump per channel (WIND'S 1200, Sacem, Giorgio, Italy) and replaced every two weeks. The water was taken from the Leutra, a 1st order rock stream near Jena (Thuringia, Germany) during 27.01.2006 to 25.05.2006 without disturbing the streambed to minimize the amount of suspended sediment.

Sediment addition. To six out of 24 channels in total, 0, 1, 5, and 15 mg cm^{-2} combusted (550 °C) silicium dioxide (SiO_2) particles (0.5 - 10 µm diameter; >80 % between 5 - 10 µm) were added respectively. Pre-experiments demonstrated that this size was embedded into the biofilm to approximately 82% in comparison to silicium dioxide particles with a particle size of 0.014-0.5 µm or 210-297 µm that were embedded into the biofilm only to 10 or 30%, respectively. The total amount of sediment for each channel was divided in six equal parts, and added at day 1, 4, 8, 11, 15 and 18. For each addition, the silicium dioxide was suspended in 24 mL of channel water. The suspension was distributed over the glass slides in 24 steps, using a pipette. Per step, 1 mL suspension was added over one glass slide respectively, with vigorous shaking of the suspension between every step. To achieve a homogeneous distribution on the biofilm, the silicium dioxide was released as high as possible in the water column, maximizing dispersal due to sedimentation of the particles in the water.

Sucrose addition and flow velocity adjustment. After 28 days, 10 mg C L^{-1}sucrose ($C_{12}H_{22}O_{11}$) was added to all flow channels. From now on, all channels had separated water circuits. Three flow channels of each treatment (channels with sediment addition and control channels without added sediment) were run in darkness, the other three continued under the light-dark cycle, all for seven more days.

At the end of the sucrose incubation, six glass slides from every treatment were transferred immediately to two flow channels (98 x 25 x 2 cm; length x width x height). This transfer was necessary as the smaller channels did not allow for a sufficient flow velocity increase. The flow velocity was adjusted to 20 and 60 cm s^{-1}, chosen to simulate a high discharge event in the Leutra stream. Bacterial abundances and inorganic biofilm mass were determined from triplicate samples of each treatment after one hour and compared to those obtained at the end of sucrose incubation.

Stream water sample analyses. Triplicate samples from the water column were filtered (Whatman, polycarbonate, 0.45 µm) during the period of 2003 to August 2005 for measurements of dissolved organic carbon (DOC), ammonia (NH^{4+}), nitrate (NO_3^-) and orthophosphate (PO_4^{3-}). Concentrations of PO_4^{3-} were determined with the ascorbic acid method (Clesceri, 1998), NO^{3-} concentrations with the ultraviolet spectrophotometric screening method (Clesceri *et al.* 1999) (Uvikon 931, Kontron Instruments, Italy). Ammonium was determined spectrophotometrically (Gadkari 1984) (Uvikon 931, Kontron Instruments, Italy). DOC was measured using a DIMATOC 100 infrared spectrometer (Dimatec Analysentechnik GmbH, Germany). Oxygen content, pH, salinity, conductivity and turbidity were measured in the water column (Horiba U 10, Multiparameter, Water Quality Meter, Kyoto, Japan). Flow velocity was measured near the tubes after removing the cylinders in 5 cm steps beginning from the river bottom to the top of the water column (Flowmate, MARSH McBirney, USA).

Laboratory experiment. Nutrient concentrations were determined from water samples taken from the stream at the beginning, two weeks after start, and end of glass slides exposure. In flow channels, samples were taken at the start, water exchanges, and the end of the experiment. Average nutrient values in the Leutra water were 0.02 ± 0.06 mg L^{-1} ammonium, 32.9 ± 4.3 mg L^{-1} nitrate, 0.16 ± 0.07 mg L^{-1} phosphate, and 2.4 ± 0.3 mg C L^{-1} DOC. Following sucrose addition, water samples of 1 mL were taken by sterile syringes from each channel five times a day (every four hours, and one eight hour interval during night), and sucrose concentrations were determined using a high pressure liquid chromatograph connected to a refractive index detector (System Gold, Beckman Coulter, USA).

Biofilm sample analyses. Biofilms from triplicate glass slides were scraped off and weighed after drying for 48 h at 60°C and a second time following combustion for 4 h at 550°C.

Determination of bacterial community. To estimate the abundance of biofilm prokaryotes, biofilms (5-6 replicates) were scraped from the glass slides using sterile glass slides and kept

1 in 50 mL Greiner tubes containing 35 mL prefiltered stream water (Whatman, polycarbonate,
2 0.2 μm pore size) and paraformaldehyde at a final concentration of 4%. To estimate the
3 abundance of water column prokaryotes, three replicates of 50 mL stream water were fixed
4 with paraformaldehyde (4% final concentration). Abundance of bacteria belonging to
5 different groups were determined by catalyzed reporter deposition fluorescence in situ
6 hybridization (CARD-FISH) (Pernthaler et al. 2004) after sonification of the biofilm samples
7 for 1 min (Sonopuls HD2200, Bandelin, Korea), because determinations with fluorescence in
8 situ hybridization (FISH) showed very low efficiency and poor reproducibility (data not
9 shown). Samples were filtered onto polycarbonate filters (GTTP, Millipore, Eschborn,
10 Germany, pore size 0.2 μm) and attached with low gelling agarose 0.1%. Cells were
11 permeabilisized with lysozyme (10 mg mL^{-1}) and achromopeptidase (60 U mL^{-1}). Horseradish
12 peroxidase (HRP) labeled oligonucleotide sequences (Biomers, Ulm) specific for the domain
13 Bacteria (Amann et al. 1990), *Alpha,- Beta,-* and *Gammaproteobacteria* (Manz et al. 1992), to
14 *Actinobacteria* (Roller et al. 1994; Berchtold et al. 1999), and *Cytophago-Flavobacteria*
15 (Manz et al. 1994) (Table S1) were linked with $Alexa_{488}$ as fluorochromic dye. Finally filters
16 were embedded into a glycerol-PBS-DAPI (1 μg μL^{-1}) mixture and cells were counted at
17 1000x magnification [Zeiss Axioplan, 450-490 FT 510 LP 515 ($Alexa_{488}$) and G 365 FT 395
18 LP 420 (DAPI)].

19 Algal community. Algal morphotypes (pennate, centrale and filamentous diatoms, coccale
20 and filamentous green algae) were quantified microscopally (Ettl 1983; Komarék and Fott
21 1983; Ettl and Gärtner 1988; Kramer 2000) after staining with Lugol's solution at 100x
22 magnification (Zeiss-Axiovert) (Utermöhl 1958). Cells were differentiated as living and dead
23 according to chlorophyll content, which was visible under the light microscope. Those cells
24 completely filled with the chloroplast were counted as live, whereas those cells with a
25 shrunken or no visible chloroplast were counted as dead.

26 Extracellular enzyme activities. Activities of β-glucosidase, β-xylosidase and alkaline
27 phosphatase were measured with artificial 4-methylumbelliferyl (MUF)substrates MUF-ß-D-
28 glycoside, MUF-ß-D-xyloside, MUF-phosphate (Sigma), for aminopeptidase Leucine-MCA
29 (L-leucine-4 methyl-coumarinyl-7-amide (Calbiochem)) was used. To 100 mg of substrate 2
30 mL of hydroximethylether (Sigma) were added in sterile Greiner-tubes to facilitate its
31 dissolution. Fifty mL of autoclaved distilled water was added and the solution was kept in the
32 freezer (-20°C) until use. Three replicates of biofilm on the glass slides at each time step were
33 transferred within minutes after sampling into 50 mL Greiner tubes with filtered (Whatman
34 glass fiber, 0.2 μm pore size) and autoclaved river water containing the artificial substrate (0.3

1 mmol L^{-1}) and incubated for two hours. Fluorescence was determined after adding 1 mL of
2 0.05 M glycine buffer (pH 10.4) (for 100 mL 80.35 mL NaOH 0.2 M and 19.64 mL Glycine
3 0.2 M) to stop the reaction and enhance fluorescence that was measured with a fluorescence-
4 spectrophotometer (Perkin Elmer, LS 50 B; em 450 nm, ex 365 nm, slit 2.5). Quantification
5 was achieved by calibration with standard MUF solutions (1-10 µmol L^{-1}) (Hoppe 1983).
6 Bacterial biomass was estimated using the converting factor $2.2*10^{-13}$ gC μm^3 (Bratbak and
7 Dundas 1984, Kemp 1990).
8 Examination of biofilm thickness. Slides were kept in 50 mL prefiltered river water
9 (Whatman, 0.2 µm) and formaldehyde (4% final concentration) and stained with the
10 fluorescence marker 5(4,6-dichlorotriazinyl) aminofluorescein (DTAF) (Schumann and
11 Rentsch 1998). After leaving out the drying step at the end of the staining procedure to avoid
12 collaboration of the biofilm, 3 samples per time step were examined using a confocal laser
13 scanning microscope (CLSM) (Zeiss LSM 510 META) at 630x magnification. Images of the
14 biofilm were analyzed using Zeiss LSM 5 Image Browser.
15 Statistical analyses. Concentration of nutrients and environmental factors listed in table 1
16 as well as bacteria numbers were analyzed using oneway ANOVA. T-tests were used to test if
17 inorganic biofilm mass, algal biomass, organic matter content or bacterial abundances differed
18 before and after the flow velocity increase. The analyses were done in SigmaStat for
19 Windows version 2.03.
20

21 **RESULTS**

22 **Stream water analyses.** The flow velocity at the natural (40 ± 30 cm s^{-1}) site and the outlet
23 (40 ± 30 cm s^{-1}) was significantly higher ($P<0.05$, one way ANOVA) than at the pool of the
24 weir (17 ± 9 cm s^{-1}). Conductivity, turbidity, ammonium-, and phosphate concentrations of
25 pool and outlet was significantly higher than at the natural site ($P<0.05$, one way ANOVA)
26 (Table 1).
27 **Abundance and structure of the bacteria community.** Colonization of glass slides in
28 May 2004 occurred rapidly after 4 hours at all sampling sites with 250,000 DAPI stained cells
29 per cm^2 respectively. After one day the abundance at the pool was significantly higher than at
30 the natural site and the outlet ($P<0.05$, one way ANOVA) (Fig 1A). In November 2003 after 1
31 day 200,000 cells per cm^2 (Natural), 240,000 cells per cm^2 (Pool), and 280,000 cm^2 (Outlet)
32 colonized the slides. After 3 days at the pool the cell abundance was enhanced which was
33 significant compared with the natural site ($P<0.05$, one way ANOVA). From day 5 on the

abundances at the pool and outlet increased but not at the natural site (Fig 1B, 2A). After 7 weeks the abundance at the natural site was significantly higher than at the weir ($P<0.05$, one way ANOVA) (Fig 2A).

The bacterial community at the natural site after 2 weeks was dominated by CF (about 60%) followed by BETA (about 30%). At the pool BETA were dominant after 2 weeks (about 65%) followed by CF (about 25%) and at the outlet BETA represented 50% of the community and CF about 40%. After 7 weeks the CF fraction at the natural site reached 70% and BETA 25%. At the pool CF and BETA were equally in proportion with 40% respectively. At the outlet CF dominated with 50% followed by BETA with 25% (Fig 3).

The abundance of Eubacteria relative to DAPI counts was between 41 and 99%. The overall abundance of cells of the examined groups relative to the counts of Eubacteria was between 13 and 60% (Table 2).

Abundance and structure of the algal community. The abundance of living algae was highest at the outlet after 2 and 7 weeks. At the natural site the abundance increased over time and at the pool and outlet it decreased (Fig 2B). Abundance of dead shells was high (about 50%) at all sites and sampling times (data not shown). Pennate diatom community at all sampling sites was mainly represented by *Navicula*, *Primularia*, *Stauroneis*, *Fragilaria, Synedra*, *Eunotia*, *Cocconeis*, *Meridion*, and *Cymbella*. At the pool *Diatom*a, and *Gomphonema* were found and *Tabellaria* at the outlet. Main representative of central diatoms was *Melosira*.

Green algae were highly abundant at all sampling sites representing nearly 50% after 2 weeks. After 7 weeks at the natural site and the outlet the proportion increased further reaching nearly 60%. Proportion of pennate diatoms was highest at the pool after 7 weeks followed by chrysophytes. At the natural site the proportion of pennate diatoms increased, chrysophytes did not change over time. At the outlet chrysophytes decreased and pennate diatoms were consistent. Centrale diatoms and filamentous greenalgae were minor at all sites after 2 and 7 weeks (Fig 4).

Biofilm Structure. Biofilm thickness at the natural site was about 80 µm after 2 and 7 weeks of exposure. At the pool the biofilm was about 200 µm thick and after 2 weeks it decreased significantly ($P<0.05$, one way ANOVA) to a thickness of 80 µm (7 weeks). At the outlet the biofilm at 2 and 7 weeks was 200 µm thick (Fig 5).

The amount of organic matter incorporated in the biofilm matrix was not different at all sampling sites and times (Fig 6A), whereas at the the pool and outlet of the weir high amounts of inorganic matter were accumulated ($P<0.05$, one way ANOVA) (Fig 6B).

1 **Extracellular enzyme activity.** Phosphatase (Phos) activity was high at all sampling sites 2 with a maximum at the natural site. Activity of beta-glucosidase (Glu), beta-xylosidase (Xyl) 3 and leucin-aminopeptidase (AP) was highest at the outlet (Fig 7A). With comprehension to 4 the bacteria biomass at the natural site the activity of Phos, Glu, and AP was significantly 5 ($P<0.05$, one way ANOVA) higher than at the other sites. The activity of Phos, Xyl, and AP 6 per bacteria biomass at the pool was higher than at the outlet (Fig 7B).

7 **Flow channels.** In the flow channels concentrations of ammonium after 4 weeks decreased 8 with exception of the addition of 1 mg cm^{-2} of inorganic sediment. Nitrate was highest with 1 9 mg cm^{-2} sediment, phosphate decreased at all treatment to one third of the concentrations 10 which were measured in the beginning. Total DOC was highest in the controls without 11 addition of sediment (Table 3).

12 **Abundance of bacteria and algal biomass.** The abundance of bacteria in the channels 13 with sediment addition at 0.7 cm s^{-1} was significantly ($P<0.05$) higher than without the 14 addition (Fig 8A). In the treatments with flow velocities of 20 and 60 cm s^{-1} the abundance in 15 biofilms with additionally 1 and 15 mg cm^{-2} sediment decreased (Fig 9A).

16 The biomass of algae with the addition of 1 and 15 mg cm^{-2} sediment was enhanced (8B). The 17 transfer to flow velocities of 20 and 60 cm s^{-1} did not change the algal biomass (Fig 9B).

18 **Biofilm thickness and organic matter content.** Biofilms at 0.7 cm s^{-1} with sediment 19 addition were thicker than without the addition (Fig. 8C). Biofilm thickness with additional 1 20 mg cm^{-2} was not reduced after the transfer into the channels with high flow velocities (Fig. 21 9C). Organic matter content at flow velocities of 0.7 and 20 cm s^{-1} and all sediment treatments 22 was enhanced (Fig 8D; 9D).

23 **Bacterial community structure.** Proportion of *Betaproteobacteria* was highest at all 24 treatments (Fig 10 A-C). In biofilms with 5 mg cm^{-2} abundance of *Gammaproteobacteria* and 25 *Cytophaga-Flavobacteria* was enhanced. With increasing sediment proportions the 26 *Alphaproteobacteria* decreased (Fig 10A-C). The percentages of Eubacteria relative to DAPI 27 counts in the channels was between 81 and 100 %, and the sum of *Alpha-, Beta-, Gamma-* 28 *Proteobacteria*, *Cytophaga-Flavobacteria*, and *Actinobacteria* relative to Eubacteria was 29 between 27 and 90% (Table 4).

30 **Sucrose reduction.** Sucrose reduction in the channels with additional sediment after 6 31 days was significant in contrast to the controls with remaining 5 mg L^{-1} at the end of the 32 experiment. Between the different sediment treatments the sucrose reduction was insignificant 33 (Fig 11).

34

1 **DISCUSSION**

2 **Colonization patterns of bacteria in the Stream Ilm.** Flow velocity and the
3 concentrations of nutrients did not affect the early colonization of glass slides by bacteria
4 which occurred rapidly within hours in the same range at all three sampling sites in the Ilm.
5 On day scale the slow flow at the pool possibly caused a faster increase of the bacteria
6 abundances. Especially the early biofilm formation is continually interacting with the water
7 column (Jefferson 2004) and so for the low numbers of bacteria at the natural site additional
8 effects as nutrient depletion and high flow velocities are possible. Furthermore the number of
9 bacteria available in the water column (data not shown) did not show any effects on the
10 number of bacteria attched on the slides at all sampling sites.
11 After two weeks of biofilm formation the high loads of sediment particles at the weir which
12 may include additional nutrients flushed out from the pool might result in high bacteria
13 numbers. The abundance of bacteria did not stagnate after 7 weeks indicating a high dynamic
14 in biofilm formation in accordance to the permanent changing conditions in the stream. At
15 this stage of biofilm formation the inner biofilm nutrient cycles may arise, making the
16 attached organisms more independent from the environmental. Epilithic biofilm matrix can be
17 effective in accumulating carbohydrase activity making the biofilm community resistant to
18 DOM fluctuations (Sinsabaugh et al. 1991) also at sites with low nutrients as the natural site.

19 **Bacterial community structure.** The bacterial community at the natural site was
20 dominated by CF which are most commonly associated with the occurrence of high molecular
21 weight organic matter and important in releasing phosphorus from organic matter (Van
22 Ommen Kloeke and Geesey 1999; Kirchman 2002). BETA are known as primary colonizer in
23 initial biofilms (10-12 days) reaching percentages of more than 50% (Manz et al. 1999) which
24 dominated the pool whereas in this study a combination of CF and BETA was found in
25 mature biofilms (7 weeks). BETA are involved in nitrogen cycling and be found in
26 environments with high DOC and nitrite/nitrate concentrations (Teske et al. 1994; Kirchman
27 2001, Zwisler et al. 2003, Gao et al. 2005). The high proportion of GAMMA at the outlet
28 indicates high nutrient conditions. This group is opportunistic in nutrient rich environments
29 (Pinhassi and Berman 2003).

30 **Colonization patterns of algae.** Green algae dominated the biofilm at all sites. This group
31 occurs in biofilms in initial phases (Sekar et al. 2004, Pohlon et al. submitted) and prefers
32 sites without light limiting conditions. In the present study the lack of shading in winter might
33 benefit the occurrence of high abundances of green algae. Another important fact was the

1 occurrence of a flood event during exposure of the glass slites which reached the 7 fold
2 discharge as the mean annual discharge of 2.44 m^3 s^{-1}. Grazers with preferences to diatoms
3 (McCormick and Stevenson 1991, Barranguet et al. 2005), seasonal changes in water
4 temperature, light and water velocity may have the same effects as flood events which reset
5 periphyton to early successional stages (Oemke and Burton 1986).
6 **Biofilm structure.** In this study biofilm thickness was not dependent on the flow velocity,
7 the bacterial abundance, and biofilm age but probably on algal abundance. Algae were highest
8 abundant at the outlet. At this site the thickest biofilm was recorded over the whole time
9 period. At the pool the drastic decrease could be caused by instability. The matrix at the pool
10 developed under slow flow velocities and was possibly more susceptible to a small flood
11 event which occurred during the exposition time (Jan. 21- Jan. 25. 2005). The incorporation of
12 high amounts of inorganic particles might have enhanced this effect. The biofilm at the outlet
13 also consisted high amounts of inorganic matter which could have intensified the thickness of
14 the matrix. At this site with high flow velocities the matrix outlasted the flood events. The
15 resulting thin biofilm at the natural site might emphasize the importance of sediment particles
16 for this parameter.
17 **Extracellular enzyme activity.** Phosphatase activity was highest at all sites indicating the
18 high demand for phosphorus in the biofilm. On the per-cell basis of the activities in the
19 biofilm the patterns of the activities changed completely. At the natural site the activity for
20 phospahtase, beta-glucosidase, and aminopeptidase was significantly higher compared with
21 the other sampling sites. The thin biofilm matrix at the natural site possibly does not provide
22 enough storage facilities for nutrients making a release of more enzymes necessary. Under
23 phosphorus limitation biofilm bacteria can produce more EPS than under nutrient availability
24 which might be an adaptive mechanism to store limiting resources in oligotrophic waters
25 (Mohamed et al. 1998), however high flow velocities as at the natural site can possibly
26 interfere with that mechanism.
27 **Flow channels.** The addition of 1 mg sediment per cm^{-2} was optimal for biofilm formation
28 and caused an enhancement of all parameters measured at flow velocities of 0.7 cm s^{-1}. Even
29 after the transfer into the flow channels with high flow velocities no effects on the biofilm
30 formation occurred. With higher sediment loads alterations in the abundance of bacteria (at 15
31 mg sediment per cm^2) or algal biomass (at 5 mg sediment per cm^2), biofilm thickness and
32 organic matter content (at 5 and 15 mg sediment per cm^2) occurred after the transfer into the
33 channels with higher flow velocities. These findings indicate that the inorganic sediment
34 particles may provide space for colonization of bacteria and algae and result in an increase of

biofilm thickness and organic matter content as well as the carbon turnover. On the other hand higher loads also cause instability. *Betaproteobacteria* might be supported by low and high sediment loads even at higher flow velocities and *Gammaproteobacteria* at intermediate sediment loads. Both bacteria groups prefer sites with high DOC contents (Kirchman 2002, Gao et al. 2005). After only one hour exposition of the biofilms at flow velocities of 20 and 60 cm s^{-1} the biofilm organisms can sustain a drastic decrease. These findings support the results from the pool at the weir in the Stream Ilm. Biofilms grown at slow flow velocities with additional high loads of inorganic particles are instable during flood events.

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Table 1. Mean values (±STADV) of abiotic parameters at the sampling sites in the Stream Ilm (asterisks indicate significant differences, P<0.05 ANOVA).

	Natural	**Pool**	**Outlet**
pH	7.2 ± 0.29	7.6 ± 0.4	7.7 ± 0.5
Conductivity (ms L^{-1})	123 ± 19	278 ± 35*	277 ± 42*
Turbidity FTU	0.24 ± 0.7	47 ± 100*	21 ± 61*
Oxygen (mg L^{-1})	9.6 ± 1.5	9.2 ± 1.9	9.3 ± 1.8
Temperature (°C)	9.4 ± 3.9	9.5 ± 3.5	9.7 ± 3.6
Ammonium (mg L^{-1})	0.22 ± 0.0	0.70 ± 0.02*	0.73 ± 0.02*
Nitrate (mg L^{-1})	8.0 ± 0.0	16.3 ± 0.6	16 ± 0.0
Phosphate (mg L^{-1})	0.15 ± 0.02	0.27 ± 0.02*	0.27 ± 0.01*
DOC (mg CL^{-1})	1.3 ± 0.8	1.9 ± 0.1	1.9 ± 0.5
Flow velocity (cm s^{-1})	40 ± 30*	17 ± 9	4 0± 30*

Table 2. Percentages of Eubacteria relative to DAPI counts and the sum of *Alpha-, Beta-, Gammaproteobacteria*, *Cytophaga-Flavobacteria*, and *Actinobacteria* relative to Eubacteria in biofilms of the Stream Ilm.

	Natural		**Pool**		**Outlet**	
Time of Exposition (weeks)	2	7	2	7	2	7
EUB relative to DAPI (%)	77	99	59	46	41	56
∑ cells of group specific probes relative to EUB (%)	50	13	40	20	60	40

Table 3. Mean (±STADV) of water chemistry parameters at the Leutra and in the flow channels

Experiment treatment	Ammonium (mg L^{-1})	Nitrate (mg L^{-1})	Phosphate (mg L^{-1})	Total DOC (mg C L^{-1})
Field site	0.02 ± 0.06	32.9 ± 4.3	0.16 ± 0.07	2.4 ± 0.3
Experiment				
Start	0.16	30.8	0.35	n.d.[#]
4 wks – control	0.07 ± 0.07	32.2 ± 5.0	0.11 ± 0.02	12.9
4 wks – 1 mg cm^{-1} sediment	0.20 ± 0.20	17.4 ± 5.1	0.13 ± 0.01	3.8
4 wks – 5 mg cm^{-1} sediment	0.10 ± 0.02	30.8 ± 3.5	0.13 ± 0.01	6.1
4 wks – 15 mg cm^{-1} sediment	0.01± 0.02	23.9 ± 2.5	0.10 ± 0.02	4.5
5 wks – control	0.07 ± 0.07	32.2 ± 5.0	0.10 ± 0.02	12.9 ± 6.8
5 wks – 1 mg cm^{-1} sediment	0.19 ± 0.18	17.4 ± 5.1	0.13 ± 0.01	3.8
5 wks – 5 mg cm^{-1} sediment	0.09 ± 0.24	30.8 ± 3.5	0.13 ± 0.01	6.1
5 wks – 15 mg cm^{-1} sediment	0.01± 0.02	23.9 ± 2.5	0.10 ± 0.02	4.5

Table 4. Percentages of Eubacteria relative to DAPI counts and the sum of *Alpha-, Beta-, Gamma- Proteobacteria*, *Cytophaga-Flavobacteria*, and *Actinobacteria* relative to Eubacteria in the flow channels with inorganic sediment addition at different flow velocities.

Sediment added	**0 mg cm^{-2}**			**1 mg cm^{-2}**			**5 mg cm^{-2}**			**15 mg cm^{-2}**		
Flow velocity (cm s^{-1})	**0.7**	**20**	**60**	**0.7**	**20**	**60**	**0.7**	**20**	**60**	**0.7**	**20**	**60**
EUB relative to DAPI (%)	95	100	81	100	100	90	88	60	87	97	72	100
∑ cells of group specific probe relative to EUB (%)	40	34	36	55	63	90	31	38	27	43	65	41

1 **Figure Legends**
2
3 **Fig. 1.**
4 Mean abundance (±STADV) of biofilm bacteria at the natural sampling site, the pool and
5 outlet of the weir in the Stream Ilm. Samples were taken within (A) 4 h and 72 h (May 2004)
6 and (B) between 1 and 14 d (Nov 2003) of exposure of glass slides. Letters above the columns
7 indicate significant differences ($p<0.05$, ANOVA).
8
9 **Fig. 2.**
10 Mean (±STADV) abundance of biofilm bacteria (A) and algae (B) at the natural site and the
11 pool and outlet of the weir in the Stream Ilm after 2 and 7 weeks of exposure. Letters above
12 the columns indicate significant differences ($p<0.05$, ANOVA).
13
14 **Fig. 3.**
15 Distribution of *Alpha- ,Beta-, Gammaproteobacteria*, *Cytophaga-Flavobacteria*, and
16 *Actinobacteria* at the natural site, the pool, and outlet of the weir in the Stream Ilm after 2 and
17 7 weeks of exposure. Proportion of other groups, not targeted by probes is not included (see
18 Table 2).
19
20 **Fig. 4.**
21 Distribution of the main groups of living biofilm algae at the natural site, the pool and outlet
22 of the weir in the Stream Ilm after 2 and 7 weeks of exposure.
23
24 **Fig. 5.**
25 Mean (±STADV) thickness of biofilms on glass slides at the natural site, pool, and outlet of
26 the weir in the Stream Ilm after 2 and 7 weeks of exposure. Letters above the columns
27 represent significant differences ($P<0.05$, ANOVA).
28
29 **Fig. 6.**
30 Mean (± STADV) of organic (A) and inorganic (B) matter incorporated in the biofilm matrix
31 after 2 and 7 weeks of exposure. Letters above the columns mark significant differences (t-
32 test).
33
34

1 **Fig. 7.**
2 Mean (± STADV) of extracellular enzyme activity in biofilms on glass slides using
3 flourigenic artificial substrates after 1 h of incubation. (A) absolute activity, (B) activity per
4 bacterial biomass. Letters above the columns represent significant differences ($P<0.05$,
5 ANOVA).
6
7 **Fig. 8.**
8 Mean (± STADV) abundance of bacteria (A), biomass of algae (B), biofilm thickness (C) and
9 organic matter content (D) in 5 weeks old biofilms on glass slides in flow channels at flow
10 velocities of 0.7 cm s^{-2} with additional inorganic sediments of 0, 1, 5 and 15 mg cm^{-2}. Letters
11 above the columns represent significant differences (t-test).
12
13 **Fig. 9.**
14 Mean (± STADV) abundance of bacteria (A), biomass of algae (B), biofilm thickness (C) and
15 organic matter content (D) in 5 weeks old biofilms on glass slides in flow channels with
16 additional inorganic sediments of 0, 1, 5 and 15 mg cm^{-2}. Biofilm samples were exposed over
17 one hour at flow velocities of 20 cm $s^{-2,}$ and 60 cm s^{-2}. Letters above the columns represent
18 significant differences (t-test).
19
20 **Fig. 10.**
21 Distribution of *Alpha-* (ALPHA)*, Beta-* (BETA)*, Gammaproteobacteria* (GAMMA),
22 *Cytophaga-Flavobacteria* (CF), and *Actinobacteria* (HGC) in 5 weeks old biofilms on glass
23 slides in flow channels with additional inorganic sediments of 0, 1, 5 and 15 mg cm^{-2}. (A) at
24 flow velocities of 0.7 cm s^{-2}. Biofilm samples were than separated and exposed over one hour
25 at flow velocities of 20 cm s^{-2} (B), and 60 cm s^{-2} (C). Proportion of other groups, not targeted
26 by probes is not included (see Table 4).
27
28 **Fig. 11.**
29 Decrease of previously added sucrose (Begin) by biofilms with additional inorganic sediments
30 of 0, 1, 5 and 15 mg cm^{-2} in flow channels over 6 days (End).
31
32
33
34

Figure 1

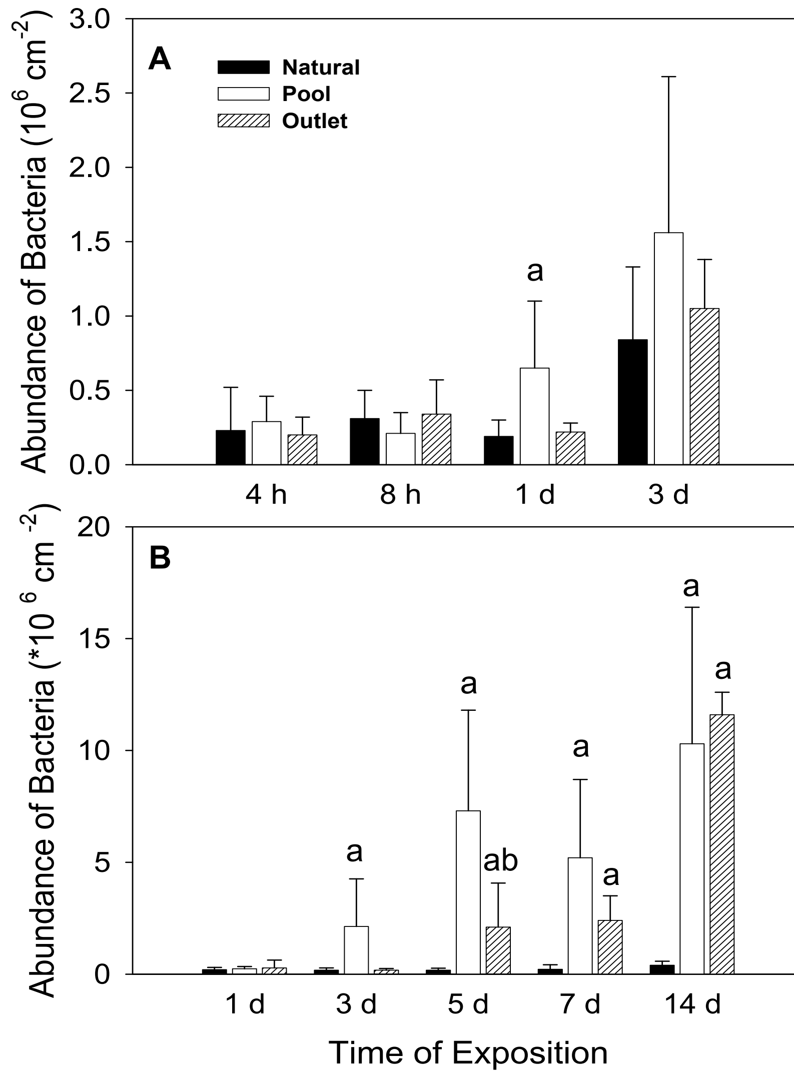
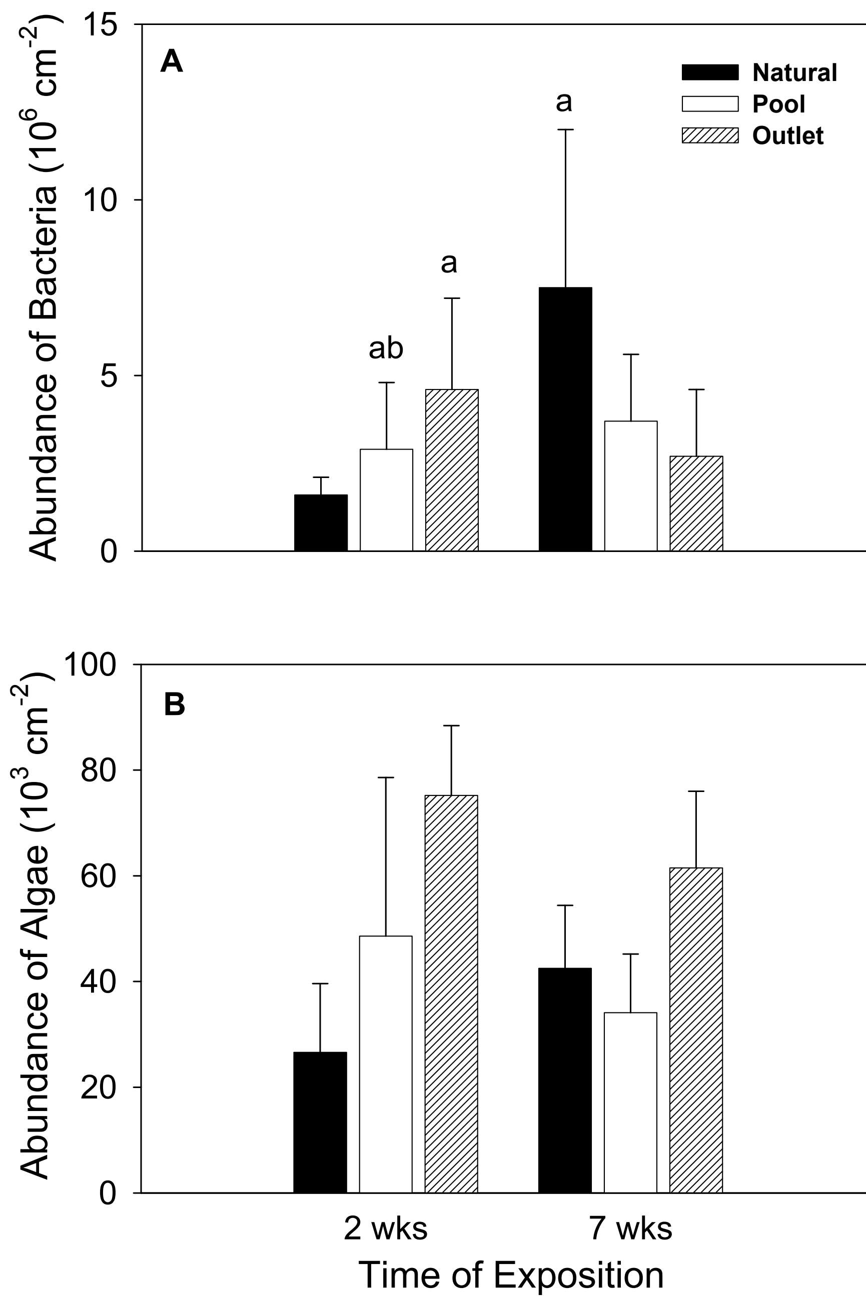


Figure 2



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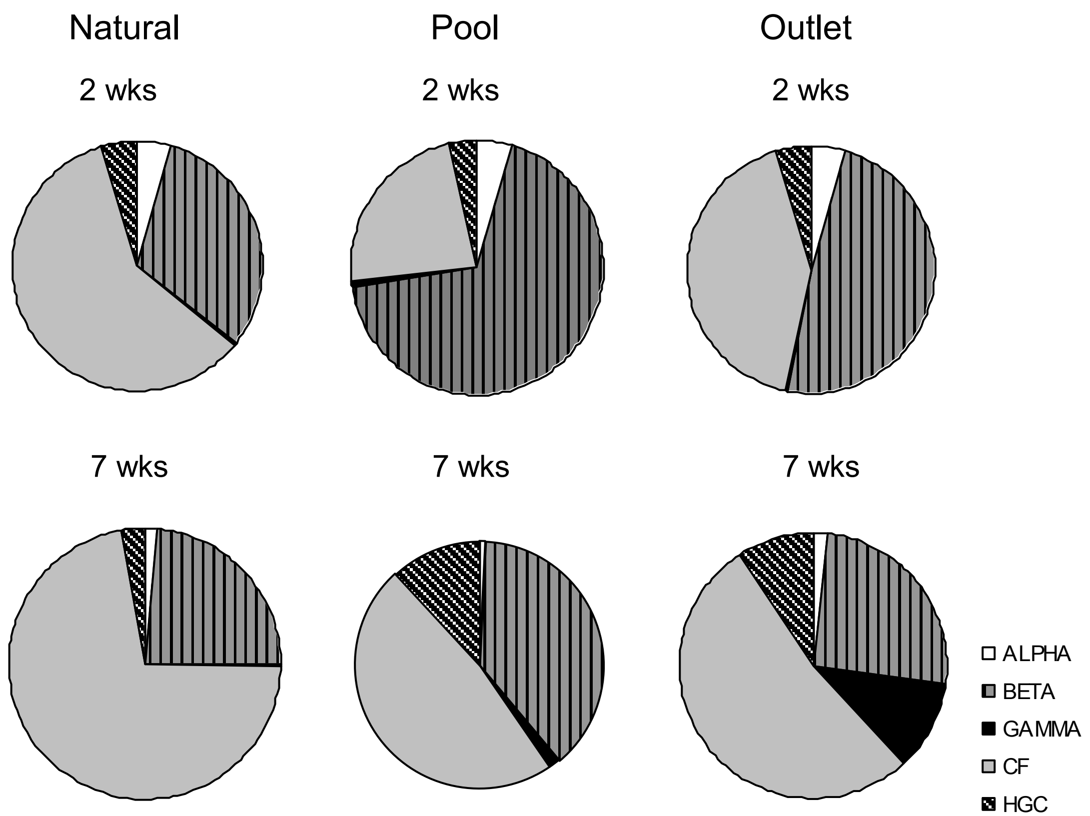

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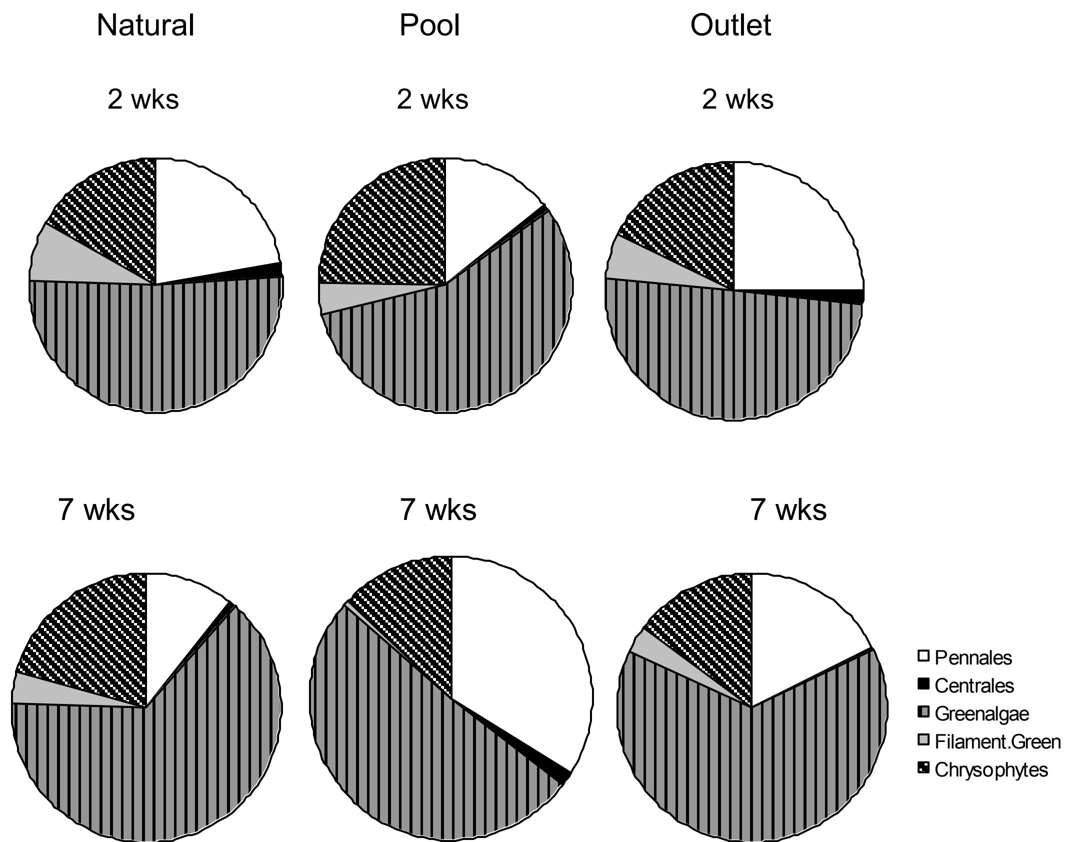

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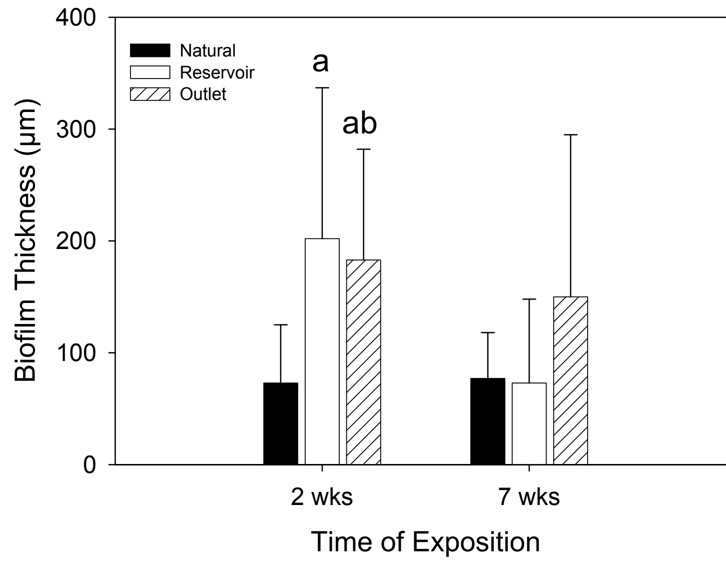
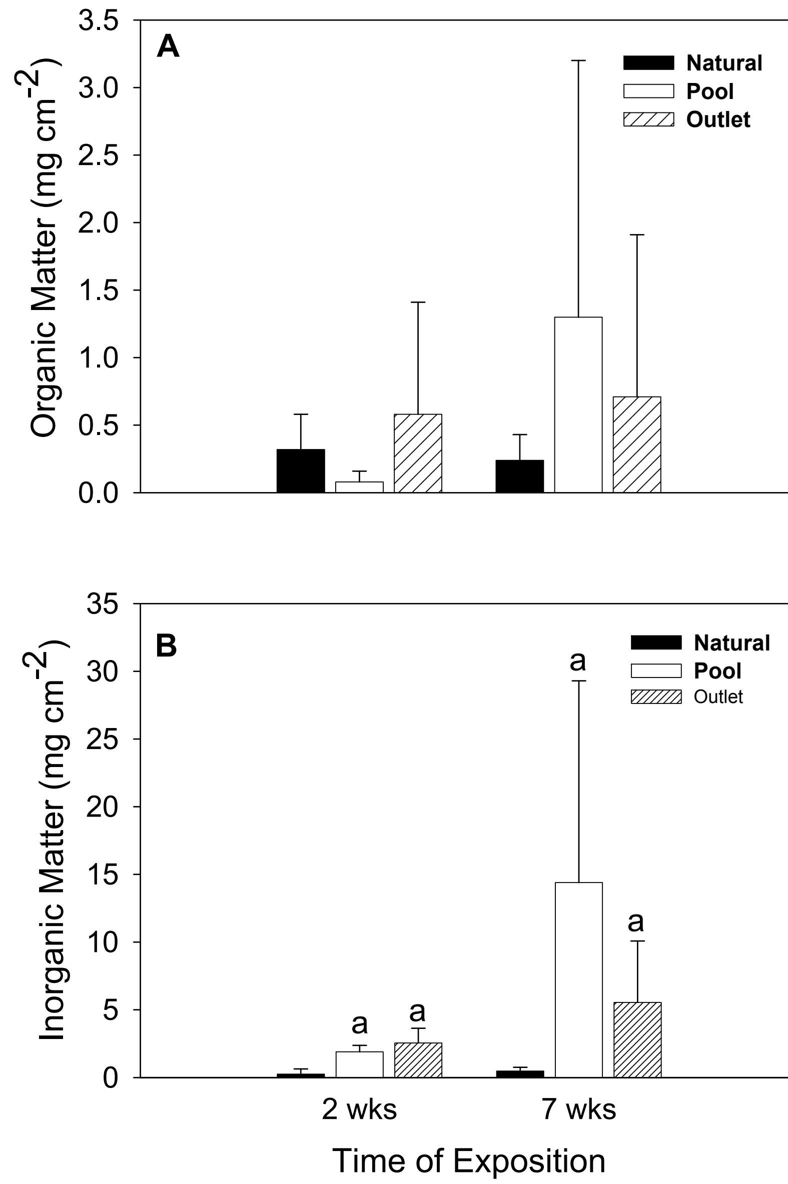


Figure 6



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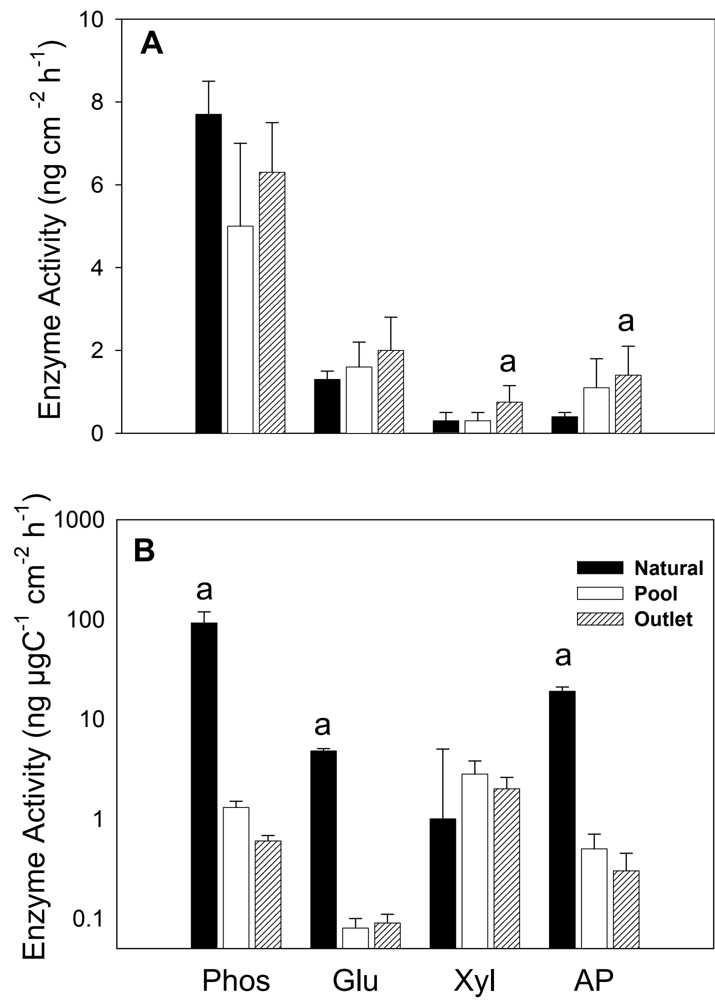


Figure 8

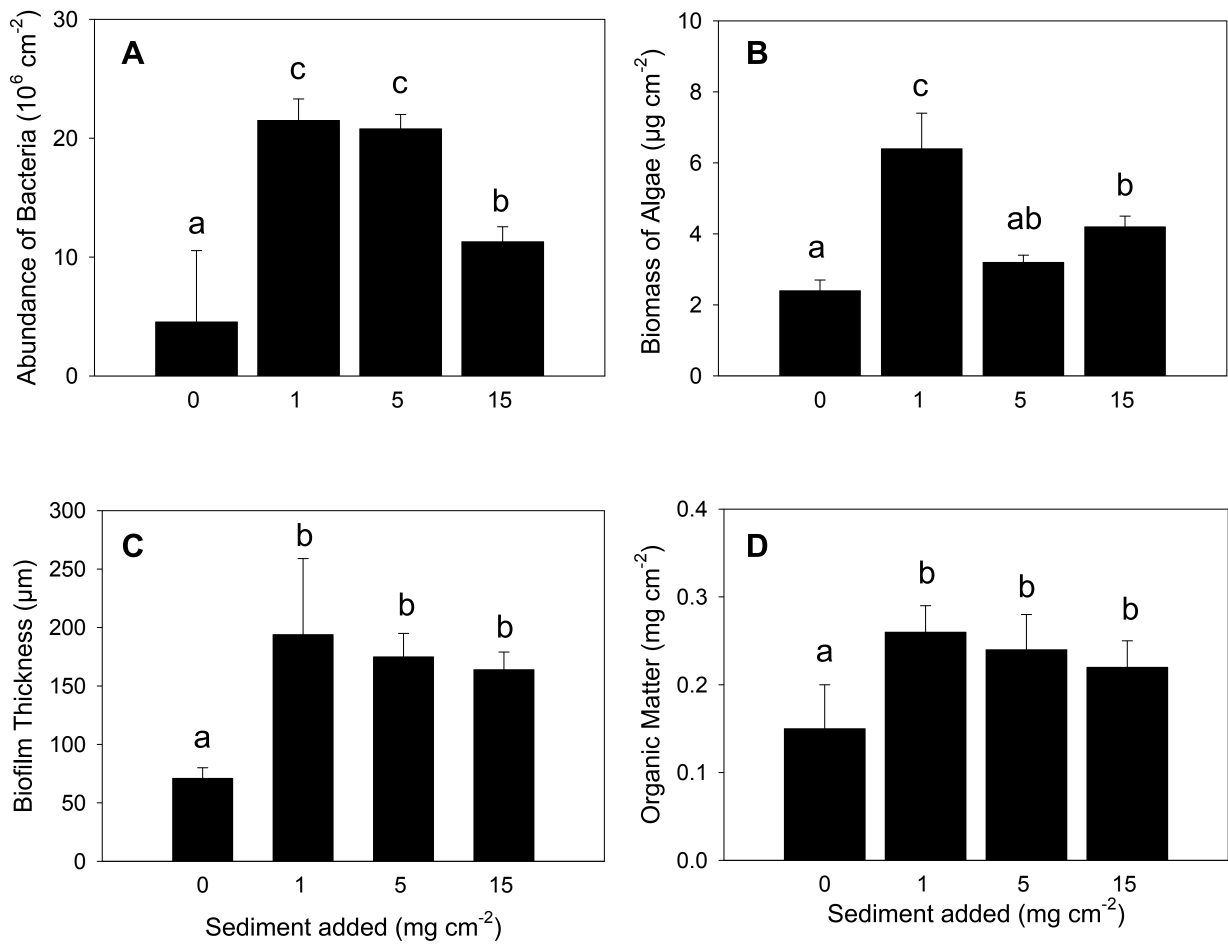


Figure 9

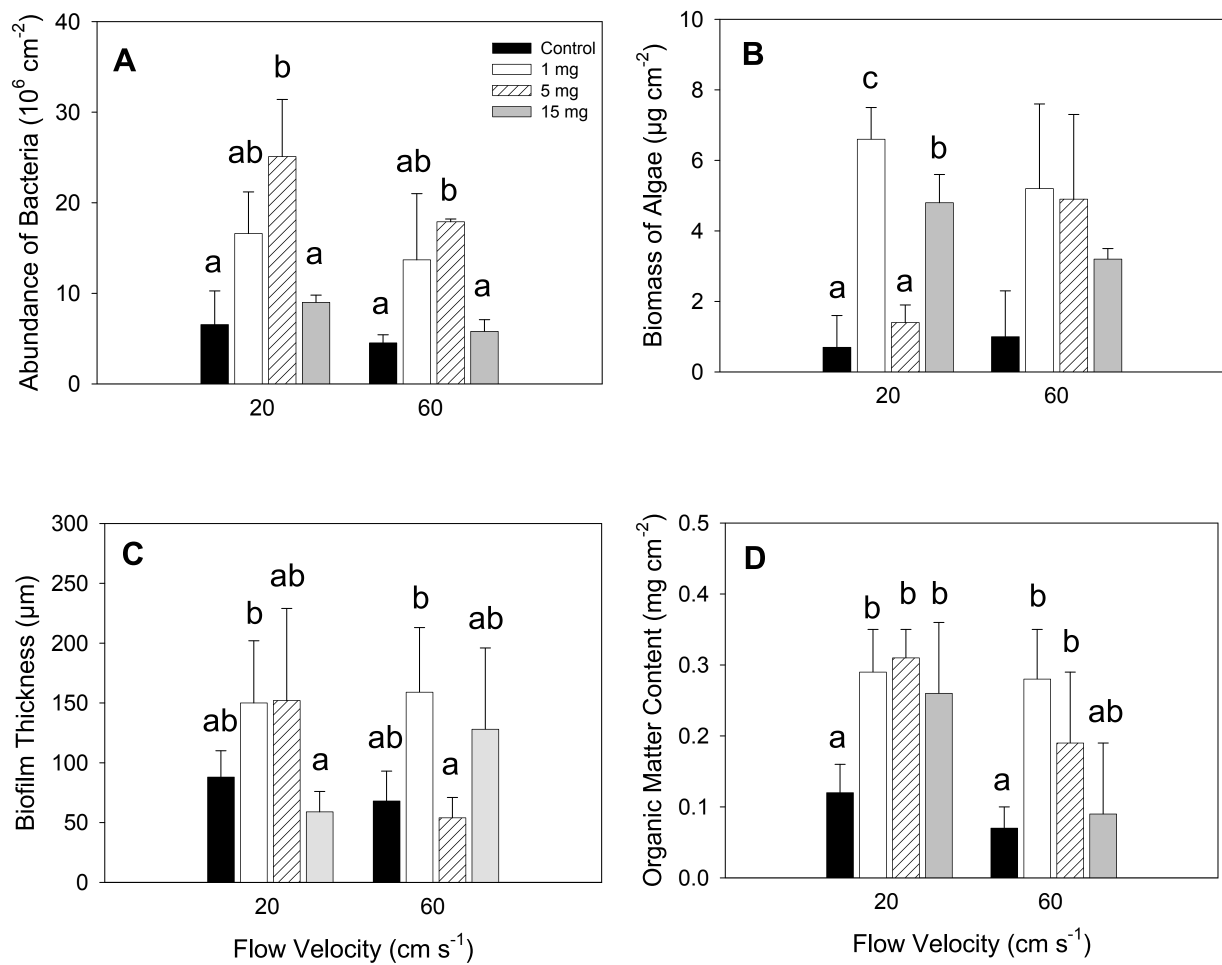


Figure 10

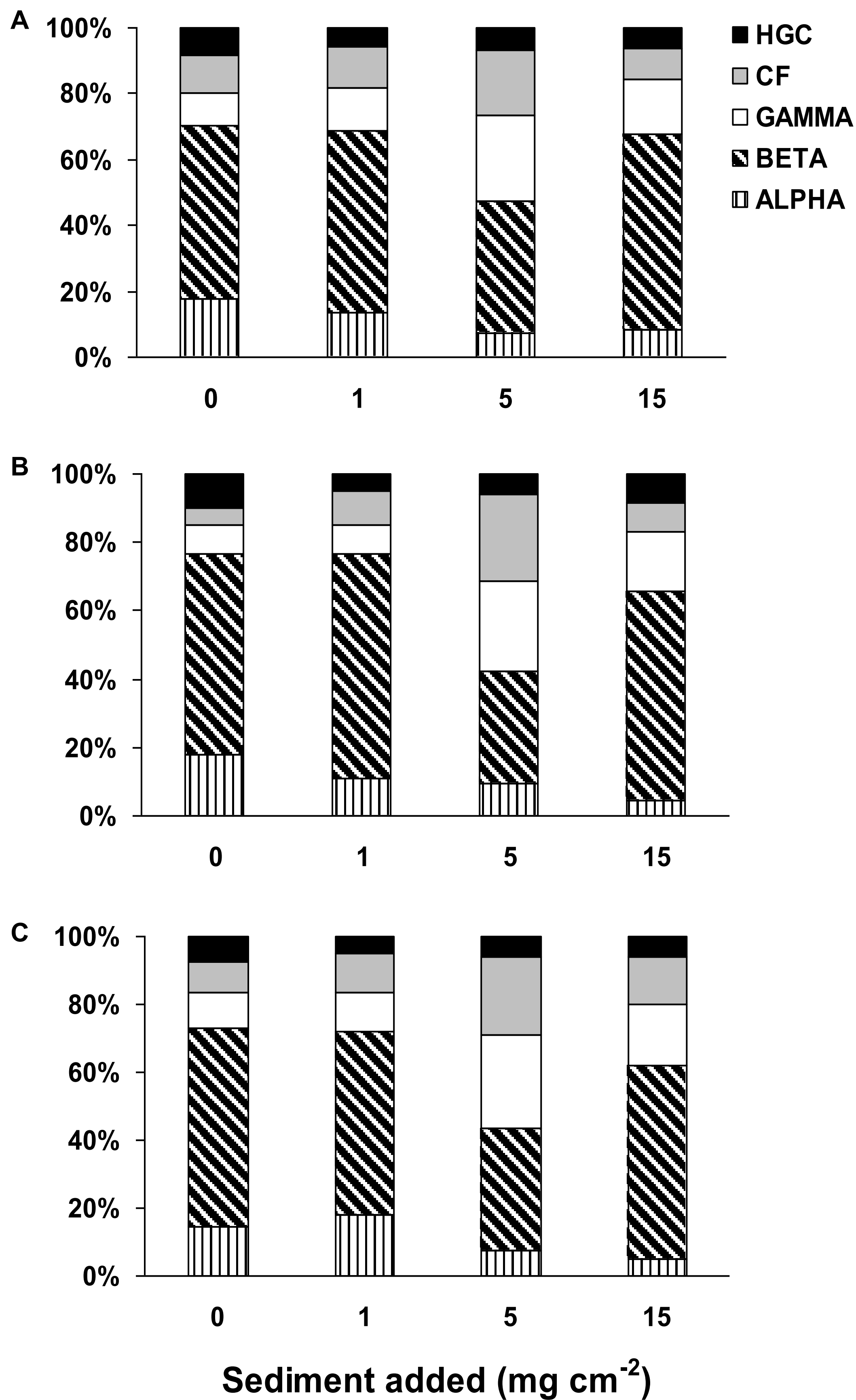
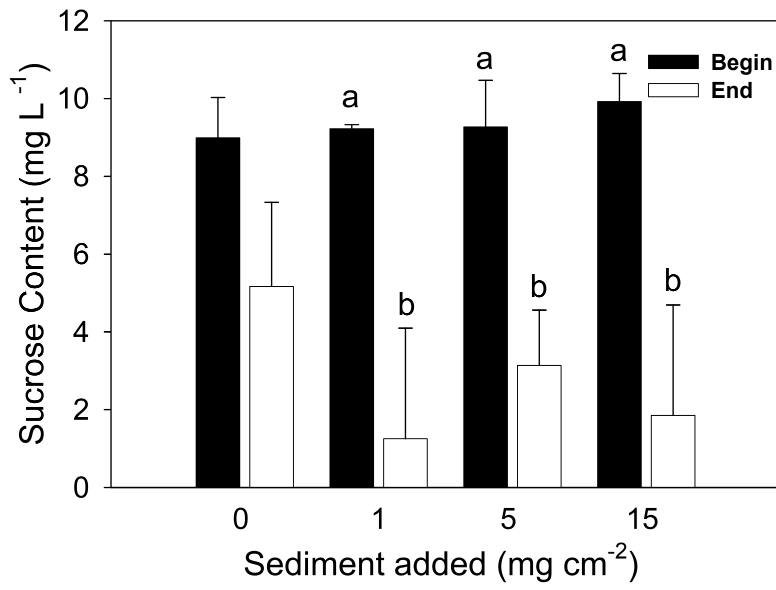


Figure 11



Supplement S1

Sequences of oligonucleotide probes and references used for CARD-FISH.

Specificy	Common Name	Sequence (5´-3´)	Reference
Eubacteria	S-D-Bact-0338-a-A-18	GCTGCCTCCCGTAGGAGT	(Amann et al., 1990)
Alphaproteobacteria	S-Sc-aProt-0019-a-A-17	CGTTCGYTCTGAGCCAG	(Manz et al., 1992)
Betaproteobacteria	L-Sc-bProt-1027-a-A-17	GCCTTCCCACTTCGTTT	(Manz et al., 1992)
Gammproteobacteria	L-Sc-gProt-1027-a-A-17	GCCTTCCCACATCGTTT	(Manz et al., 1992)
Cytophaga-Flavobacteria	S-P-CyFla-0319-a-A-18	TGGTCCGTGTVAGTAG	(Manz et al., 1994)
Actinobacteria	HCG69a	TATAGTTACCACCGCCGT	(Roller et al., 1994) (Berchtold et al., 1999)

1 # Influence of incorporated inorganic sediment on stream
2 # biofilms

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Abstract

Benthic bacteria are responsible for the bulk part of carbon cycling in stream ecosystems, usually considered to live either as biofilms attached to stone or rock surfaces or in-between fine streambed sediments. The effects of fine, inorganic sediments incorporated into attached biofilms have received relatively little attention, although it might represent a large part of the biofilm mass. In this study, the effects of incorporated inorganic sediment on the bacterial community structure, sucrose utilization and abrasion resistance of flow channel grown biofilms were investigated. Biofilms with 5 mg cm^{-2} added silicium dioxide had 4.3 to 6.9 times higher eubacterial abundances compared to biofilms without added sediment. Abundances of Cytophaga – Flavobacteria and γ-Proteobacteria were enhanced, and those of α-Proteobacteria reduced by sediment addition. Decrease rates of added sucrose were 1.5 to 1.7 fold higher in the sediment treatments compared to the control treatments, likely due to the increased eubacterial abundances. A part of the observed differences could be due to an enhanced shading effect of the sediment particles on autotrophic biofilm organisms. In contrast to control biofilms, biofilms with added sediment lost significant amounts of bacteria and sediment under an increased flow velocity. Thus, given the spatial and temporal variability of sedimentation in streams, incorporation of inorganic particles in biofilms could be an important ecosystem factor.

Introduction

Bacterial communities play an important role in the carbon cycle of stream ecosystems. They constitute an important part of heterotrophic biomass (36%) and the major fraction of heterotrophic production (71%) in a stream (Marxsen, 2006). Besides their classical role as decomposers, they are an important food source for consumers ranging from protists to invertebrates and fish. Allochthonous organic matter is usually the main energy source in streams, and heterotrophic bacteria provide a link between this organic matter and higher trophic levels (Meyer, 1994; Augspurger et al., 2008).

The majority of stream bacteria live attached to the streambed (Geesey et al., 1978), either in sediments or as biofilms attached to stones. Biofilms consist of microorganisms embedded in a matrix of extracellular polymeric substances (EPS) exposed to permanent sedimentation of particles from the water column. Inorganic particles will accumulate on the biofilm and are subsequently incorporated into the matrix. Deposited sediment can exceed organic biofilm mass in gravel streams and affect ciliate colonization (Risse-Buhl and Küsel, 2008) and patch selection by grazing invertebrates (Suren, 2005). However, their influence on biofilm structure and function is unclear.

In stream sediments, the structure and function of microbial communities is affected by the sediment particles. Sand and silt particles are preferentially colonized. Bacterial biomass is higher in finer grained particles (e. g. Bott and Kaplan, 1985 and Meyer-Reil 1994), although higher abundances are reported on larger standardized glass particles (Santmire and Leff, 2007). Microbial assemblages are significantly correlated to sediment particle size in hyporheic zones (Sliva and Williams, 2005), and differences between bacterial communities in sediments and biofilms grown on stones are higher than within samples of one habitat (Beier et al., 2008). Metabolic activity differs between biofilms on sand and rock substrata (Romaní and Sabater, 2001), and biofilm metabolism is affected by the physical habitat heterogeneity of streambeds, i.e. the variance of substratum size (Cardinale et al., 2002).

Natural microbial communities are structured by multiple interacting forces, and their composition can have functional significance (e.g. Cavigelli and Robertson, 2000; Horz et al., 2004; Bell et al., 2005; Monson et al., 2006). Species sorting in response to local environmental factors is a key determinant of aquatic bacterial community composition (Van der Gucht et al., 2007). Factors affecting microbial communities in streams include temperature (Hullar et al., 2006), inorganic nutrients and dissolved organic carbon (DOC) (Olapade and Leff, 2005; Hullar et al., 2006; Beier et al., 2008), as well as the quality of total

1 organic matter (Fazi et al., 2005). Biofilm community composition can also be influenced by
2 autochthonous carbon sources (Olapade and Leff, 2005; Hullar et al., 2006), supporting the
3 possibility of a strong internal coupling of primary producers and heterotrophs in biofilms.
4 Biogeography has only a weak effect (Van der Gucht et al., 2007), although a distance effect
5 along a stream can be observed (Beier et al., 2008), probably due to the unique unidirectional
6 flow in lotic systems.

7 The flow regime is a key factor in stream ecosystems, and transport and sedimentation
8 of particles is governed by the flow velocity. Streambed heterogeneity results in spatial
9 variations in the flow pattern, and biofilms can adapt their internal structure to resist shear
10 stress under higher flow velocities (Beyenal and Lewandowski, 2002). Nevertheless, temporal
11 discharge peaks, e.g. following snowmelts or heavy rainfalls, can result in biofilm abrasion
12 due to overwhelming shear stress, resetting biofilm growth.

13 The aim of this study was to elucidate the effects of inorganic particles incorporated
14 into the matrix of biofilms. We hypothesized that (1.) added sediment particles increase the
15 available substrate surface for bacteria, and thus lead to higher abundances. (2.) Accumulation
16 of inorganic particles in the matrix changes the microhabitat in biofilms, which results in an
17 altered bacterial community structure. (3.) Furthermore, shading due to the sediment particles
18 will lower primary production, resulting in a decreased production of algal exudates. Hence,
19 utilization of carbon sources from the water column should increase. (4.) The incorporated
20 sediment will destabilize the EPS matrix, making it more vulnerable to increased shear stress
21 under higher flow velocities. In a pre-experiment, biofilms were grown simultaneously in
22 flow channels and in a stream to evaluate the comparability of flow channel biofilms and their
23 natural counterparts. In the main experiment, biofilms were grown under light in flow
24 channels, one half of them receiving regular sediment additions. After four weeks, sucrose
25 was added to all channels. Channels were run for one more week, now either with or without
26 a light source. Finally, these biofilms were exposed to an increased flow velocity to test their
27 stability under increased shear stress.

28

29 **Methods**

30 *Biofilm growth*

31 Stream biofilms were grown for five weeks on glass slides at two pool sites in the
32 Leutra; a 1st order rock stream near Jena (Thuringia, Germany). Flow velocities at normal
33 discharge varied between < 0.01 m s^{-1} in larger pools up to 0.2 m s^{-1} in riffles. Twelve glass
34 slides were exposed in plastic frames at both pool sites. Laboratory biofilms were grown in

flow channels (50 x 16 x 4 cm; length x width x height) containing 24 glass slides in a climate chamber at 13 °C in a 12 h light-dark cycle. Photosynthetic active radiation approximated 100 µmol m^{-2} s^{-1} above the water surface during the light period. Flow velocity in all channels was adjusted to 0.01 m s^{-1}, and channels were connected to one pump reservoir. Five litres of Leutra water per channel were circulated using one rotary pump per channel (WIND'S 1200, Sacem, Giorgio, Italy) and replaced every two weeks. The water was taken from the stream without disturbing the streambed to minimize the amount of suspended sediment. In the pre-experiment, biofilms were grown in three flow channels simultaneously with the exposition of slides in the Leutra.

Sediment and sucrose addition

In the main experiment, 5 mg cm^{-2} combusted (550 °C) silicium dioxide (SiO_2) particles (0.5 - 10 µm diameter; >80 % between 5 - 10 µm) were added to six out of twelve channels in total. The total amount of sediment for each channel was divided in six equal parts, and added at day 1, 4, 8, 11, 15 and 18. For each addition, the silicium dioxide was suspended in 24 ml of channel water. The suspension was distributed over the glass slides in 24 steps, using a pipette. Per step, 1 ml suspension was added over one glass slide respectively, with vigorous shaking of the suspension between every step. To achieve a homogeneous distribution on the biofilm, the silicium dioxide was released as high as possible in the water column, maximizing dispersal due to sedimentation of the particles in the water. After 28 days, 10 mg C L^{-1}sucrose ($C_{12}H_{22}O_{11}$) was added to all flow channels. From now on, all channels had separated water circuits. Three flow channels of each treatment (channels with sediment addition and control channels without added sediment) were run in darkness, the other three continued under the light-dark cycle, all for seven more days.

Biofilm abrasion under higher flow velocity

At the end of the sucrose incubation, six glass slides from every treatment, respectively, were transferred immediately to two flow channels (98 x 25 x 2 cm; length x width x height). This transfer was necessary, as the smaller channels did not allow for a sufficient flow velocity increase. The flow velocity was adjusted to 0.60 m s^{-1}, chosen to simulate a high discharge event in the Leutra stream. Bacterial abundances and inorganic biofilm mass were determined from triplicate samples of each treatment after one hour and compared to those obtained at the end of sucrose incubation.

Biotic parameters

Eubacterial abundance and eubacterial population structure were determined at the end of the pre-experiment and immediately before and after sucrose incubation in the main experiment. Samples were taken in triplicate by scraping the biofilm off one glass slide into filter sterilized 3.6 % formaldehyde. α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Cytophaga-Flavobacteria, and Aktinobacteria were quantified to determine changes in the community structure. These groups were selected due to their abundance in streams and their preference for different organic compound classes (Santmire and Leff, 2007), and detected with catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) (Pernthaler et al., 2002; Pernthaler et al., 2004), which gives better results compared to FISH in sediments (Fazi et al., 2005). Group specific horseradish peroxidase (HRP) labeled oligonucleodite 5´-3´ sequences (Biomers, Ulm, Germany) and a NON probe were used (Table 1) with $Alexa_{488}$ as fluorochromic dye. Euacteria were enumerated with CARD-FISH and compared to counts after staining with 4',6-diamidino-2-phenylindoldihydrochloride (DAPI) (Porter and Feig, 1980). The difference between eubacterial counts and the sum of α-, β-, γ- Proteobacteria, Cytophaga – Flavobacteria, and Actinobacteria counts was calculated to represent the abundance of Eubacteria not belonging to these groups, and is hereafter referred to as 'other Eubacteria'. Additionally, after the sucrose incubation chlorophyll *a* (Chl *a*) concentrations of biofilms were determined spectrophotometrically following an acetone extraction (Clesceri, 1998), and biofilms were examined using a confocal laser scanning microscope (400 x magnification; Leica TCS SP). DAPI counts were used to determine bacterial abundances after the abrasion test.

Abiotic parameters

Inorganic biofilm content was determined at the end of the pre-experiment, and after sucrose incubation and at the end of the abrasion test in the main experiment. Triplicate samples were weighted after drying for 48 h at 60 °C and following combustion for 4 h at 550 °C. Incorporation of added sediment was calculated as the difference in inorganic biofilm content between the sediment addition and control treatments. Water samples were taken from the stream at the beginning, two weeks after start, and end of glass slides exposure. In flow channels, water samples were taken at the start, immediately before and after water exchanges, and at the end of the experiment. Ammonium, nitrate and phosphate were determined photometrically (Clesceri, 1998). DOC was measured using an infrared spectrometer (DIMATOC 100, Dimatec Analysentechnik GmbH, Germany). Average nutrient values in the Leutra water were < 2.8, 444.1 ± 111.2, 2.8 ± 1.2, and 2.4 ± 0.3 for

1 ammonium, nitrate, phosphate and DOC, respectively. After sucrose addition, water samples
2 of 1 mL were taken by sterile syringes from each channel five times a day (every four hours,
3 and one eight hour interval during night). Concentrations of sucrose were determined using a
4 high pressure liquid chromatograph connected to a refractive index detector (System Gold,
5 Beckman Coulter, USA) (Reiche et al., 2008).
6
7 **Results**
8 *Leutra biofilms vs. flow channel biofilms*
9 Inorganic biofilm content of the Leutra biofilms was 8.3 to 10.3 times higher
10 compared to flow channel biofilms (Figure 1A). Leutra biofilms contained 1.4 to 1.9 times
11 more eubacteria compared to flow channel biofilms ($p<0.05$; one way ANOVA), with
12 abundances of $95.3 \pm 10.0 \times 10^6$ cells cm^{-2} and $106.4 \pm 6.1 \times 10^6$ cells cm^{-2} in biofilms of the
13 two Leutra sites and $54.9 \pm 12.9 \times 10^6$ cells cm^{-2} in the flow channel biofilm (Figure 2A).
14 Eubacterial counts represented 82 to 112 % of the DAPI counts. β-Proteobacteria was the
15 most abundant group targeted with CARD-FISH (Table 2), γ-Proteobacteria reached almost
16 as high abundances in the Leutra biofilms. Both Leutra sites had similar community
17 compositions, whereas the community in the flow channel biofilm had a lower proportion of
18 γ-Proteobacteria (6.8 % vs. 12.8 and 13.8 % in the field site biofilms) and Cytophaga –
19 Flavobacteria (0.7 % vs. 2.7 and 3.1 % in the field site biofilms). Nutrient concentrations in
20 the flow channel water were similar to the Leutra water (see appendix A).
21 *Community structure in 4 week old biofilms*
22 Inorganic mass of biofilms with 5 mg cm^{-2} sediment added was similar to the
23 inorganic mass of biofilms grown in the Leutra (Figure 1A and 1B), whereas in channels
24 without additional sediment the amount of inorganic biofilm mass was 11.9 to 15.2 times
25 lower ($p<0.05$; one way ANOVA). Eubacterial counts represented 74.7 to 97.7 % of the
26 DAPI counts. Eubacterial abundances in biofilms with added sediment were 5.3 to 6.9 times
27 higher compared to those in control biofilms ($p<0.05$; one way ANOVA) (Figure 3A).
28 Eubacterial community structure differed between biofilms with added sediment and control
29 biofilms (Figure 3B). Relative abundances of γ-Proteobacteria and Cytophaga – Flavobacteria
30 were higher in biofilms with added sediment (18.1 % vs. 7.5 %, and 11.6 % vs. 5.4 %,
31 respectively), and α-Proteobacteria and other Eubacteria had lower relative abundances in
32 biofilms with added sediment (4.8 % vs. 8.2 %, and 39.7 % vs. 52.5 %, respectively), while
33 β-Proteobacteria and Aktinobacteria had similar relative abundances in biofilms with and
34 without sediment addition (22.2 % vs. 23.0 %, and 3.6 % vs. 3.4 %, respectively).

1 *Influence of sucrose addition*

2 Sucrose concentrations decreased 1.7 fold and 1.5 fold faster in channels with added
3 sediment in the light and dark treatment, respectively (*p<0.05*; one way ANOVA) (Figure 4).
4 There was a trend for a faster decrease in channels without light, but no significant differences
5 could be detected. The decrease rates are equivalent to a per cell decrease rate of 20.7 x 10^{-9}
6 mg C h^{-1} and 22.9 x 10^{-9} mg C h^{-1} in the light and dark treatment of the control channels, and
7 5.0 x 10^{-9} mg C h^{-1} and 6.6 x 10^{-9} mg C h^{-1} in the light and dark treatment with sediment
8 addition. Resulting from the different sucrose decrease rates, DOC concentrations were lower
9 in channels with added sediment (see appendix A) than in control channels, while inorganic
10 nutrients did not differ between treatments (*p<0.05*; one way ANOVAs).

11 In the light treatment, eubacterial abundances increased 1.9 fold in biofilms with
12 sediment addition compared to a 3.0 fold increase in biofilms without sediment addition
13 (Figure 3C). In the dark treatment, eubacterial abundances increased 2.4 fold in biofilms with
14 sediment addition compared to a 2.5 fold increase in biofilms without sediment addition
15 (Figure 3C). Eubacterial abundances were still 4.3 to 5.0 times higher in biofilms with added
16 sediment compared to those without sediment addition (*p<0.05*; one way ANOVA), but there
17 was no difference in eubacterial abundances between the respective light and dark treatments
18 (*p>0.05*; one way ANOVA). In channels with sediment addition, abundances of the
19 specifically targeted groups did not increase much, if at all. They were in the range of 0.8 to
20 1.3 times of their abundance after 4 weeks (Table 2). Hence, in biofilms with sediment
21 addition not specifically targeted eubacteria accounted mostly for the observed increase in
22 total abundances (Figure 3C and 3D). In the control channels increase in abundances was
23 more evenly spread among the groups. Strong increases were detected in α-Proteobacteria
24 (2.3 and 2.2 fold increase with and without light, respectively), β-Proteobacteria (2.7 and 2.2
25 fold increase with and without light, respectively), Aktinobacteria (2.7 and 4.6 fold increase
26 with and without light, respectively), and other Eubacteria (3.4 and 2.9 fold increase with and
27 without light, respectively) (Table 2). Cytophaga – Flavobacteria increased strongly only in
28 light (4.4 fold increase vs. 1.2 fold increase without light), and γ-Proteobacteria had the
29 lowest increase (1.7 and 1.1 fold increase with and without light, respectively) (Table 2).

30 After 5 weeks, Chl *a* content did not differ between treatments, except for lower
31 concentrations in the control dark treatment (*p<0.05*; one way ANOVA). Chl *a*
32 concentrations were 0.039 ± 0.005 µg cm^{-2} and 0.016 ± 0.002 µg cm^{-2} in control channels with
33 and without light, respectively, and 0.053 ± 0.009 µg cm^{-2}, and 0.046 ± 0.004 µg cm^{-2} in
34 channels with sediment addition with and without light, respectively. Microscopy revealed

1 that inorganic particles covered almost the entire surface in the sediment treatments, whereas
2 only a few particles were present in the control treatments. Biofilms rarely covered the whole
3 area in the microscope pictures in all treatments, but sediment particles in the control
4 treatment (if present) where always covered with a dense biofilm.
5 *Effects of increased flow velocity*
6 Abrasion was visible in biofilms with added sediment almost immediately after
7 exposure to the increased flow velocity, loosing lobate parts of several mm^2 in size. After five
8 to ten minutes, visible abrasion stopped. In control biofilms, no abrasion was visible during
9 the whole time. After one hour, biofilms with added sediment had lost in average 34.1 % and
10 51.4 % of incorporated sediment, and 25.5 % and 60.7 % of their bacteria in the light and dark
11 treatment, respectively ($p<0.05$; t-tests) (Table 3). No differences were found in biofilms
12 without added sediment ($p>0.05$; t-tests).
13
14 **Discussion**
15 *Flow channel biofilms vs. stream biofilms*
16 Bacterial communities in stream biofilms differ from those in the water column
17 (Besemer et al., 2007), and thus mostly independent during biofilm development. The initial
18 biofilm colonizers, however, seem to be selected stochastically from the water column
19 (Jackson et al., 2001). In this study, Leutra biofilms had similar bacterial community
20 structures compared to simultaneously grown flow channel biofilms initiated with Leutra
21 water. The detected shift in γ-Proteobacteria and Cytophaga – Flavobacteria as well as the
22 difference in total eubacterial abundances was also observed in the main experiment between
23 channels with and without sediment addition. In contrast to the flow channel biofilms, Leutra
24 biofilms were exposed to the natural occurring sedimentation. Hence, this is likely a result of
25 the higher sediment content present in the Leutra biofilms, and flow channel biofilms seemed
26 to represent natural biofilm community structures well.
27 *Sediment influence on biofilm communities*
28 Surfaces in aquatic environments are rapidly colonized by bacteria (Costerton et al.,
29 1995). The added sediment particles provided new, clean surfaces for colonization, and
30 biofilms in those treatments had higher bacterial abundances compared to control channels.
31 Particles present in the control biofilms seemed to be favored spots for biofilm growth. It
32 could be that elevated spots provide more favorable nutrient and resource conditions, and
33 hence are actively colonized by bacteria (Klausen et al., 2003).

1 In the sediment treatments, autotrophic activity might be reduced due to enhanced
2 shading by the added sediment particles, making relatively more inorganic nutrients available
3 for heterotrophic organisms. Abundances of Cytophaga – Flavobacteria and γ-Proteobacteria
4 were enhanced in these channels, and both groups can be associated with high concentrations
5 of nitrate and DOC (Kirchman 2002; Gao et al., 2005). α-Proteobacteria, in streams more
6 abundant in low nutrient habitats and correlated to algal exudates (Gao et al., 2005), were
7 reduced by sediment addition. However, the eubacterial groups responding to the sucrose
8 addition differed strongly between biofilms with and without sediment addition, and seemed
9 not to be related to the described nutrient preferences. Thus, the detected community structure
10 could not reveal all differences in community composition, which reveals the limitations of
11 the coarse taxonomic resolution of groups. At the same time, it highlights the differences
12 between bacterial communities present in biofilms with and without added sediment.

13 *Sediment influence on sucrose uptake*

14 The faster sucrose decrease was likely related to the overall higher eubacterial
15 abundances in biofilms with added sediment, as both per cell decrease rates and increase in
16 eubacterial abundances were higher in control biofilms. Algal exudates can be a significant
17 carbon source for biofilms even with high sugar concentrations present in the water. Self
18 shading, however, can result in lower photosynthesis rates (Boston and Hill, 1991; Guasch
19 and Sabater, 1995) and it seems plausible that sediment particles enhance this effect. As
20 hypothesized, sucrose utilization was higher in channels with added sediment. However,
21 decrease rates did not differ between light and dark (i.e. without primary production)
22 treatments, suggesting that production of algal exudates was overall low. On the other hand,
23 this effect might have been countered by the higher increase in bacterial abundances in the
24 control treatment; release of algal exudates might not have resulted in lower sucrose
25 utilization, but in higher growth rates. Nevertheless, the observed differences in sucrose
26 utilization seemed to mostly result from the enhanced eubacterial abundances in biofilms with
27 added sediment.

28 *Sediment influence on abrasion*

29 In sediments, high microbial biomass supports sediment stability (Yallop et al., 2000). In
30 biofilms, high sediment density might decrease biofilm stability, as abrasion occurred in
31 biofilms with added sediment, but not in control biofilms. Sediment particles could be
32 obstacles in the biofilm matrix, preventing the formation of a coherent and thus stable
33 structure. Furthermore, biofilms with added sediment had a higher biomass and contained
34 more inorganic particles per area, and consequently should have been thicker compared to the

1 control biofilms. The resulting shear stress from the water flow would be higher in thicker
2 biofilms and could lead to increased abrasion. Therefore, at least a part of the induced
3 instability by sediment particles could be an indirect effect as they enhanced biofilm growth
4 in the first place.

5 *Consequences for stream ecosystems*

6 Stream biofilms are hybrids somewhere between biofilms (i.e. microorganisms
7 attached to a surface) and colonized streambed sediments, depending on the amount of
8 sediment incorporated in the matrix. Sedimentation rates depend on the local flow velocities,
9 which are determined by the physical topography of the streambed, riffle-pool sequences,
10 straightenings, weirs, and the streambed slope. The heterogeneity of the topography in turn
11 can affect biofilm community structure and function (Cardinale et al., 2002). Consequently,
12 variation in incorporated inorganic sediment particles resulting from this heterogeneity could
13 be an important factor involved in these processes.

14 Temporal variation in sedimentation comes with discharge fluctuations, which, in turn,
15 can be mediated by large physical structures like weirs (Pohlon et al., 2007). Additionally,
16 discharge peaks might also reset biofilm growth due to abrasion, especially in areas with high
17 sedimentation. Thus, regions which favor bacterial growth due to sedimentation under normal
18 discharge are more vulnerable to increased flow velocities, whereas regions of lower
19 sedimentation might be more resistant to discharge fluctuations.

20

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4

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Table 1: Sequences of oligonucleotide probes used for bacterial community analysis.

Taxon	Common Name	Sequence (5´-3´)	Reference
Eubacteria	S-D-Bact-0338-a-A-18	GCTGCCTCCCGTAGGAGT	(Amann et al. 1990)
α- Proteobacteria	S-Sc-aProt-0019-a-A-17	CGTTCGYTCTGAGCCAG	(Manz et al. 1992)
β- Proteobacteria	L-Sc-bProt-1027-a-A-17	GCCTTCCCACTTCGTTT	(Manz et al. 1992)
γ- Proteobacteria	L-Sc-gProt-1027-a-A-17	GCCTTCCCACATCGTTT	(Manz et al. 1992)
Cytophaga-Flavobacteria	S-P-CyFla-0319-a-A-18	TGGTCCGTGTVAGTAG	(Aman et al. 1995)
Aktinobacteria	HCG69a	TATAGTTACCACCGCCGT	(Amann et al. 1995)
NON	NON338	ACTCCTACGGGAGGCAGC	(Wallner et al. 1993)

Table 2: Abundances (mean ± standard deviation) of bacterial groups in biofilms targeted with CARD-FISH. In the pre-experiment, abundances were determined in 5 week old biofilms simultaneously grown in two pools of the Leutra stream and in laboratory flow channels with Leutra water. In the main experiment, light grown biofilms had been separated after 4 weeks to either continued growth under light or darkness for one more week.

Treatment	α- Proteobacteria [10^5 cells cm^{-2}]	β- Proteobacteria [10^5 cells cm^{-2}]	γ- Proteobacteria [10^5 cells cm^{-2}]	Cytophaga – Flavobacteria [10^5 cells cm^{-2}]	Actinobacteria [10^5 cells cm^{-2}]
Pre-experiment					
Leutra pool 1	62.65 ± 2.93	166.52 ± 33.91	122.13 ± 11.30	25.57 ± 19.85	0.84 ± 0.28
Leutra pool 2	74.95 ± 18.58	151.40 ± 15.94	147.05 ± 12.86	33.48 ± 3.24	0.95 ± 0.55
flow channels	47.76 ± 13.04	84.87 ± 24.08	37.15 ± 14.25	3.69 ± 1.11	1.05 ± 0.43
Main experiment					
4 weeks					
control	1.25 ± 0.49	3.54 ± 1.44	1.16 ± 0.44	0.87 ± 0.90	0.51 ± 0.13
sediment	4.42 ± 1.35	20.51 ± 3.12	16.82 ± 2.64	10.85 ± 2.42	3.34 ± 0.10
5 weeks					
control light	2.91 ± 0.29	8.64 ± 2.73	1.65 ± 0.79	1.86 ± 1.18	1.41 ± 0.28
control dark	2.69 ± 0.76	8.52 ± 3.13	1.49 ± 0.50	1.62 ± 0.80	2.26 ± 0.60
sediment light	17.38 ± 7.27	69.95 ± 20.38	16.20 ± 0.70	15.46 ± 2.30	7.57 ± 0.83
sediment dark	17.79 ± 9.39	74.82 ± 32.99	17.65 ± 2.20	14.66 ± 9.90	3.56 ± 2.26

Table 3: Inorganic sediment content and bacterial abundances of 5 week old biofilms with and without added sediment before and after an one hour increase in flow velocity from 0.01 m s^{-1} to 0.6 m s^{-1}. After 4 weeks, light grown biofilms had been separated to either continued growth under light or darkness. Values represent means standard deviation.

Treatment	Unit	before flow increase	after flow increase
Inorganic Sediment			
control light	mg cm^{-2}	0.35 ± 0.08	0.19 ± 0.09 ^{ns}
control dark	mg cm^{-2}	0.30 ± 0.09	0.15 ± 0.10 ^{ns}
sediment light	mg cm^{-2}	4.32 ± 0.38	3.22 ± 0.48 *
sediment dark	mg cm^{-2}	4.60 ± 0.17	1.81 ± 1.45 *
Bacterial abundances[#]			
control light	10^6 cells cm^{-2}	4.55 ± 1.21	4.52 ± 0.85 ^{ns}
control dark	10^6 cells cm^{-2}	4.31 ± 0.42	3.97 ± 0.30 ^{ns}
sediment light	10^6 cells cm^{-2}	20.81 ± 3.60	13.72 ± 2.18 *
sediment dark	10^6 cells cm^{-2}	18.34 ± 5.76	8.91 ± 4.17 *

^{ns} no significant difference ($p>0.05$; t-test) before and one hour after the flow velocity increase.

* significant difference ($p<0.05$; t-test) before and one hour after the flow velocity increase.

[#] DAPI counts

1 **FIGURE LEGENDS**

2 Figure 1: Inorganic sediment content in 5 week old biofilms grown (A) simultaneously
3 in the Leutra stream and in laboratory flow channels with Leutra water, and (B) in laboratory
4 flow channels with Leutra water with or without 5 mg cm^{-2} sediment addition. After 4 weeks,
5 channels were separated either under light or dark conditions. Different letters indicate
6 significant differences ($p<0.05$;one way ANOVA).

7

8 Figure 2: Eubacterial abundances (A) and eubacterial community structure (B)
9 determined by CARD-FISH in 5 week old biofilms grown simultaneously in the Leutra
10 stream and in laboratory flow channels with Leutra water. Different letters indicate significant
11 differences ($p<0.05$;one way ANOVA).

12

13 Figure 3: Eubacterial abundances (left column) and eubacterial community structures
14 (right column) determined by CARD-FISH in 4 week old, light grown biofilms (upper row),
15 with or without 5 mg cm^{-2} sediment addition. Ten mg C L^{-1} sucrose was added after 4 weeks,
16 and channels were separated either under light or dark conditions for one more week (lower
17 row). Different letters indicate significant differences ($p<0.05$;one way ANOVA).

18

19 Figure 4: Sucrose decrease rates detected in flow channels containing biofilms with or
20 without added sediment. Biofilms were grown under light conditions for 4 weeks. At this
21 time, 10 mg C L^{-1} sucrose were added and decrease rates determined over one more week
22 under either light or dark conditions.

23

24

Figure 1

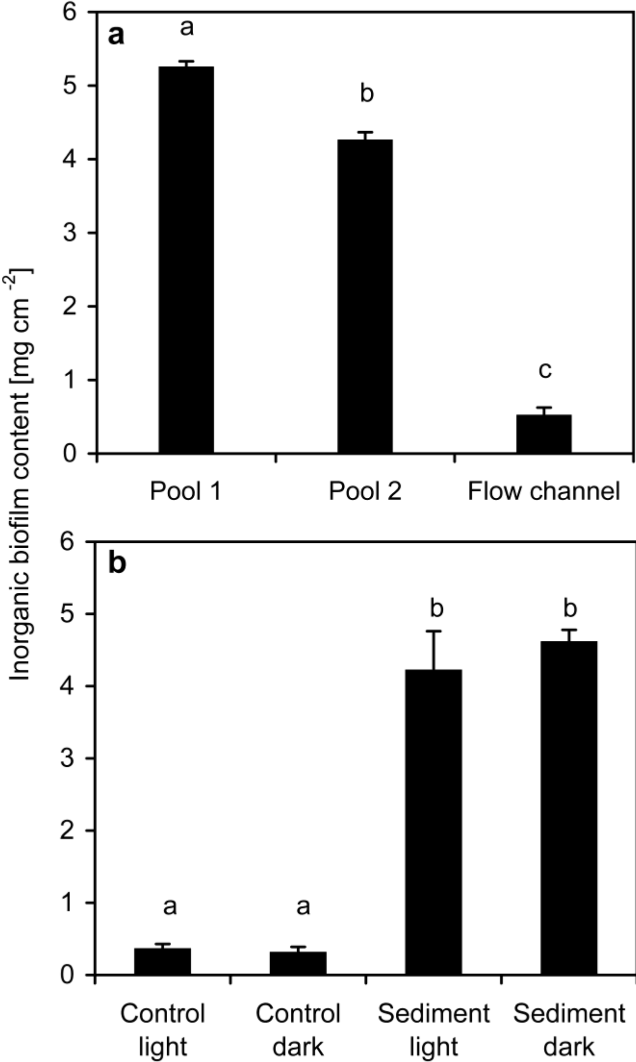


Figure 2

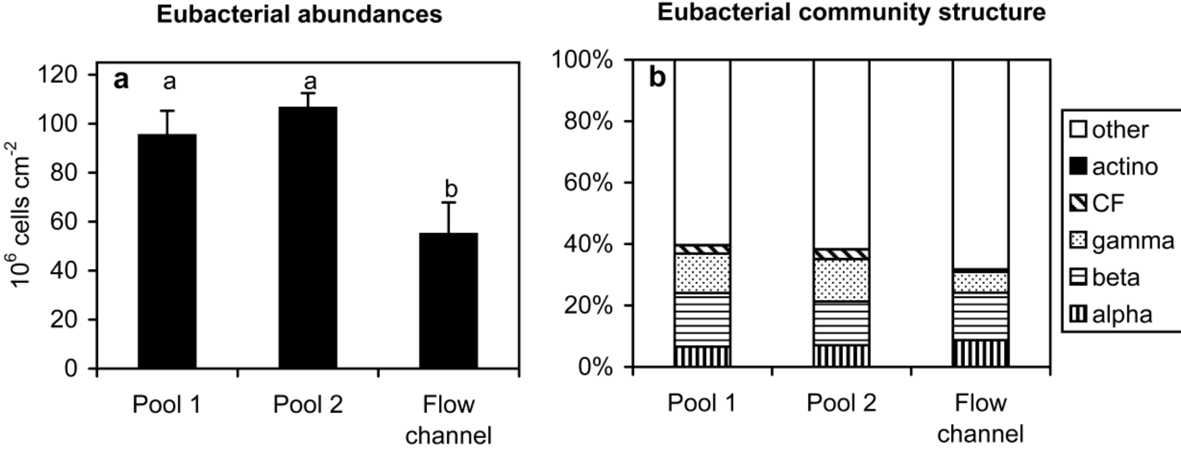


Figure 3

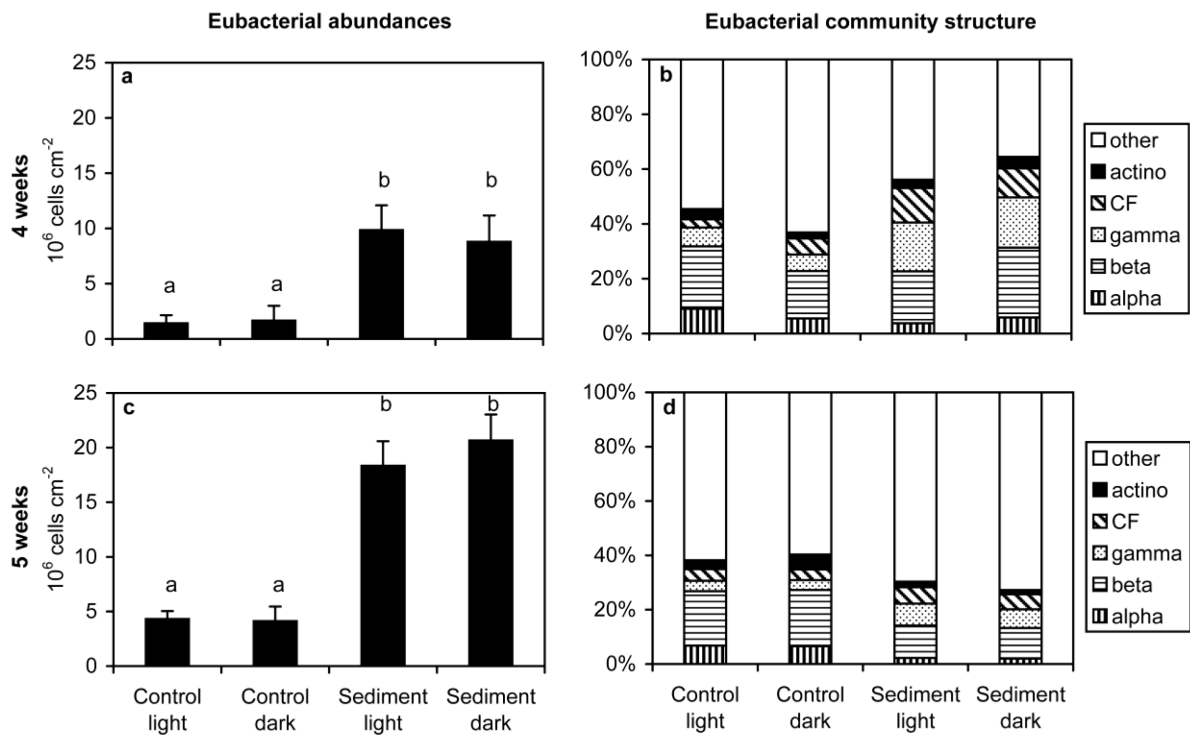


Figure 4

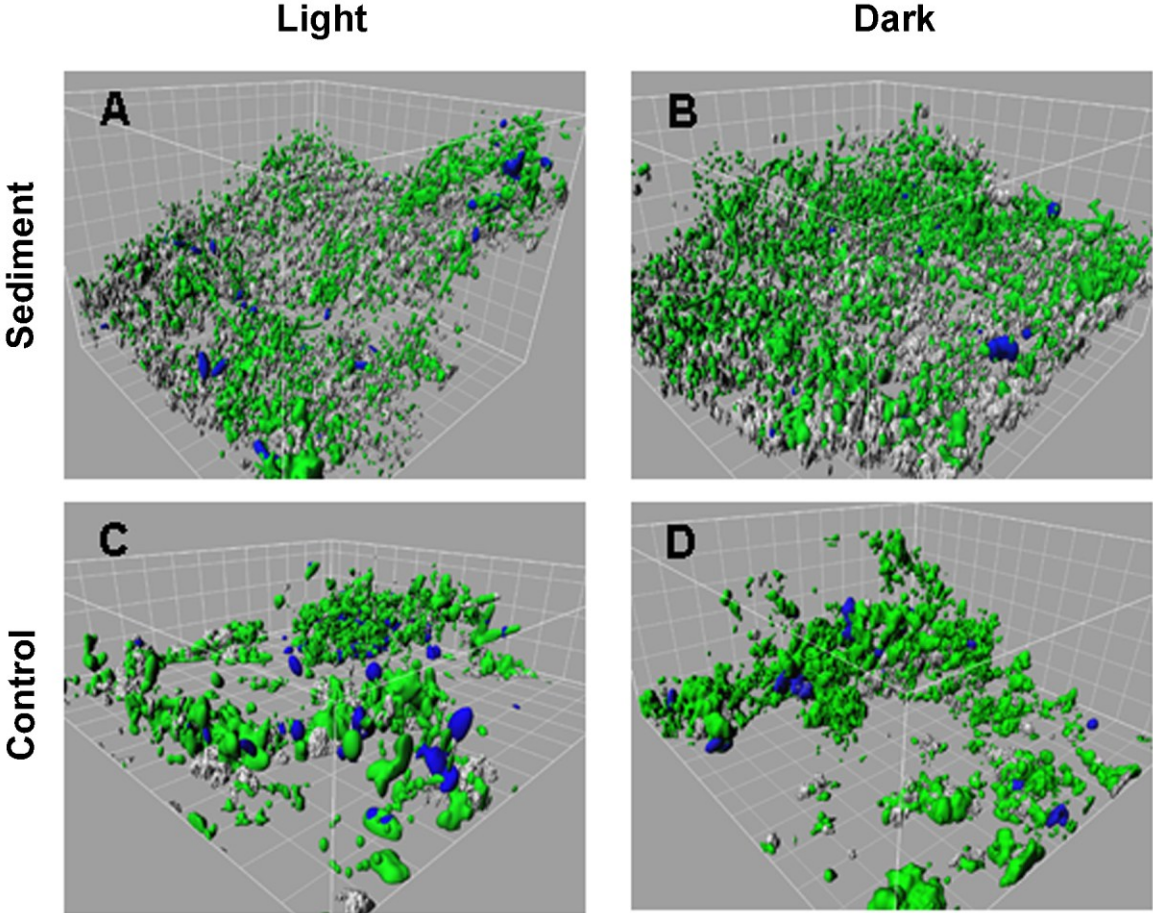
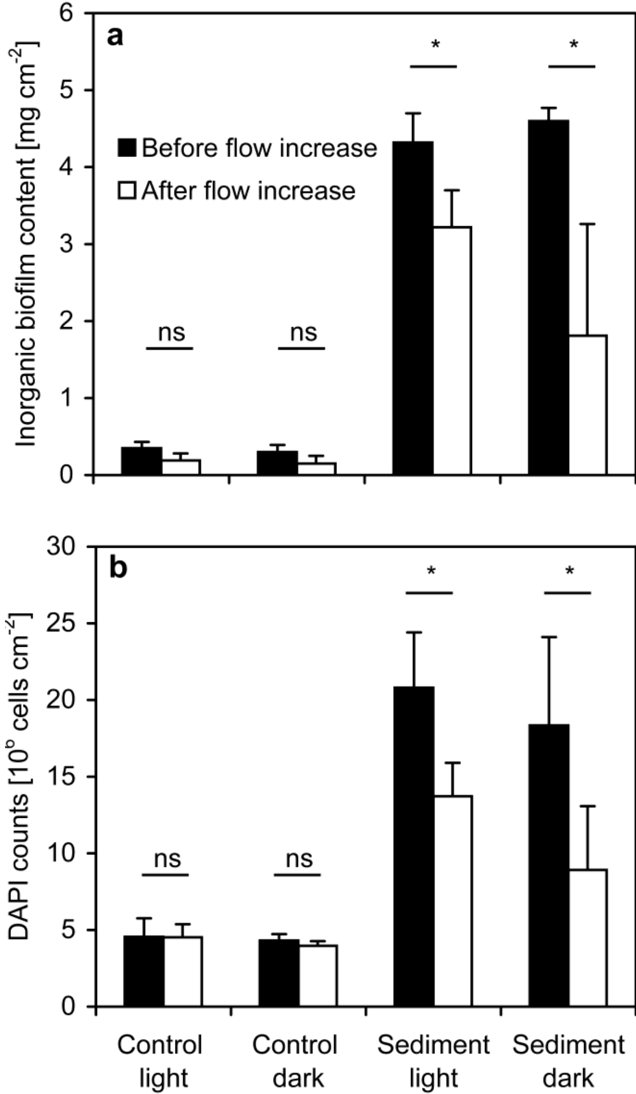


Figure 5



Appendix A

Table A1: Water chemistry parameters. Water samples were taken from the stream at the beginning, two weeks after start, and end of glass slides exposure. In flow channels, water samples were taken at the start, two and four weeks after start, and at the end of the experiment. In the main experiment, light grown biofilms with and without added sediment had been separated after 4 weeks to either continued growth under light or darkness, and 10 mg C L^{-1} sucrose added after 4 weeks of biofilm growth. Five week data represents remaining sucrose at the end of the experiment.

	Ammonium [µmol L^{-1}]	Nitrate [µmol L^{-1}]	Phosphate [µmol L^{-1}]	Total DOC [mg C L^{-1}]	Added sucrose [mg C L^{-1}]	DOC - sucrose [mg C L^{-1}]
Pre-experiment						
Field site	< 2.8	444.1 □ 111.2	2.8 □ 1.2	2.4 ± 0.3	-[#]	-[#]
Flow channel	< 2.8	402.3 □ 54.0	0.9 □ 0.1	2.5 □ 0.2	-[#]	-[#]
Main experiment						
Start	< 2.8	308.8	3.6	n.d. [§]	0	n.d.
2 weeks	< 2.8	352.5	3.7	n.d.	0	n.d.
4 weeks	8.6	497.4	3.7	12.2	10.0	2.2
5 weeks – control light	4.0 □ 4.1	519.1 □ 80.8	1.2 □ 0.2	12.9 □ 6.8	5.1 □ 2.8	7.8 □ 5.5
5 weeks – control dark	3.8 □ 1.8	553.3 □ 92.7	1.5 □ 0.2	7.7 □ 2.6	4.7 □ 2.5	3.0 □ 1.7
5 weeks – sediment light	5.1 □ 1.3	497.1 □ 55.6	1.4 □ 0.1	6.1 □ 1.3	3.1 □ 2.8	3.0 □ 1.5
5 weeks – sediment dark	3.1 □ 2.8	463.9 □ 66.4	1.2 □ 0.3	5.1 □ 0.1	1.4 □ 1.3	3.7 □ 1.3

[#] No sucrose added in this experiment.

[§] not determined.

Enhanced Abundance of Biofilm Bacteria at Small Weirs in the Stream Ilm (Thuringia, Germany)

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Biofilm, bacteria, small weirs, stream

Abstract

In urban areas dam constructions regulate the discharge of streams by reducing the peak flows. The effects of dams on the stream morphology and the organisms are dependent on dam size. In the current study the impact of small weirs on the abundance of bacteria and invertebrates as well as the nutrient conditions in a stream has been investigated. In streams biofilm bacteria degenerate particulate organic matter and transform it into biomass which provides an important resource for higher organisms. These processes are dependent on the flow which is a major determinant in streams. Glass slides were exposed at three weirs in close proximity and a natural sampling site. Biofilm bacteria abundance, abiotic parameters, and nutrients were estimated after 14 days in June, August, October, December 2003 and March 2004. A single weir was chosen for estimations of the flow pattern and the invertebrate community structure. With the exception of March 2004 the flow velocity at the natural site was significantly higher than at the pools of the weirs. At the pools of the weirs the flow velocity was lower than at the corresponding oulets. At the natural site the range of bacterial abundance was between 0.6 and 4.0 $*10^6$ cells cm^{-2}, at the pools between 0.3 and 74 $*10^6$ cells cm^{-2} and at the outlets between 0.3 and 81 $*10^6$ cells cm^{-2}. The abiotic parameters at the different sampling sites were not different with the exception of the conductivity which was lower at the natural site. The ammonium and nitrate concentrations increased over time at all sampling sites. The flow patterns clearly show a unification of the flow at the pool. The outlet was denoted by a large pool behind the weir and a lage riffle adjacent. At the natural site small poos and riffles alternated. These results indicate that small weirs alter the flow pattern of a stream and the abundance of bacteria.

Introduction

More than 50% of large rivers in the northern hemisphere are affected by dams. Impacts of dams on the ecosystem up-and downstream is often originated by inundation, flow manipulation, and fragmentation (Nilsson et al., 2005). Dams vary in size, structure and function. Many of the effects of dams on the biophysical regime are related to the dam size operational mode. Dam size (height, width) strongly influences many environmental factors such as thermal stratification and thermal regime modification which influence biotic variables, barrier effects to biota migration, and sediment transport (Poff and Hart, 2002). A three-level hierarchy catches the effects of dams on the river. First order effects include water quality, sediment load, and flow regime and cause second order effects as the changes in channel cross-section, bed-sediment movement, and primary production which lead to the third order effects in changes of the macroinvertebrate community and other biota (Arle, 2005; Nichols et al., 2006).

The serial discontinuity concept describes changes of physical parameters, biological phenomena at the population, community, and ecosystem level caused by regulation over the entire longitudinal stream profile (Ward and Stanford, 1983). For example, coarse particulate organic matter is trapped in the reservoir of large dams which leads to a local increase of respiratory activity of heterotrophic organisms. The higher demand for oxygen under low flow velocity conditions initiates anoxia in deeper water layers causing a shift in the entire biological community. In contrast, the increase of the light intensity at the stream bottom behind a dam due to a clarification of the turbid streams might stimulate photosynthetic productivity (Ward and Stanford, 1983).

Small weirs influence local velocity patterns and sediment composition (Stanley et al., 2002; Magilligan et al., 2003), particulate organic matter budget (Wagner et al., 2003), and fish and macroinvertebrate communities (Söderström, 1987; Benstead et al., 1999; Grubbs and Taylor, 2004; Santucci et al., 2005). Flow regulation by dams and weirs also stabilize seasonal water levels which might promote growth of filamentous algae at the expense of bacteria in biofilms (Sheldon and Walker, 1997). All factors affect the turnover efficiency of organic and inorganic matter in biofilms, which are autotrophic and heterotrophic organisms attached to surfaces (Beveridge et al., 1997). Biofilm structure and function is tightly related to the flow velocity. Under a laminar flow cell transport to solid surfaces and mass transfer of nutrients are the limiting steps during colonization. Thus, the extent of biofilm growth increases with increasing flow velocity. However, when the flow velocity increases to conditions of turbulence, biofilm erosion or sloughing of the adhered biomass occurs (Costerton et al.,

1995). The abundance of microorganisms (number of cells cm^{-2}) is lower under conditions of turbulence than under laminar flow (Franca and Cravo, 2000) and fluctuating water levels and turbidity appear to maintain biofilm communities in a state of early succession (Sheldon and Walker, 1997).
How small weirs affect the stream biota or whether additional effects of dams in a close proximity exist are the qustions of this study. Biofilm and stream water bacteria as well as physical and chemical parameters at three weirs in close proximity have been investigated. The main focus was on the effects of impoundment and the resulting alteration in flow velocity on the abundance of biofilm bacteria. Additionally the flow patterns, at one of the three weirs have been investigated. The results have been compared with findings from natural sites of the stream.

Methods

Sampling sites

Biofilm samples were taken in the 3rd order Stream Ilm (Thuringia, Germany). At Griesheim (stream km 103) three low head weirs are situated in close proximity (< 1 km). The three natural sites were chosen at Manebach (126 km, Natural 1), 1 km upstream of the first weir (104 km, Natural 2), and three km down stream of the first weir (100 km, Natural 3) at Griesheim (Fig 1).

Sampling

Biofilm samples for bacteria enumeration were taken after 14 days of exposure on June 20th, August 13th, October 22nd and December 2nd 2003, and March 12th 2004.

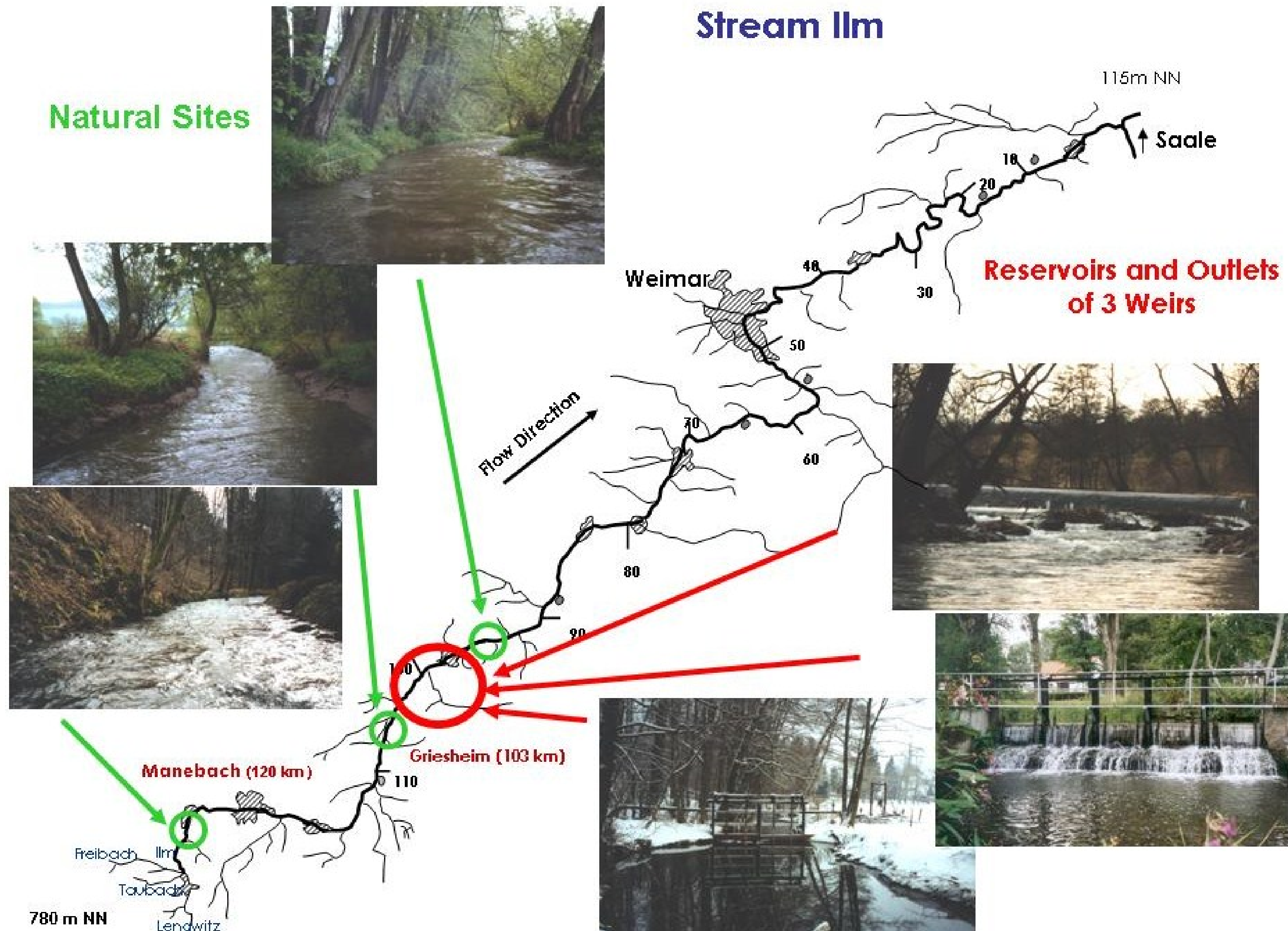

Fig. 1. Course of the Stream Ilm. Green arrows mark the natural sampling sites and red the weirs.

The first weir (Pool 1 and Outlet 1) is situated in the main stream of the Ilm. The construct includes two steps of concrete with overflowing water flow (Fig 2).

Fig. 2. The first weir is situated at the main stream of the Stream Ilm. The water flow passes the barrier atop the wall.

The sampling site of the second weir (Pool 2 and Outlet 2) is located at the upstream site of a tributary short behind the separation from the main stream. The flow passes underneath the two wooden gates which can be opened (Fig 3).

Fig. 3. The second weir is situated at a tributary of the stream. The water flow passes underneath the wooden gates

The third weir (Pool 3 and Outlet 3) is situated down stream the brunch approximately 100 m distant from the second weir. It consits of 5 wooden gates and the water flow passes underneath (Fig 4).

Fig. 4. The third weir is situated at the end of the tributary. The the water flows passes underneath the gates.

Measurement of environmental factors

Triplicate samples from the water column were filtered (Whatman, 0.45 µm pore size) for determination of ammonia (NH^{4+}), and nitrate (NO^{3-}). Concentrations of NO^{3-} were determined using the ultraviolet spectrophotometric screening method (Clesceri, 1998) (Uvicon 931, Kontron Instruments, Italy). NH^{4+} was determined spectrophotometrically (Gadkari, 1984)(Uvicon 931, Kontron Instruments, Italy). Oxygen content, pH, salinity, conductivity and turbidity were measured in the water column (Horiba U 10, Multiparameter, Water Quality Meter, Kyoto Japan). Flow velocity was measured (Flowmate; MARSH McBirney) at all tubes after removing the baskets in 5 cm steps beginning from the river bottom up to the top of the water column. For the contour plots flow velocity was measured in 5 cm steps from the river bed on up to the water surface and horizontally in 500 cm steps in flow direction and transversal.

Sample collection and enumeration of bacteria (DAPI)

Samples were collected after exposition of glass slides in a sampling cylinder of punched (diameter of the holes 5 mm) stainless steal basket (diameter 8 cm, length 16 cm) for 6 glass slides fixed horizontally on tubes above the river ground (Pohlon et al. submitted). Five to six replicates were taken at each sampling site (Fig 1, Chapter 1). Biofilms were

scraped of the slides using sterile glass slides and kept in a 50 ml Greiner tube adding formaldehyde with a final concentration of 4%. At each sampling time and site, three replicates of stream water were taken and fixed with formaldehyde (4% final concentration). Presonicated biofilm samples and water samples (3-12 replicates) were stained with DAPI (1µg ml^{-1}) (Porter and Feig, 1980) before counting at 1000 x magnification (Zeiss Axioplan).

Statistical analyses

Bacteria abundances of the different sampling sites, flow velocities as well as the physico-chemical data were analyzed using one way ANOVA (SigmaStat). Abundance of biofilm bacteria at all sampling sites were correlated with the abundances of the corresponding stream water bacteria as well as the corresponding flow velocities (SigmaStat).

Results

Physical and chemical parameters

There were no effects of the weirs on the water quality data. Oxygen, pH, turbidity, and temperature were not different. Conductivity at Natural 1 was significantly lower than at the other sampling sites. This sampling site is situated close to the spring with different geological conditions than all the other sampling sites (Table 1).

Table 1. Mean (±STDAV) of physical parameters at the sampling sites (Cond=conductivity, Turb= Conductivity, Temp=temperature), asterisks indicate significant differences (ANOVA, P<0.05).

Site	pH	Cond µs cm^{-1}	Turb	O_2 mg l^{-1}	Temp °C
Natural 1	7.2±0.3	123±19 *	0.2±0.7	9.6±1.5	9.4±3.9
Natural 2	8.2±1.2	306±53	12±21	9.3±1.4	8.7±6.8
Natural 3	7.8±0.2	316±45	15±22	9.3±1.5	8.4±6.3
Pool 1	7.3±0.5	292±58	1.0±0.8	10.3±1.1	7.5±5.0
Pool 2	7.6±0.4	278±35	47±100	9.2±1.9	9.5±3.5
Pool 3	7.7±0.7	300±61	2.2±2.2	9.7±1.3	8.1±5.2
Outlet 1	7.2±0.4	289±56	1.0±2.0	10.8±1.0	7.7±5.4
Outlet 2	7.7±0.5	276±42	21±62	9.3±1.8	9.7±3.6
Outlet 3	7.1±0.7	294±61	1.0±1.2	10.4±1.2	8.2±5.2

Concentration of nitrogen and ammonium increased over time at all sampling sites with no significant differences between natural sites, pool, and outlets of the weirs (Table 2).

Table 2. Mean (±STDAV) of nitrate – and ammonium concentrations at all sampling sites and dates.

Site	Date	NO_3 µmol l^{-1}	NH_4 mmol l^{-1}
Natural 1	June / 20 / 03	17.2±0.2	0.1±0.03
	August / 13 / 03	30.0±4.1	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	24.7±7.9	0.6±0.6
	March / 3 / 04	75.9±82.7	2.7±3.7
Natural 2	June / 20 / 03	27.4±2.2	0.6±0.08
	August / 13 / 03	29.1±4.7	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	67.9±24.5	3.9±4.4
	March / 3 / 04	105.0	<0.1
Natural 3	June / 20 / 03	19.7±6.5	0.4±0.02
	August / 13 / 03	39.2±3.3	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	73.4±17.0	0.7±0.2
	March / 3 / 04	102±5.6	9.1±9.8
Reservoir 1	June / 20 / 03	34.1±2.0	0.5±0.04
	August / 13 / 03	27.4±1.8	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	63.6±11.4	0.3±0.2
	March / 3 / 04	99.1±6.8	7.2±8.6
Reservoir 2	June / 20 / 03	n.d.	n.d.
	August / 13 / 03	34.6±9.6	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	75.5±9.7	n.d.
	March / 3 / 04	105±2.1	6.7±8.2
Reservoir 3	June / 20 / 03	19.7±6.5	0.4±0.02
	August / 13 / 03	36.0±5.7	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	75,0±6.8	0.5±0.4
	March / 3 / 04	102±5.6	9.6±8.8
Outlet 1	June / 20 / 03	30.8±4.0	0.5±0.04
	August / 13 / 03	25.2±6.0	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	24.7±7.9	2.6±3.0
	March / 3 / 04	75.9±82.7	7.5±9.8
Outlet 2	June / 20 / 03	n.d.	n.d.
	August / 13 / 03	40.8±7.1	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	67.9±24.5	2.0±0.3
	March / 3 / 04	105.0	9.7±7.5
Outlet 3	June / 20 / 03	n.d.	n.d.
	August / 13 / 03	29.2±8.3	<0.1
	October/ 22 / 03	n.d.	n.d.
	December / 12 /	73.4±17.0	0.8±0.5
	March / 3 / 04	102±5.6	12.7±14.7

Discharge and flow pattern

The discharge of the Stream Ilm during the sampling time was below mean annual values (2.45 m^3 per s). At 2003 the number of days below mean discharge was with 42 days very high (mean value of annual days below mean discharge is 19). The discharge on June 20th was 0.51 m^3 s^{-1}, on August 13th 0.161 m^3 s^{-1}, on October 22nd 0.6 m^3 s^{-1}, on December 2nd 2003 0.85 m^3 s^{-1}, and on March 12th 2004 0.85 m^3 s^{-1}. Between January 12th and February 20th 2004 the discharge was high with 2 flow peaks of 5 and 11 m^3 s^{-1}.

Flow velocity

The mean flow velocity at the pools was lower (10 fold) than at the natural sites and the outlets. The velocities at the natural sites and the outlets varied between 20 and 40 cm per second and were not different in magnitude and variability. The flow velocity at Pool 2 was higher than at the other pools from October 2003 on. In August 2003 the flow velocity at Outlet 1 and 2 was reduced due to the extreme low water level in summer 2003 (Table 3).

Table 3. Mean flow velocity (±STADV) at the sampling sites and dates. Asterisks indicate significant differences between sampling sites (P>0.05, ANOVA).

	Jun 20, 2003	Aug 13, 2003	Oct 22, 2003	Dec 2, 2003	Mar 12, 2004
Natural 1	43 (±10) *	13 (±8.1) *	25 (±13) *	39 (±17) *	22 (±19)
Reservoir 1	2.2 (±3.5)	0.7 (±2.5)	3.5 (±2.4)	5.2 (±4.8)	7.2 (±3.5)
Reservoir 2	13 (±7.1)	2.2 (±4.7)	9.7 (±7.3)	12 (±7.0)	19 (±12)
Reservoir 3	5.5 (±3.4)	3.4 (±2.2)	5.2 (±2.0)	6.5 (±2.4)	8.3 (±5.8)
Outlet 1	18 (±10)	2.6 (±1.3)	26 (±13) *	29 (±6.7) *	26 (±9.7)
Outlet 2	37 (±19) *	38 (±17) *	26 (±13) *	30 (±1.8) *	25 (±25)
Outlet 3	42 (±13) *	19 (±21) *	37 (±16) *	26 (±6.3) *	55 (±18) *

At the Natural 1 small patches of slow and fast flow velocities alternated (Fig 5). The mean (±STADV) flow velocity was 51 (±3) cm s^{-1}. Pool 2 was characterized by an uniformity of slow flow velocities with a mean value of 17 (±9) cm s^{-1} (Fig 6). At Outlet 2 a large pool behind the barrier occurred followed by a single large riffle. The mean flow velocity at this site was 42 (±22) cm s^{-1} (Fig 7).

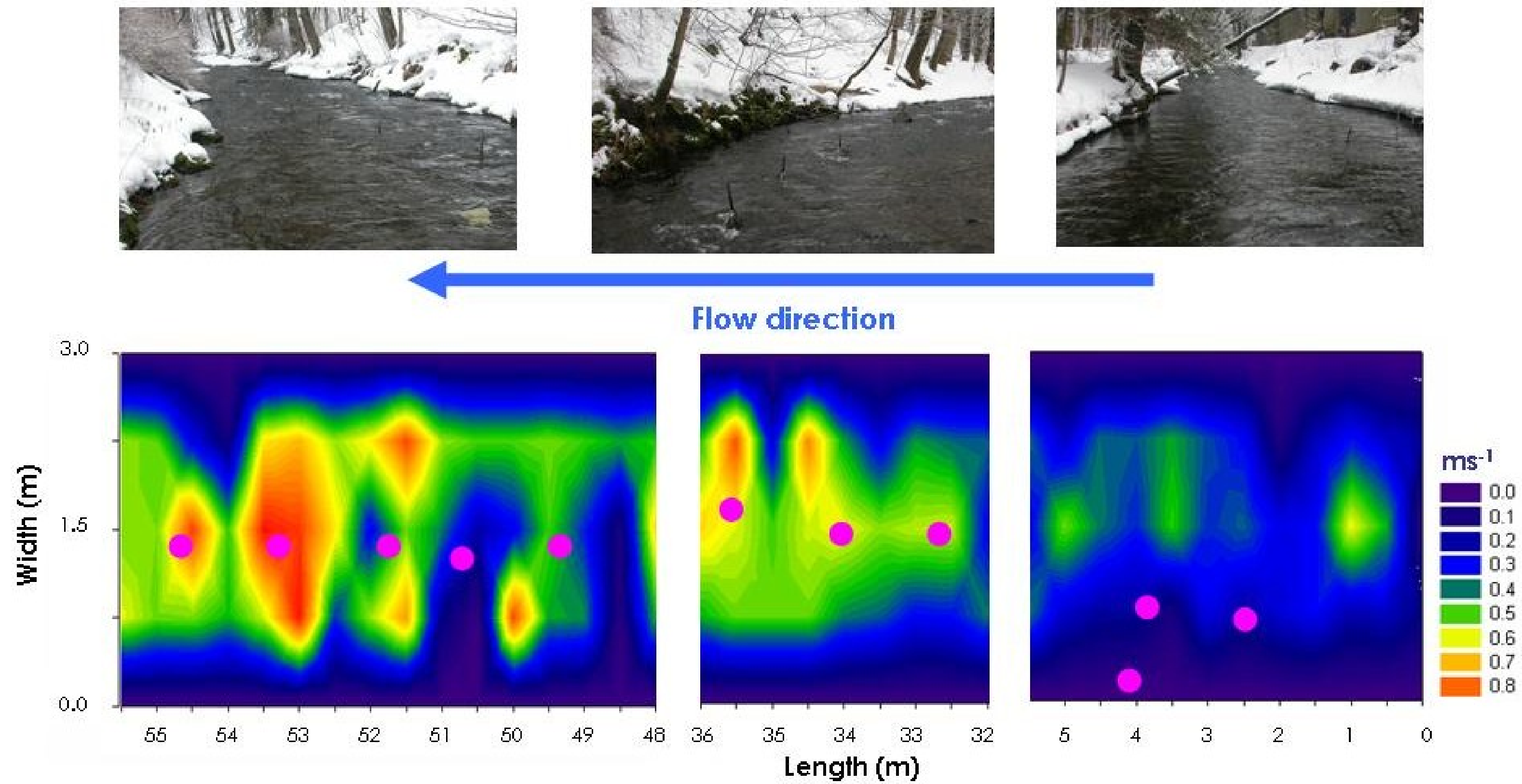

Fig. 5. Contour plot of the mean flow velocities (ms^{-1}) measured from the river bed surface up to the head water in 5 cm steps vertically and in 0.5 m steps in flow direction as well as transversal at the natural site in Manebach (Natural 1). Pink dots mark the positions of the sampling baskets.

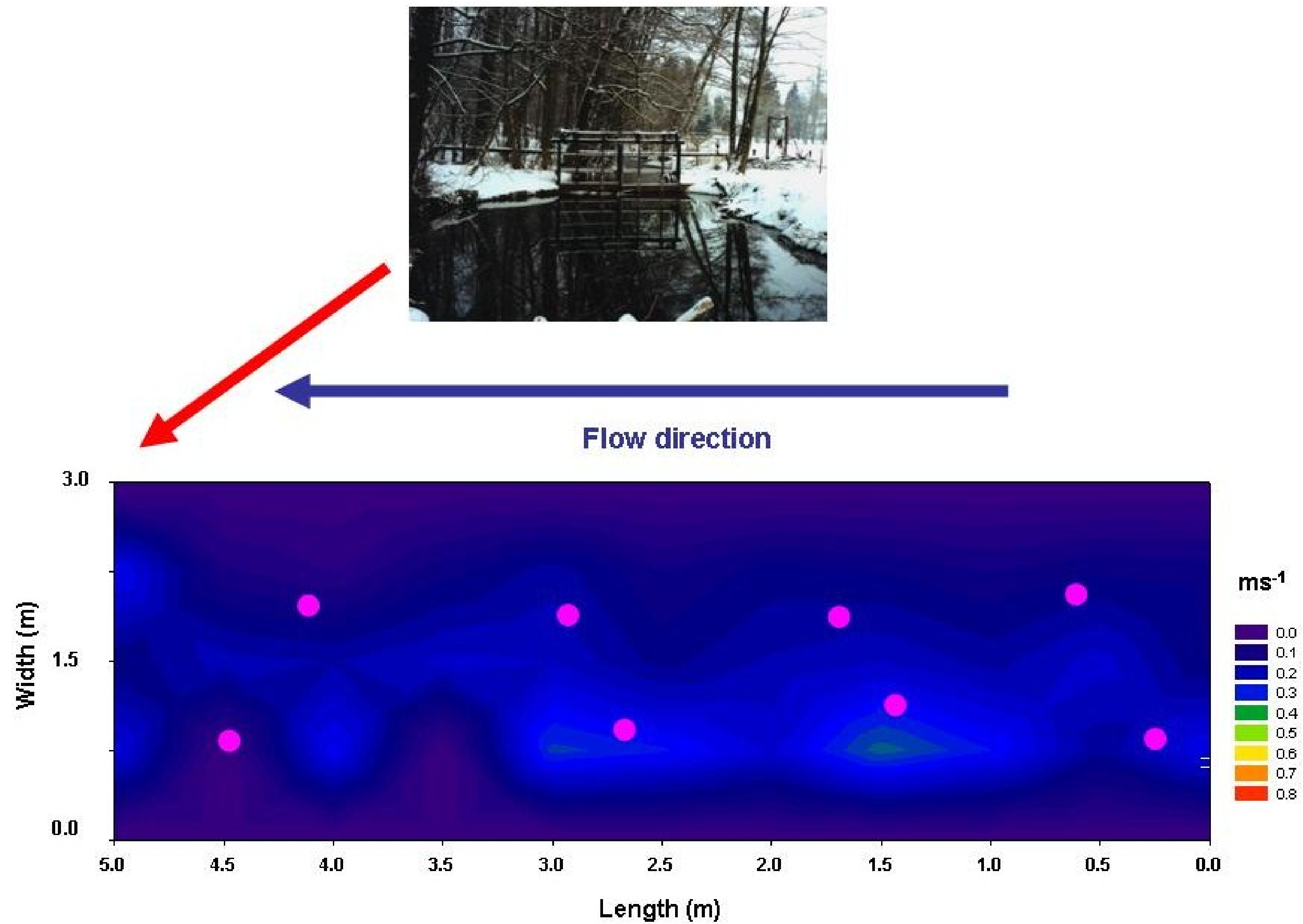

Fig. 6. Contour plot of the mean flow velocities (ms-1) measured from the river bed surface up to the head water in 5 cm steps vertically and in 0.5 m steps in flow direction as well as transversal at the weir 2 in Griesheim (Pool 2). Pink dots mark the positions of the sampling baskets.

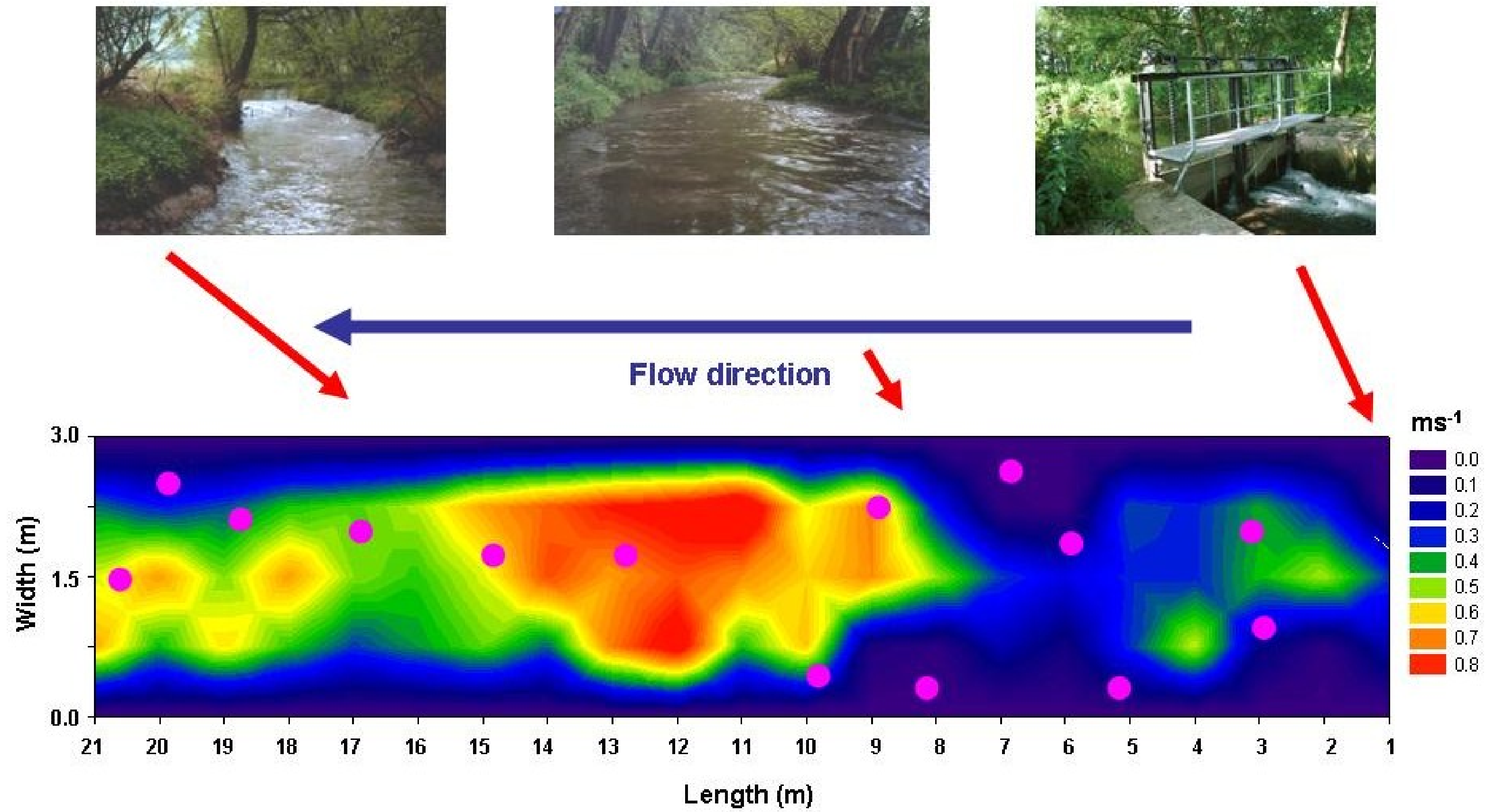

Fig. 7. Contour plot of the mean flow velocities (ms-1) measured from the river bed surface up to the head water in 5 cm steps vertically and in 0.5 m steps in flow direction as well as transversal at the weir 2 in Griesheim (Outlet 2). Pink dots mark the positions of the sampling baskets.

Abundance of Bacteria

The mean bacterial abundance at the natural site over the entire time period varied between 0.6 and 4.0 $*10^6$ cells cm^{-2} at the pools between 0.3 and 74 $*10^6$ cells cm^{-2} and at the outlets between 0.3 and 81 $*10^6$ cells cm^{-2}.

At Natural 1 on June 20th, October 22nd, and March 12th the abundance was in the same order of magnitude. On August 13th the abundance was 6-fold and on December 2nd two-fold higher (Fig 8A). Only on March 12th 2004 the mean (±STADV) abundance of biofilm bacteria at Natural 2 and 3 was measured with 0.46 (±0.2) and 0.43 (±0.2) bacteria * 10^6 per cm^3 respectively.

At Pool 2 and 3 on June 20th the abundances of biofilm bacteria was 6- and 7-fold ($P<0.05$ ANOVA) higher than at Pool 1 respectively. At Pool 3 on August 13th the abundance was significantly ($P<0.05$ ANOVA) (15- and 9-fold, respectively) lower than at Pool 1 and 2. On December 2nd the abundance at Pool 3 was significantly ($P<0.05$ ANOVA) reduced (5- and 3-fold) again (Fig 8B).

At Outlet 1 on August 13th the abundance was significantly (27-and 62-fold, respectively) higher than at Outlet 2 and 3 and on December 2nd 8-and 4-fold lower than Outlet 2 and 3, respectively. At March 12th the abundance at all three outlets was lowest (Fig 8C).

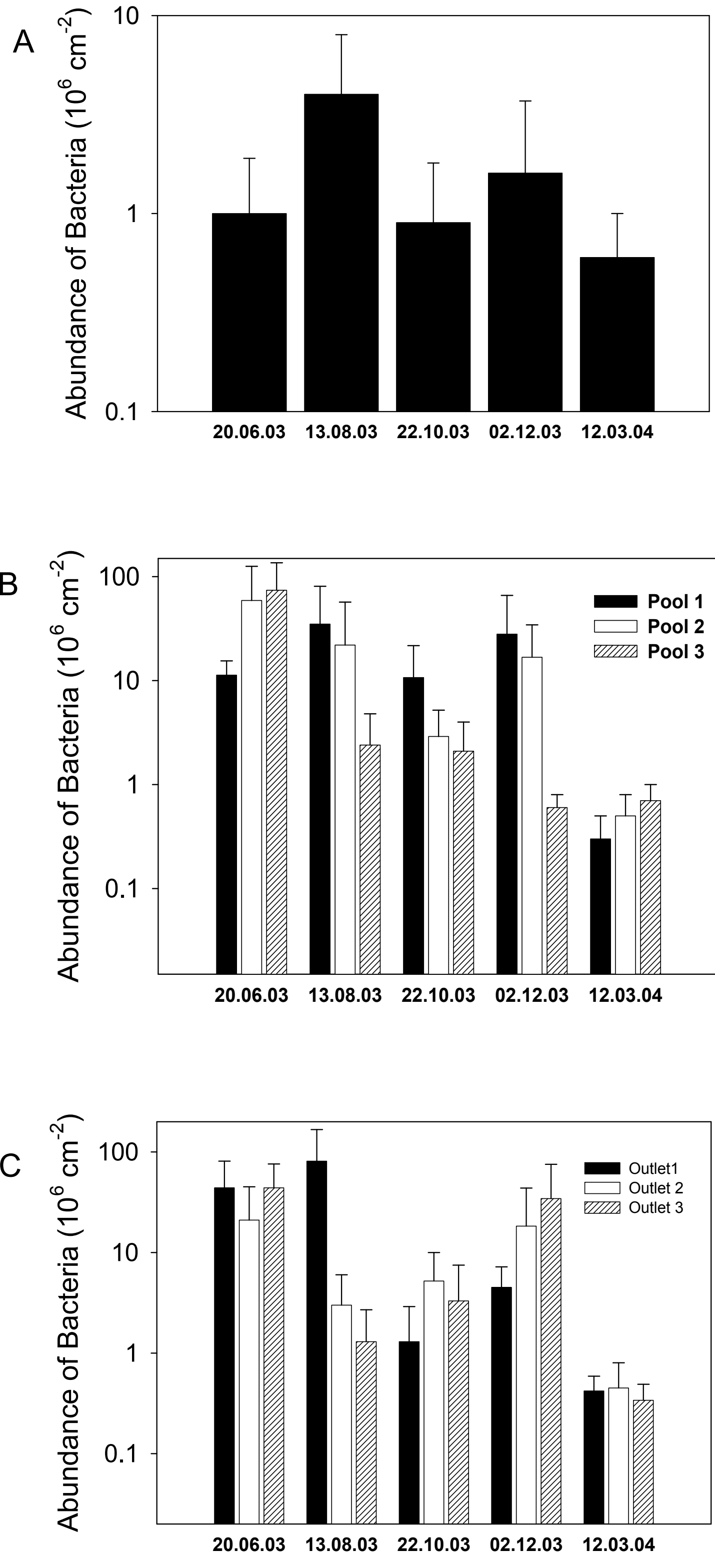

Fig. 8. Mean (±STDAV) bacterial abundance in biofilms at the natural site 1 (Manebach) (A), the pools of three weirs (B), and the corresponding outlets (C).

The magnitude of the bacterial number in the water column at all sampling sites was equally with two exceptions: March 12th 2004, the abundance at Natural 2 was significantly higher than at Natural 1 and 2, and August 13th, possibly regarding to the low water level at this time the abundance of bacteria in the stream water was high (Table 4).

Table 4. Mean (±STDAV) of stream water abundance at all sampling sites and dates.

Sampling Date	**20.06.2003**	**13.08.2003**	**22.10.2003**	**02.12.2003**	**12.03.2004**
Natural 1	0.83 (±0.25)	4.54 (±1.62)	0.22 (±0.31)	1.08 (±0.68)	0.35 (±0.15)
Natural 2	2.03 (±0.54)	1.94(±0.64)	0.63 (±0.28)	0.46 (±0.45)	2.24 (±1.41)
Natural 3	4.03 (±3.18)	3.02 (±.1.60)	4.68 (±4.53)	1.15 (±1.15)	1.29 (±0.97)
Reservoir 1	7.03 (±0.82)	14.04 (±3.91)	2.68 (±4.53)	0.49 (±0.39)	1.02 (±0.00)
Reservoir 2	2.29 (±0.02)	15.97 (±3.90)	2.01 (±2.44)	1.52 (±1.13)	1.82 (±0.42)
Reservoir 3	4.79 (±2.52)	14.05 (±2.21)	1.33 (±0.56)	1.38 (±0.51)	1.62 (±0.10)
Outlet 1	2.83 (±0.00)	16.84 (±8.25)	10.45 (±13.3)	1.07 (±,0.17)	1.42 (±0.18)
Outlet 2	3.06 (±0.76)	13.92 (±0.92)	0.49 (±0.37)	1.14 (±0.47)	1.12 (±0.25)
Outlet 3	3.17 (±1.66)	13.13 (±1.38)	1.34 (±0.39)	0.55 (±0.52)	0.50 (±0.32)

Discussion

Flow Velocity

The flow patterns (Fig 5-7) clearly show a reduction of the flow velocity and an unification at the pool of the weir whereas at the outlet no mosaic like pattern of the flow exists as shown at the natural site. In contrast to the natural site with a higher number of small riffles and pools the outlet was dominated by one large pool short behind the weir followed by one large riffle. The slow flow velocity and the appearance of Megaloptera as a typical colonizer of muddy sediments only at the weir sites support the assumption that this sites function as sedimentation traps. The presence of more than 55 weirs along 130 km in the Stream Ilm builds a longitudinal pattern of large pools in front of the barriers followed by large riffle like structures at the outlets. Due to the decrease of the flow velocity, the pools might exhibit zones of sedimentation.

No consistent flow patterns at the three weirs could be observed during the sampling season. Flow velocities at the pool of the second weir (Pool 2) differed at three sampling times from the other pools. The mode how the flow passes the barrier has no effect on the flow velocities up- and down stream of the barriers. The stream water passes the first weir atop the wall (overflow) and the weirs 2 and 3 underneath. The fact that there were no differences of the chemical and physical values indicate that low-head dams do not alter chemical attributes of the stream water, which has been shown in other studies for temperature and dissolved oxygen (Stanley et al., 2002).

Abundance of bacteria

The bacterial abundance was independent from the flow velocity at all sampling sites. The tenfold higher abundance of biofilm bacteria at all weir sites compared to the natural sites might be caused by the sediment trapping at the pools which also provides nutrients (Stanley and Doyle, 2002) for the biofilm organisms. This finding can be supported by the abundance of bacteria in the stream water which were not different at natural- and weir sites. In August 2003 the low number of bacteria was possibly by caused by the low water level during the extreme dry summer.

Restoration purposes

In straightened rivers and streams which conquer high altitute difference as the Ilm small weirs can play a key role as zones with a reduced flow. Straightening increases flow velocities and results in shorter turnover lengths, but also results in a reduced stream length. However, the increased turnover capacity in straightened zones has to be balanced with the loss of streambed area, i.e. turnover area. Thus, reservoirs in front of small weirs seem to be important zones of transient storage for organic matter processing. Especially under flooding conditions, artificial pool sites like impoundment areas might be important “refuge zones” for microbial biofilm activities.

Flow is a major determinant of biotic composition in rivers. During dam construction invertebrate abundance and diversity can decrease as a reflection of the changes in flow, substrate, temperature, and a reduction in substrate heterogeneity together with an accumulation of sediment (Boon, 1988). Dam construction can also result in shifts of microbial community structures as biofilms which in turn can alter the turn over in the river section concerned. On the other hand artificial weirs provide a successful tool to re-establish the riffle-pool character in regulated river (Gordon et al., 1996). Probably an enhancement of the water passage to reduce the first order effects of the dam can be a first step to re-establish “natural-like” conditions for the community.

For restoration purposes each weir needs to be evaluated individually. Long term investigations are necessary to make sure how the environment has been changed after dam constructions and how the community can be supported. The lack of streams without human impacts as constructions of different feature makes this evaluation more difficult. Definitely it is clear that it is not advisable to remove all artificial structures without a detailed examination of all community levels.

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Querying the Obvious: Lessons from a Degraded Stream

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Abstract

A detailed assessment of degradation issues is essential for the development of reasonable restoration strategies. The assessment may be a difficult task when fluxes of organic matter and energy are concerned, which are primarily mediated by microorganisms. In small streams, biofilms are hot spots of trophic interactions. Small weirs cause small-scale changes of flow velocity, which affects the formation, structure, and function of biofilms. Weirs are superficially considered as disturbing cross barriers that should immediately be removed for the restoration of riparian systems. However, our empirical studies of weirs in the stream Ilm, Germany, and conceptual modeling approaches revealed a rather beneficial effect because weirs compensate the loss of natural retention structures in straightened rivers. Longer processing time of particulate organic matter in the weir reservoirs may have a positive effect on biofilm productivity and nutrient cycling in aquatic ecosystems. This is a striking example of thorough investigations that resulted in a complete and surprising reassessment of a degradation situation, and for a case in which uninformed gut feeling decisions about management plans would have had detrimental effects.

Key words: **conceptual model, disturbance, microbial activity, biofilm, stream ecosystems, weirs.**

Introduction

The first step before any restoration management plan could be developed is indeed to determine the state of degradation. Apparently, this statement is trivial because degraded systems are supposed to be easy to identify. But in fact, revealing the very reasons for degradation is a rather demanding task. Degradation may be due to hidden pathways that are not immediately obvious, but that need to be carefully scrutinized for reasonable decisions about promising restoration strategies. Even more so, reliance on superficial characteristics of a degraded system could be dangerously misleading and may result in not only unsuccessful but also detrimental management actions.

In particular, this caution applies to the fluxes of energy and matter through ecosystems that are mainly mediated by microorganisms. The scientific knowledge about the microbiology in aquatic ecosystems is still rather incomplete, the methods for quantitative investigations are demanding, and even the systematic classification of organisms is challenging. The essential turnover processes in streams take place in the sediment or at the water–sediment interface, that is, in a changing environment that is anything but easily assessable. Here, we present our experiences from studies in the Ilm, a third-order hard water stream in Thuringia, Germany, which resulted in a surprising reassessment of an "obvious" degradation situation.

Degradation of Running Water Ecosystems

All major rivers worldwide are largely influenced by human impacts (Giller 2005), which change the lotic character, affect the ecosystem structure and function, alter the habitat heterogeneity, and fragment riparian zones (Jansson et al. 2000; Giller 2005). More than 50% of the rivers in the northern hemisphere are affected by dams (Nilsson et al. 2005), which modify flow regimes, interrupt sediment transport, deteriorate water quality, and break biological continuity (Ward & Stanford 1979; Petts 1984). Large dams alter the river continuum (Vannote et al. 1980) by disturbing the spiraling of resources (nutrients and organic matter) and disconnecting upstream and downstream reaches.

The vast majority of dam structures in Central European stream systems, however, are weirs with a hydraulic head not greater than 5 m (Poff & Hart 2002), which in contrast to large dams have only small or moderate effects (Hart et al. 2002). Weirs do not substantially alter the natural discharge regime but rather affect the local flow velocity patterns, sediment composition (Magilligan & Nislow 2001; Stanley et al. 2002), and particulate organic matter (POM) budgets (Wagner 2003). Barrier effects of weirs on movement and population dynamics of migratory fish species are evident (Mills 1989; Lewis 1991; Morita & Yamamoto 2002), but the consequences for microbial and

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macroinvertebrate communities are less well documented (Pringle 2003; Arle 2005).

Rivers cause a permanent discontinuous transition between transport and storage of organic matter, largely in the form of dissolved organic matter (DOM) (Wetzel 1992). According to the microbial loop concept (Azam et al. 1983), the transfer of energy and matter to higher trophic levels in aquatic ecosystems is largely mediated by microorganisms, which convert DOM into POM (Kerner et al. 2003). In small streams, the retention of DOM mainly occurs in biofilms (Schwoerbel 1994; Fischer et al. 2002). Biofilms are complex assemblages of bacteria, fungi, algae, micro- and meiofauna within a polysaccharide matrix (Lock 1981; Lock et al. 1984) and are formed at any submerged surfaces such as stones, plants, and roots (Zubkov & Sleigh 1999). Biofilms are hot spots for the turnover of organic matter in small streams because the majority of bacteria lives attached to the streambed (Geesey et al. 1978). Biofilm bacteria display higher sugar assimilation rates (Fletcher 1986) and higher enzyme activities (Romaní & Sabater 1999*b*) compared to planktonic bacteria. Biofilms provide an important food resource for higher grazing organisms like aquatic snails (Sheldon & Walker 1997; Lawrence et al. 2002).

Effects of Weirs on Aquatic Communities

Weir reservoirs create distinct physical conditions, which differ considerably from free-flowing natural reaches (Baekken et al. 1981; Stanley et al. 2002), but chemical and thermal differences often occur only locally (Santucci et al. 2005). Our studies of weirs in the Ilm confirmed that although flow velocity was reduced in the reservoirs (0.10 ± 0.02 m/second) compared to the outlet and natural sites (0.35 ± 0.10 m/second), pH, oxygen content, turbidity, conductivity, and temperature in the water column were not affected. However, because the Ilm is heavily fragmented by more than 50 weirs on an entire length of only 137 km, cumulative effects may occur. So in a series of studies, we tried to disentangle the complex interaction between altered flow velocity, the formation and function of biofilms, and the benthic invertebrate communities near weirs.

As a general pattern, the abundance of microorganisms and the accumulation of biomass in biofilms are negatively correlated with increasing flow velocity (up to 0.30 m/second) (Lau & Liu 1993; Battin et al. 2003*a*, 2003*b*). At even higher flow velocity and turbulence, biofilm erosion or sloughing of the adhered biomass occurs (Characklis 1990; Costerton et al. 1995). On the other hand, uptake of DOM is primarily limited by diffusion through the laminar sublayer or by processes within the biofilm (Gantzer et al. 1989). Thus, the higher DOM content and nutrient availability in stream water during high discharge periods might cause higher extracellular enzyme activities of biofilms (Romaní & Sabater 1999*a*). Consequently, bacterial productivity may increase during high discharge periods. Bacterial turnover depends on both external and internal carbon supply because algae colonizing biofilms release extracellular organic carbon that can be rapidly used by bacteria (Sundh & Bell 1992).

The next higher trophic level in biofilms are protists, such as heterotrophic flagellates and ciliates, which feed on bacteria and algae (Azam et al. 1983). Increasing flow velocity results in higher contact rates between planktonic protists that pass the biofilm (Hunt & Parry 1998). Peritrich ciliates on surfaces benefit from the enhanced advection of prey at increasing flow velocity (Shimeta et al. 2001), and thus the clearance rate of some benthic bacterivorous ciliates may improve. In general, grazing pressure from ciliates and flagellates on bacteria in rivers is low (0.02−1.67%) (Gücker & Fischer 2003). Higher organisms such as ostracodes predominantly consume algae and extrapolymeric substances, while unspecific feeders like snails and mayflies can efficiently reduce the biofilm thickness (Lawrence et al. 2002).

In our study, the biofilm abundances of bacteria, heterotrophic flagellates, and ciliates in weir reservoirs were similar to those in the respective outlets but slightly higher than at natural sites. Extracellular enzyme activities were highest at outlet sites, but biofilm thickness and chlorophyll *a* content were enhanced at reservoirs, indicating a tight mutualistic interaction between the bacterial production and the photosynthetic activity of algae and/or cyanobacteria. Thus, at low flow velocities, the high potential release of extracellular organic carbon by algae and the limited diffusion of nutrients from the water column into the biofilm might restrict the efficiency of allochthonous DOM turnover in the reservoir. However, enhanced enzymatic and microbial activity in the water might compensate for organic matter processing.

Only few invertebrates feeding on biofilms were present in the reservoirs, and so carbon and energy flow from biofilms to invertebrate communities might be small. Biomass of invertebrates was similar in the reservoirs and natural sites but slightly higher in the outlet. In general, the aquatic community appeared to be not significantly affected by the weirs. However, invertebrate species diversity was reduced ahead of the weirs, and detritivorous collector–gatherers dominated (88% of all invertebrates; Arle 2005), as is confirmed by other studies (Stanley et al. 2002; Santucci et al. 2005). Within the reservoirs, the benthos normally undergoes a succession toward lentic life forms, but these changes are locally restricted and appear to have no effect on downstream reaches.

Cross barriers can reduce the longitudinal connectivity by preventing or impeding the migration of organisms throughout the stream system (Pringle 2003) that lead to fragmentation of the habitat and isolated populations (Pechlaner 1986; Winston et al. 1991; Drinkwater & Frank 1994; Marchant & Hehir 2002). Depending on size and operational type, small low-head weirs might also act as barriers to some invertebrate species (Watters 1996;

Cortes et al. 1998; Benstead et al. 1999; Conception & Nelson 1999; Stanley et al. 2002). We observed a slightly modified invertebrate downstream drift within the weir reservoirs of the Ilm, but the barrier effect was unimportant for the maintenance of diverse invertebrate communities upstream and downstream of the weir (Arle 2005). The strong spatial restriction of impacts from each single weir probably explains the absence of any notable cumulative effect of multiple weirs on invertebrate communities even in the heavily fragmented Ilm.

Another effect of weirs is the retention of POM. Many headwater streams like the Ilm are energetically dependent on allochthonous organic material (Fisher & Likens 1973; Cummins 1974). Large amounts of POM are stored in the reservoir and detritivorous collector–gatherers dominate during low discharges. Only major floods can reset the system (Fjellheim et al. 1993). Trapping of POM in reservoirs of large dams leads to a local increase of respiratory activity of heterotrophic organisms (Ward & Stanford 1983). A similar increase might occur in the reservoir of weirs. The reservoirs in the Ilm in fact contained higher POM standing stocks than outlet and natural sites (Arle 2005). Therefore, we hypothesized that in straightened, homogeneously structured streams, that is, with reduced size of riparian corridors and in absence of natural retention zones, multiple weirs may compensate the loss of natural retention structures because POM as the energy base for stream biota will be longer retained in the system.

Modeling Disturbed Streams

The interactions between flow velocity and nutrient cycling among the different trophic levels of aquatic systems are hard to unravel, and so modeling is an appropriate tool to reveal the causal relationships and the expected outcome from different scenarios. DOM spiraling modeling suggests that uptake lengths for DOM increase with decreasing flow velocity (Kaplan & Newbold 2003). Rapid uptake of labile DOM from the stream water will result in a greater concentration difference between water column and biofilm and, thus, will be more strongly influenced by turbulent mixing than the uptake of less labile DOM (Kaplan & Newbold 2003). Classic approaches like the river continuum concept (Vannote et al. 1980) and the nutrient spiraling concept (Elwood et al. 1983) consider riverine ecosystems as continuous ecosystems. However, due to structural alteration, many stream systems worldwide are far away from their natural character.

To describe DOM turnover efficiency in regulated streams, we developed a conceptual model that regards the effect of physical (e.g., temperature, flow velocity) and biotic (e.g., growth rates, trophic interactions) parameters, as well as their synergistic interactions (e.g., growth rates depend on temperature). In the presence of weirs, flow velocity and POM storage are altered. Under normal discharge, the decrease of flow velocity will lead to a decrease of DOM uptake (Kaplan & Newbold 2003) and, thus, the DOM turnover efficiency of the stream system also decreases. The retention of POM in the reservoir, however, may stimulate bacterial transformation, providing an additional DOM source for the biofilm. Under high discharge conditions, sediment particles will be mobilized in unregulated stream reaches, leading to a detachment of benthic biofilms and a temporary reduction of DOM turnover. Because weirs mediate the discharge regimes and lower upstream flow velocities, biofilm detachment might be prevented in the reservoirs, creating spots with continuing DOM turnover. Major floods, however, will lead to a depletion of POM and to a reset of the aquatic community also in the reservoirs.

From implementing different flow regimes into our model, we found support for the hypothesis that decreased DOM turnover efficiency in weir reservoirs might be counteracted by increased residence time of organic matter. In addition, the high abundance of detritivorous collector–gatherers provides a direct trophic link from organic material to invertebrates. The increased residence time and the channeling of organic matter directly to the invertebrate community are important for straightened streams with severely reduced natural retention capability.

Consequences for Restoration Ecology

The complexity of running water ecosystems requires sufficient analyses of the functional and structural parameters before restoration management plans are set up (SER 2004). To evaluate the impact of human alterations on the ecological "health" of rivers and streams, biomonitoring approaches are used (Bunn & Davies 2000). Large amounts of money have been spent on restoration projects (Roni et al. 2002) but their success often remains unclear. Therefore, a standard program for restoration approaches is required (Giller 2005), in which the optimal conditions for the targeted ecosystem have to be evaluated and necessary measurements have to be defined (Palmer et al. 2005). Additionally, conceptual hypotheses and models should be employed to set up the aims of the proposed activities (Jansson et al. 2005).

Weirs were widely used in river regulation to control floods and to provide power, for example, for generating electricity. In river restoration, dam removal is commonly seen as an obvious and relatively inexpensive option when the ecological health of the system is of prime consideration (Santucci et al. 2005). However, artificial weirs provide an efficient tool to reestablish the riffle-pool character in regulated rivers (Gordon et al. 1996). Straightening increases flow velocities, resulting in not only shorter turnover lengths but also in a reduced stream length. Therefore, the increased turnover capacity in straightened zones has to be balanced with the loss of turnover area and the faster downstream transport of nutrients. Multiple weirs might increase the POM retention capacity and

improve nutrient cycling, which are indicated from studies of their natural analogs, that is, debris dams and beaver dams. Reservoirs of small weirs can be key zones of transient storage for organic matter processing, and as artificial pool sites, they might be valuable "refuge zones" especially under flooding conditions.

We are still far away from a complete understanding of the effects of weirs on aquatic communities and organic matter turnover in streams, which is a prerequisite for efficient management of these structures. The recent development of molecular techniques and other sophisticated methods have enabled aquatic ecologists to get a first glimpse into the microbial "black box." However, to define the optimal conditions for the targeted ecosystem, much more research is needed to thoroughly understand the structure–function relationships of microbial biofilms, the hot spot of organic matter turnover in small streams. Our case studies in the stream Ilm indicated that the "obvious degradation issue" of fragmentation by weirs should rather be considered beneficial because it compensates the loss of natural retention structures due to straightening. If a complete removal of weirs is nonetheless considered, it has to be necessarily combined with (1) the restoration of the entire riparian zone and (2) the recreation of a heterogeneous channel structure with a variety of natural retention structures. Uninformed gut feeling decisions for weir removal without considering the retention function of the structures would have had detrimental effects.

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General Discussion

Dynamic of Stream Ecosystems

The main hallmark of stream ecosystems is the down stream flow which requires adaptive efforts from the entire biota (Schönborn 1992). For microorganisms the EPS matrix fulfills this requirement by protecting the community against shear stress and acting as storage room for nutrients and enzymes (Freeman and Lock 1995, Ramasamy and Zang 2005). Furthermore annual drought and flood events cause significant impacts on the stream ecosystem in extreme circumstances the loss of the habitat (Blenkinsopp and Lock 1994, Galle 2004; Cheng et al. 1997). Associated with the current high loads of organic and inorganic matter become relocated dependent on the water mass and land use (Walling 2006; Wang 2008; Battin et al. 2008) including the associated microorganisms. It is predictable that the stream biofilm is stressed by this continuously changing chemical and physical environment. Therefore it is advantages for the biofilm organisms that the colonization of spaces offered occurs rapidly.

Colonization Patterns of Bacteria and Algae in the Stream Ilm

Early colonization of empty spaces offered in the Stream Ilm in Manebach (Natural Site 1) in August 2005 occurred rapidly by bacteria and algae emphasizing the effort for settlement of both organism groups. Green algae dominated the early biofilm (24 h) and diatoms in later stages as described in other studies (Sekar et al. 2002; Sekar et al. 2004). The first day of colonization by bacteria was denoted by the appearance of the examined groups (12 h) and an enrichment of GAMMA and BETA after 24 h. Between 1 and 7 days the major increase of the bacterial abundance occurred (Fig 1) and the community was dominated by CF, GAMMA and BETA. In contrast to earlier findings (Manz et al. 1999), in the beginning of biofilm formation CF dominated the community and not BETA and ALPHA. Surprisingly HGC occurred in high proportions at all time steps. This group usually accounts for less than 3% of the community (Olapade and Leff 2004). The proportion of the community composition changed further and until 5 months all investigated groups appeared in percentages of about 10 to 25%. These results suggest that the succession of the bacterial community in the current study did not terminate until 7 days (Araya et al. 2003), (Chapter 1).

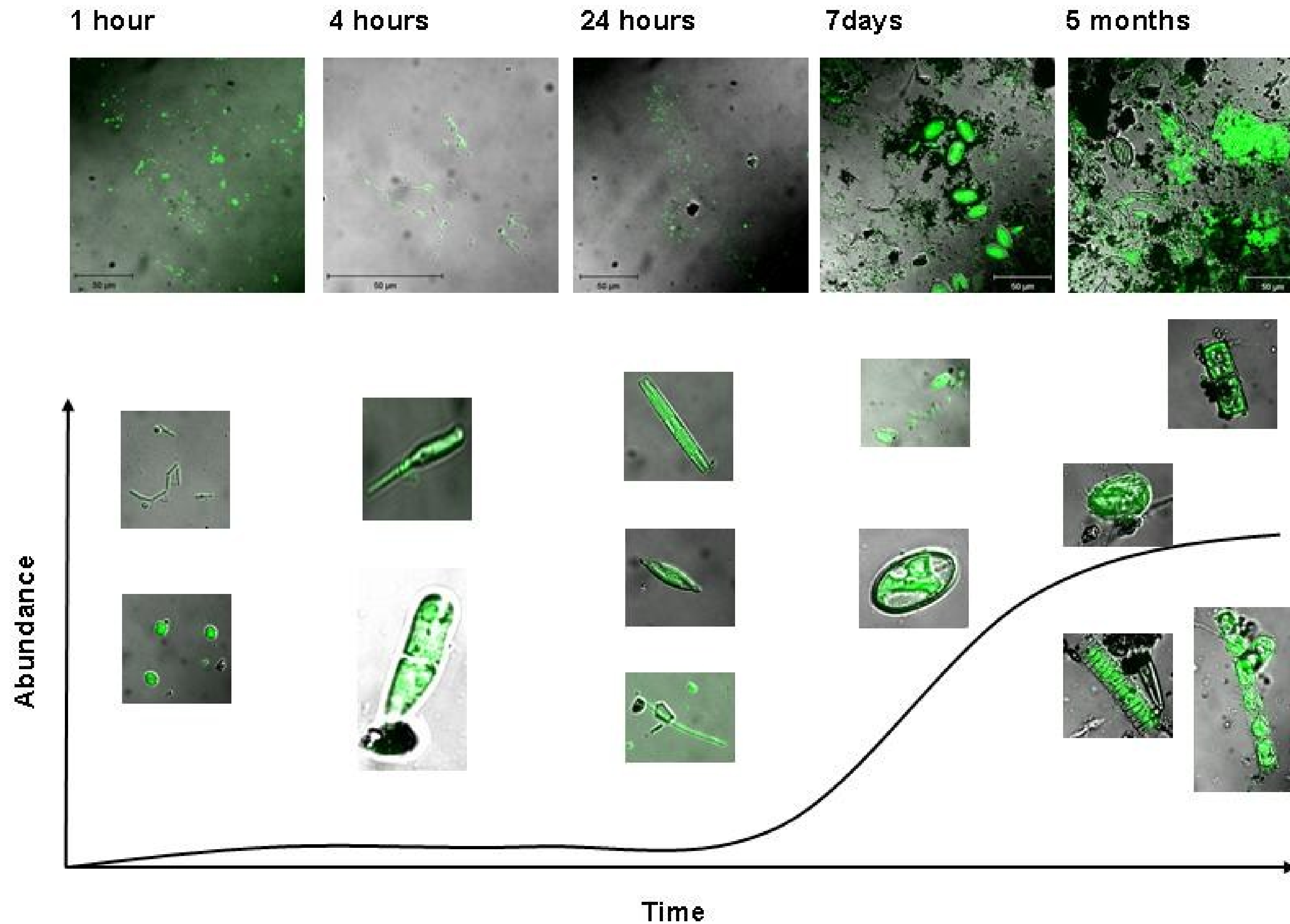

Figure 1. CLSM images of the colonization patterns of biofilms in the Stream Ilm. Within the first day a thin biofilm was developed. Single bacteria cells and motile green algae appeared (1 h) and after 4 h stalked diatoms colonized the surface. After 24 h filamentous bacteria and pennate diatoms were detectable. The highest increase of the abundance of both organism groups occurred between 1 and 7 days and the cells associated into large cell cluster. After 5 months filamentous algae and protozoans were abundant.

In February 2005 the community structure of algae after 2 and 7 weeks at all sampling sites was dominated by green algae suggesting that the biofilm community remained in an early succession state. During the exposition time several flood events occurred (with a 7 fold increase of the mean annual discharge) and green algae are successful primer colonizers (Chapter 1). The bacterial community at all sampling sites and times of exposition was dominated by CF and BETA in different proportions because the bacteria community composition changes during the seasons of the year with highest numbers of CF and BETA in winter (Olapade and Leff 2004). The difference in the proportion of the dominant groups according to the sampling sites with different flow velocities may indicate that besides the seasonal changes other physical or / and chemical parameters affect the bacteria community structure in the stream, (Chapter 2).

Impact of the Flow Velocity and Sedimentation on the Microbial Community in Stream Biofilms

The biofilm structure and function is largely related to the current velocity (e.g. Battin et al. 2003). In this study the early (4 and 8 h) bacterial abundance at the pool (17 cm s^{-1}), outlet (40 cm s^{-1}), and the natural site (40 cm s^{-1}) was of the same magnitude. Only in day scale the bacteria seemed to be affected by high flow velocities because at the pool the abundance was significantly enhanced. After 14 days at the pool and outlet of the weir the abundance of bacteria regardless of the different velocities, was higher than at the natural site. This effect might be caused by high nutrient concentrations in the water column of the weir sites but also by high amounts of inorganic material incorporated in the biofilm matrix (Fig 2).

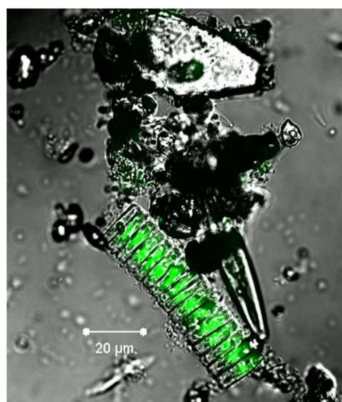

Figure 2. CLSM image of sediment particles (white and black) incorporated in the biofilm matrix of biofilms grown at the pool of a weir in the Stream Ilm.

Sediment particles provide space for colonization (Fig 3) and may increase the capacity for an enhancement of the number of bacteria and algae in biofilms. Furthermore the addition of 1, 5, and 15 mg cm^{-1} sediment particles in flow channels at a flow velocity of 0.7 cm s^{-1} resulted in an increase of the bacterial abundance, algal biomass, biofilm thickness, organic matter content, and sucrose turnover. But the transfer of these biofilms into flow channels with 20 and 60 cm s^{-1} for only 1 h led to instability in biofilms with additional 5 and 15 mg cm^{-1} sediment. The community composition in the Stream Ilm at the natural site was dominated by CF and at the pool and outlet of the weir by BETA or the combination of CF and BETA. After 7 weeks at the outlet GAMMA were enhanced. In the flow channels with sediment addition CF and GAMMA were enhanced and ALPHA reduced. Proportions of CF and BETA were high at sites with low and high sediment loads as well as in the flow channels. But it seems that GAMMA are competitive at sites with sediment loads. There is evidence that the addition of 1 mg cm^{-1} sediment in biofilms grown in the flow channels was optimal. As shown in the Stream Ilm with about 10 mg cm^{-1} of inorganic sediment incorporated in the matrix the biofilm thickness at the pool was largely decreased after the flood event and those at the sites with high flow velocities were not affected irrespectively of the amount of sediment incorporated. Biofilms grown at slow flow velocities with high amounts of inorganic matter incorporated can not resist events with high flow velocities. So the addition of sediment may provide space for colonization and additional nutrients but also cause instability leading to loss of biomass (Chapter 2 and 3).

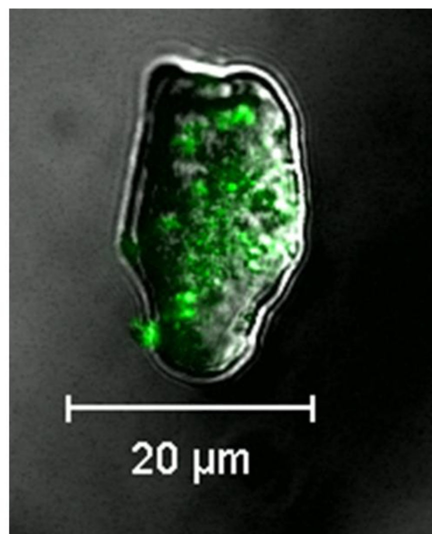

Figure 3. CLSM image of a sediment particle colonized by bacteria in biofilms from the pool of the Stream Ilm.

Evaluation of the Biofilm Structure in the Stream Ilm

Ripple like- and honeycomb structures of biofilms (Okabe et al. 1998; Battin et al. 2003) may be beneficial for the biofilm community in streams. In the current study biofilm formation started with single cells and small colonies (1 h) over a formation of streamers (4 h) up to big cell cluster (7 d and 5 mo) (Fig 1). Additionally high amounts of sediment flocks were incorporated (Fig 2). The resulting biofilm was patchy. In some areas the biofilm cover reached high altitudes but also larges areas were covered by a thin matrix (Fig 4). Organic and inorganic sediment particles incorporated may cause the patchy environment. Sediment particles are subjected to small scale fluid motions of the stream close to the biofilm surface and might be flushed out easily. Especially big sediment particles might be vulnerable to high flow velocities. Thus a certain pattern could not bee detected (Chapter 1 and 2).

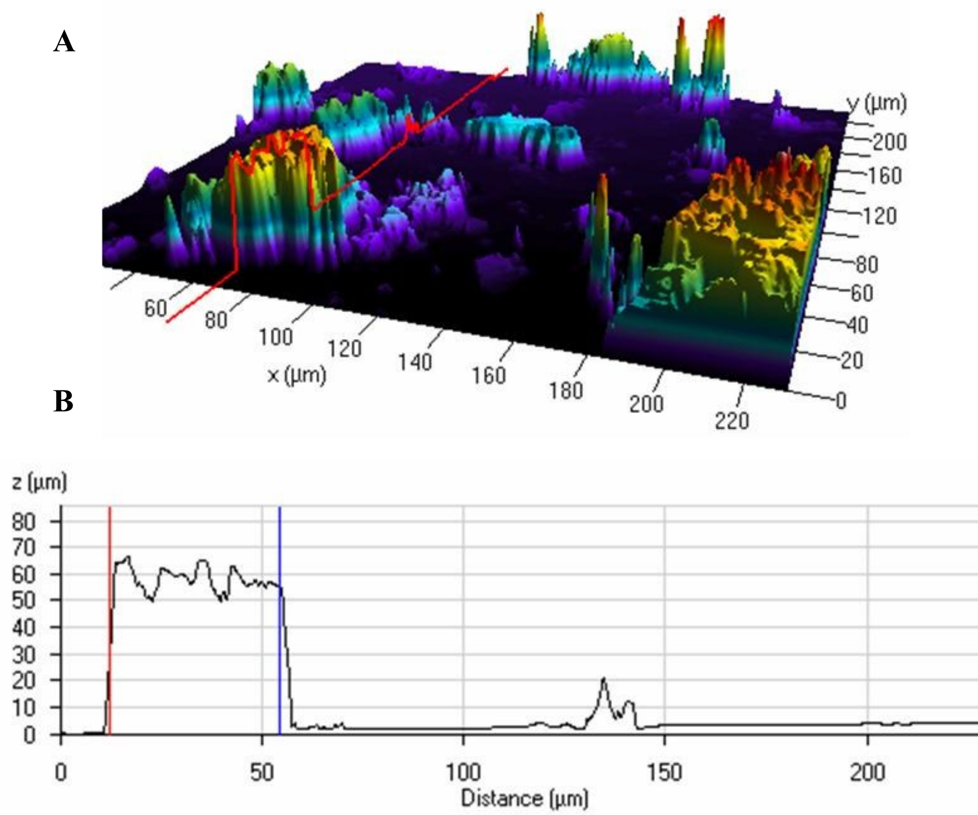

Figure 4. (A) CLSM image of a biofilm grown in the Stream Ilm. Patches of low (black) and high (red) altitude biofilms alternate. (B) Profile of the section marked by the red line.

Measurements of the extracellular enzyme activity

In this study the extracellular enzyme activities were measured at very early states of biofilm formation. The enzymatic activity is linked to biofilm organisms (Hoppe 1983) accordingly with the appearance of high numbers of bacteria and algae at these early stages the alkaline phosphatase activity could be measured already after 4 hours as well as the beta-glucosidase after 8 hours. Phosphate is a limiting nutrient in aquatic environments (Mohamed et al. 1998) and the release of high concentrations of alkaline phosphatase by the organisms might be necessary for the demand of phosphate during early stages of biofilm formation. The switch of the beta-xylosidase : beta-glucosidase ratio from very low 0.1 to 0.4 in 5 months old biofilms indicate a change in organic matter resources utilization by the biofilm community. High ratios indicate the importance of hemicellulose of allochthonous origin (Romani and Sabater 2000) which might be incorporated in the biofilm matrix (Chapter 1).

At the natural site and the weir the alkaline phosphatase activity was 4-times higher as the other enzymes measured emphasizing the demand of phosphorus for the organisms. The differences in the enzyme activity due to the flow – and sediment conditions at the sampling sites were low, beta-xylosidase and alkaline phosphatase activity was high at both weir sites. But referred to the bacteria cell numbers the activities of alkaline phosphatase, beta-glucosidase and aminopeptidase were significantly higher at the natural site. The biofilm from this site was thin at the entire time period of sampling and the amount of incorporated material was low. Nutrient concentrations were significantly lower for ammonium and phosphate in the water column. These high per cell enzymatic activity might be a feature for a high turnover necessity of high molecular weight nutrients at this site. Bacteria at the weir sites might be benefited by sedimentation which causes an enhancement of the abundances of bacteria and algae and probably which provide additional low molecular weight nutrients (Chapter 1 and 2).

Flow pattern and the bacterial community at small weirs in the Stream Ilm

The flow velocities in front of the three small weirs investigated in the Stream Ilm were reduced and the flow patterns of the second weir clearly showed a unification of the current. Straight behind the barrier of the second weir a large pool occurred and adjacent a large riffle. Thus the impact of the weirs is not restricted to the area in front of the barrier, also behind. At the natural sampling site in Manebach the current was dominated by high flow velocities with a high diverse flow pattern, so small pools and riffles alternated.

Overall the bacteria cell numbers were one dimension higher at the weir sites compared with the natural site indicating an enhancement at the weirs possibly due to sedimentation.
The bacterial community structure in 5 months old biofilms at the natural site was denoted by an nearly balanced proportion of the investigated groups whereas at the pool CF and GAMMA dominated and at the outlet CF, GAMMA, and BETA (Fig 5). As shown in the flow channels GAMMA are enhanced at sites with high amounts of sediment incorporated.

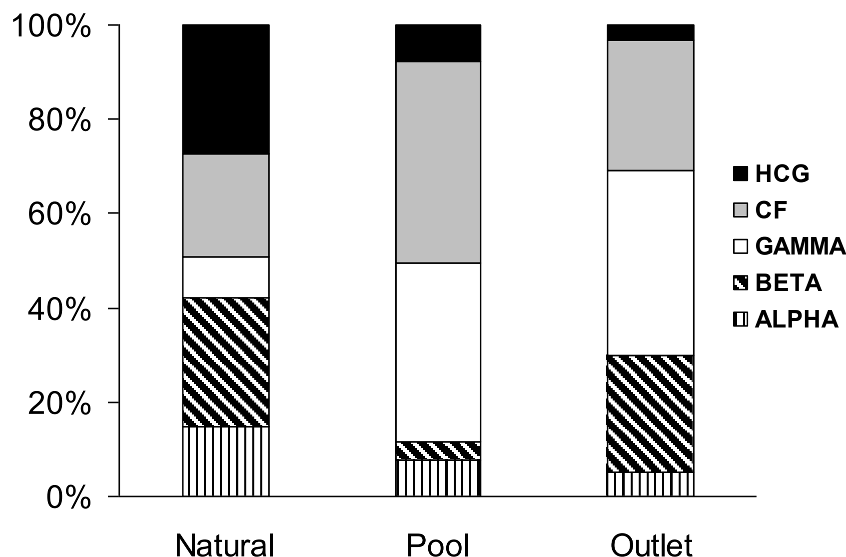

Figure 5. Proportion of the bacterial community in 5 months old biofilms at the natural site and the pool and outlet of a weir in the Stream Ilm in August 2005.

Small weirs might be important for stream ecosystems as storage for nutrients in small scales. A serial impoundment of a stream by small weirs may despite of the negative impact as barriers for higher organisms, locally slow down the flow velocity and build a series of large pools and riffles. Especially in straightened streams small weirs may be important sites for processing of organic material by entrapment of particulate organic matter (POM) (Chapter 5 and 6).

Conclusion

Colonization of empty spaces offered in a stream occurs rapidly by bacteria and algae. The early enzymatic activity measured emphasizes high turnover activities from the beginning of biofilm formation on. After only 7 days the major "work" in biofilm formation has been done. (*Hypothesis and Question 1*).

The barrier slows the steam flow down and causes sedimentation at this site. This effect is not restricted to the pool. At both weir sites high amounts of inorganic sediment incorporated enhance the bacterial abundance and within the bacterial community *Gammaproteobacteria*. Bacteria at these sites are less active by exhibiting a lower per cell enzymatic activity as at the natural site (*Question and Hypothesis 2*).

The incorporation of amounts up to 15 mg cm^{-2} of inorganic sediments is advantages for the biofilm community by enhancing the abundance and the turnover. However biofilms grown at a slow flow velocity with 5 and 15 mg cm^{-2} sediment particles incorporated also suffer loss. Sediment loads of about 1 mg cm^{-2} are optimal (*Question and Hypothesis 3*).

Small weirs generate longitudinal series of pools and riffles in high altitudes. The over all influence of weirs in the Stream Ilm on the microbial community structure and function seems to be positive. Only biofilms grown at slow flow velocities might be affected by loosing biomass after flood events. The longer residence time of the sediments in front of the weir might be beneficial for the biofilm community and their consumers by providing additional organic matter. However small weirs do not replace natural barriers as trees and stones although in streams modified by human impacts as straightening it might be the best and mostly only option to slow the current down (*Question and Hypothesis 4*).

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Summary

Biofilms are assamblages of bacteria, algae, fugi, and protozoans embedded in a polymeric matrix (EPS). In small streams biofilms cover plants and stones and are hot spots in carbon turn over by transferring particulate organic material into biomass and providing a resource for higher organisms. The microorganisms hydrolyse high molecular weight organic material by extracellular enzymes which can be stored within the matrix. Because of the discharge coupled with sediment transport in streams, biofilms are exposed to a permanent changing environment. In extreme circumstances floods and droughts affect the biofilm community and turnover. But also human impact as straightening and dam construction might affect the biofilm in a stream. In the current study the impact of the flow velocity and sedimentation on the bacterial and algal community in the Stream Ilm (Thuringia, Germany) has been investigated. Furthermore the enzymatic activity and biofilm structure was measured. The early colonization patterns of bacteria and algae on artificial surfaces have been investigated and a small weir was used to determine the impact of the flow velocity on the structure and function of the biofilm community. Finally the impact of different amounts of inorganic sediment on the bacterial community structure and carbon turnover was investigated in flow channels in the laboratory. Bacteria and algae colonize surfaces very quickly and the early turnover is denoted by the release of extracellular enzymes within hours. The colonization follows a successional pattern. The pioneer community of algae is dominated by greenalge and the bacteria by *Cytophaga-Flavobacteria*. In streams small weirs lead to a reduction of the flow velocity and enable sedimentation of organic and inorganic material. Biofilms grown under slow flow velocities and at sites with high sediment loads as weirs are thicker and contain higher numbers of bacteria and algae. Inorganic sediment particles provide space for colonization and might also contain additional nutrients. But the stability of biofilms grown at slow flow velocities with increasing amounts of inorganic sediment incorporated decreases. Biofilms grown at high flow velocities with low fractions of inorganic sediment incorporated are thin and the extracellular enzyme activity per cell is higher than at the sites with high sediment loads indicating a demand for nutrients at this sites. It seems that *Beta*- and *Gammaproteobacteria* are enhanced and *Alphaproteobacteria* reduced in biofilms with high inorganic sediment loads incorporated. The impact of sedimentation on the microbial biofilm by a small weir is not restricted to the pool of the weir but also to the outlet despite of the predominantly high flow velocities at this site. Because of small weirs the down flow of a

stream is local reduced and unified, but in streams with high flow velocities or in straightened streams small weirs provide zones of sedimentation leading to retention. Besides the fact that weirs act as barriers for the up and down stream movement and transport they also increase the turn over fascilities of particulate organic matter accumulated at this sites and promote the carbon transport into higher trophic levels.

Zusammenfassung

Biofilme sind Gemeinschaften von Bakterien, Algen, Pilzen und Protozoen eingebettet in einer Matrix aus polymeren Substanzen (EPS). Sie besiedeln in Fließgewässern die Oberflächen von Steinen und Pflanzen und spielen eine wichtige Rolle im Kohlenstoffkreislauf des Gewässers. Mittels extrazellulärer Enzyme können sie allochthones organisches Material hydrolysieren, zersetzen und in Biomasse umsetzen und somit für höhere trophische Ebenen verfügbar machen. Durch die Fließgewässerdynamik sind Biofilme ständig verändernden Bedingungen ausgesetzt bis hin zu Extremzuständen wie Austrocknung und Flutereignissen, wobei auch anthropogene Veränderungen der Fließgewässerstruktur, wie Wehre, eine Rolle spielen. Gegenstand der vorgelegten Arbeit war es, die Besiedlungsdynamik und Populationsstruktur der Bakterien- und Algengemeinschaft auf künstlich exponierten Oberflächen aufzuklären und einen Einblick in den Stoffumsatz dieser Biofilme in den jeweiligen Stadien der Entwicklung zu geben. An einem Wehr wurde der Einfluss von Fließgeschwindigkeit und Sedimentation auf die Struktur der Bakterien- und Algengemeinschaft und der enzymatischen Aktivität untersucht. Dieser experimentelle Ansatz wurde in einem Fließrinnenexperiment im Labor nachgestellt, um explizit den Einfluss von unterschiedlichen Sedimentmengen auf die Biofilmstruktur und den Kohlenstoffumsatz zu untersuchen. Die Besiedlung von ausgebrachten Oberflächen durch Bakterien und Algen in der Ilm erfolgt sehr schnell und sukzessiv, wobei der mikrobielle Stoffumsatz durch eine hohe enzymatische Aktivität bereits zu Beginn der Besiedlung gesichert wird. Die Pioniergemeinschaft der Algen ist von Grünalgen und die der Bakterien von *Cytophaga-Flavobakterien* geprägt. Kleine Wehre sind Barrieren und führen zu einer Verringerung der Fließgeschwindigkeit im Fließgewässer und ermöglichen somit die Sedimentation von organischem und anorganischem Material. Biofilme, die in Bereichen mit hoher Sedimentation und / oder langsamen Fließgeschwindigkeiten wachsen, sind dicker und weisen eine höhere Anzahl an Bakterien und Algen auf. Allerdings nimmt die Stabilität insbesondere in Biofilmen, die bei langsamen Fließgeschwindigkeiten gewachsen sind mit steigender Sedimentmenge ab. Offenbar werden bei hohen Sedimentmengen, akkumuliert in der Matrix, *Beta-* und *Gammaproteobakterien* begünstigt und *Alphaproteobakterien* reduziert. Der Einfluss von Sedimentation auf Biofilme an einem kleinen Wehr ist nicht nur auf den Bereich vor der Barriere beschränkt, sondern erstreckt sich auch noch in den Bereich hinter der Barriere ungeachtet der dort vorherrschenden hohen Fließgeschwindigkeiten. Durch kleine

Wehre wird die Fließgeschwindigkeit in dem gesamten Staubereich künstlich herabgesetzt und vereinheitlicht, doch insbesondere in Gewässern mit sehr hohen Fließgeschwindigkeiten oder bei Begradigung können diese Wehre zu Sedimentation und zu Retention führen. Unter Restorationsgesichtspunkten stellen Wehre Barrieren dar, die den Durchfluss des Gewässers behindern, aber auch Zonen von Gewässerberuhigung. Erhöhte Sedimentation und die Möglichkeit von hohem mikrobiellem Stoffumsatz in diesen Bereichen des Fließgewässers können den Kohlenstofftransfer zu höheren Trophieebenen begünstigen.

Eigenständigkeitserklärung

Ich versichere an Eides statt, dass ich die von mir vorgelegte Dissertation selbständig angefertigt habe und nur die von mir angegebenen Quellen und Hilfsmittel verwendet habe. Die Bestimmungen der Promotionsordnung der Biologisch-Pharmazeutischen-Fakultät der Friedrich-Schiller-Universität Jena sind mir bekannt. Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen und Dritte erhielten weder unmittelbar noch mittelbar geldwerte Leistungen, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Die Dissertation oder Teile davon wurden noch nicht als Prüfungsarbeit an der Friedrich-Schiller-Universität Jena oder an einer anderen Einrichtung für eine staatliche oder andere wissenschaftliche Begutachtung eingereicht.

Jena, den 24.08.2009

Ort, Datum

Elisabeth Pohlon

Hervorgegangene und geplante Publikationen

Alle Kapitel der Dissertation wurden bzw. werden als Publikationen bei internationalen Fachzeitschriften eingereicht. Mein Beitrag an der Erstellung der vorliegenden fünf Manuskripte und Publikationen gestaltete sich wie folgt:

Elisabeth Pohlon, Jürgen Marxsen, and Kirsten Küsel. *Pioneering bacterial and algal communities and potential extracellular enzyme activities of stream biofilms.* Submitted to **FEMS Microbial Ecology** (03.06.2009, accepted with major revions):
Erstellung des Manuskriptes, Planung und Durchführung der Freiland– und Laborarbeit sowie Auswertung der Daten; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch Jürgen Marxsen und Kirsten Küsel.

Elisabeth Pohlon[1], Anne Huchel[1], Clemens Augspurger[1,2,], Jürgen Marxsen[3,4] and Kirsten Küsel[1*]. *Distinct flow velocities and sedimentation affect microbial biofilms in streams.* To be submitted.

Erstellung des Manuskriptes, Planung und Durchführung der Freiland– und Laborarbeiten sowie Auswertung der Biofilmdaten aus der Ilm; Entwicklung der Fließrinnen in Zusammenarbeit mit Jürgen Marxsen; Betreuung des Fließrinnenversuchs in Kooperation mit Clemens Augspurger; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch Jürgen Marxsen und Kirsten Küsel.

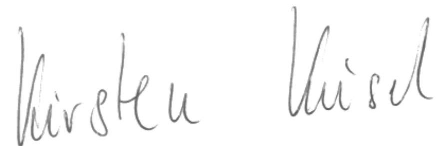
Anne Huchel, Clemens Augspurger, **Elisabeth Pohlon**, Thomas R. Neu, and Kirsten Küsel *Influence of incorporated inorganic sediment on stream biofilms.* **FEMS Microbial Ecology**. (to be submitted).

Einweisung und Betreuung der Ermittlung der Großgruppen der Bakteriengemeinschaft mittels CARD-FISH und Diskussion der entsprechenden Daten sowie Korrektur des Manuskriptes; der wesentliche Anteil der Planung und Durchführung sowie Erstellung des Manuskriptes erfolgte durch die Koautoren.

Elisabeth Pohlon and Kirsten Küsel *Enhanced abundance of biofilm bacteria at small weirs in the stream Ilm (Thuringia, Germany).* **Restoration Ecology** (to be submitted):
Erstellung des Manuskriptes, Planung und Durchführung der Freiland– und Laborarbeit sowie Auswertung der Daten; konzeptionelle Abstimmung und Planung der Arbeiten sowie Überarbeitung des Manuskriptes durch Kirsten Küsel.

Elisabeth Pohlon, Clemens Augspurger, Ute Risse-Buhl, Jens Arle, Marlene Willkomm, Stefan Halle, and Kirsten Küsel *Querying the Obvious: Lessons from a Degraded Stream:* **Restoration Ecology 14**, 312-316:
Erstellung des Manuskriptes; konzeptionelle Abstimmung und Überarbeitung des Manuskriptes durch die Koautoren.

Bestätigung des Eigenanteils an den Manuskripten:



Jena, den 24.08.2009

Ort, Datum

Kirsten Küsel

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2000 - 2002	Project work at the Hydrolabor Schleusingen, Bauhaus University Weimar: "Efficiency control of five fish passes at the Werra and the Schmalkalde in South Thuringia".
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