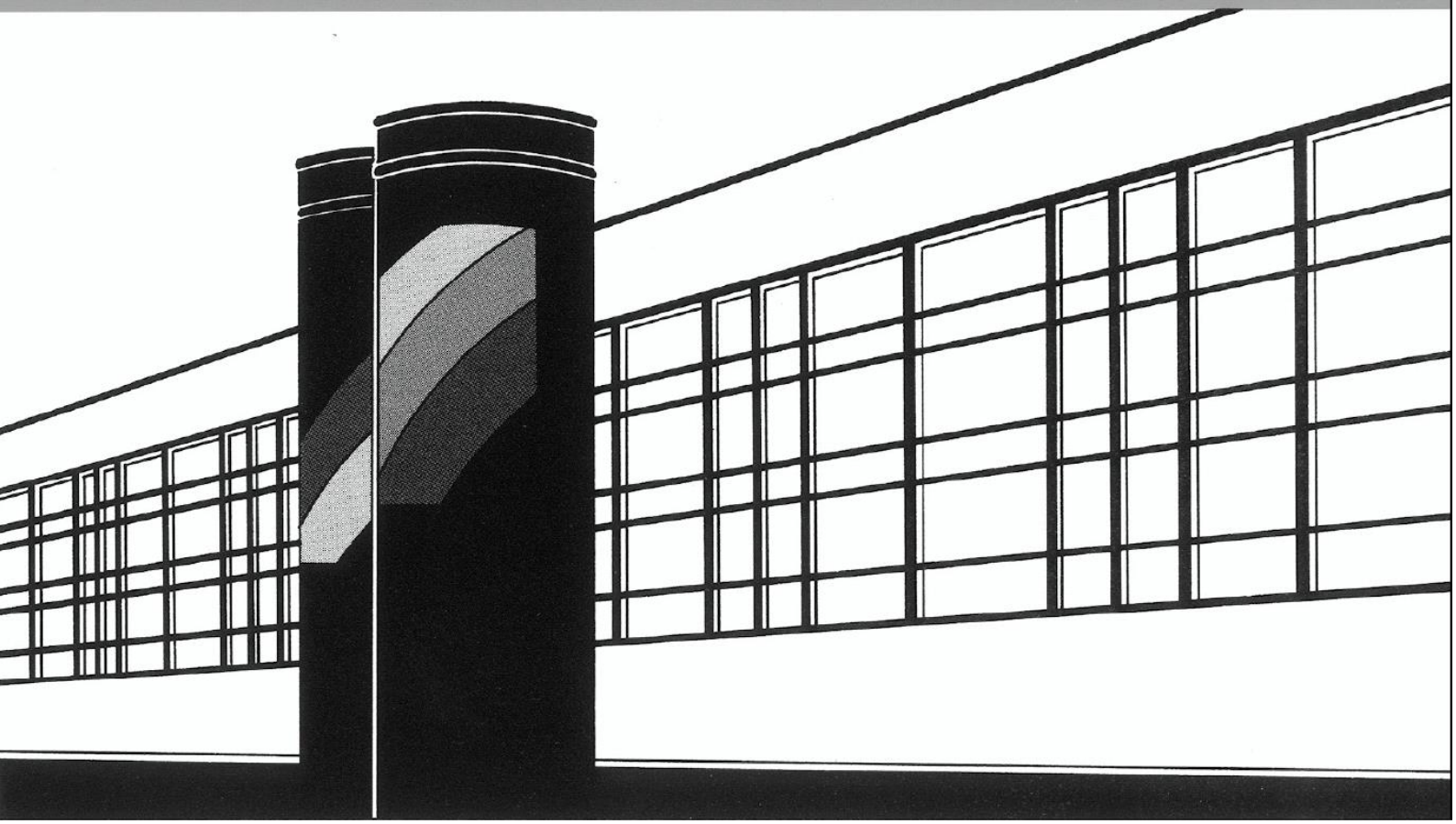
Universität Stuttgart

Institut für Wasser- und Umweltsystemmodellierung

Mitteilungen



Heft 258 Holger Schmidt

Microbial stabilization of lotic fine sediments

Microbial stabilization of lotic fine sediments

von der Fakultät Bau- und Umweltingenieurwissenschaften der
Universität Stuttgart zur Erlangung der Würde eines
Doktor-Ingenieurs (Dr.-Ing.) genehmigte Abhandlung

vorgelegt von

Holger Schmidt
aus Eggenfelden, Deutschland

Hauptberichterin: Prof. Dr.-Ing. Silke Wieprecht
Mitberichter: PD Dr. rer. nat. Werner Manz

Tag der mündlichen Prüfung: 10. November 2017

Institut für Wasser- und Umweltsystemmodellierung
der Universität Stuttgart
2018

Heft 258

Microbial stabilization of lotic fine sediments

von
Dr.-Ing.
Holger Schmidt

Eigenverlag des Instituts für Wasser- und Umweltsystemmodellierung der Universität Stuttgart

D93 Microbial stabilization of lotic fine sediments

Bibliografische Information der Deutschen Nationalbibliothek
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://www.d-nb.de abrufbar

Schmidt, Holger:
Microbial stabilization of lotic fine sediments, Universität Stuttgart. - Stuttgart: Institut für Wasser- und Umweltsystemmodellierung, 2018

(Mitteilungen Institut für Wasser- und Umweltsystemmodellierung, Universität Stuttgart: H. 258)
Zugl.: Stuttgart, Univ., Diss., 2018
ISBN 978-3-942036-62-7

NE: Institut für Wasser- und Umweltsystemmodellierung <Stuttgart>: Mitteilungen

Herausgegeben 2018 vom Eigenverlag des Instituts für Wasser- und Umweltsystem-modellierung

Druck: Document Center S. Kästl, Ostfildern

Danksagung

Zu allererst möchte ich mich bei Prof. Dr. Ing. Silke Wieprecht bedanken. Ohne Ihren Weitblick wäre diese etwas ungewöhnliche Doktorarbeit an Ihrem Lehrstuhl wohl nicht zustande gekommen. Darüber hinaus möchte ich mich dafür bedanken, dass Sie irgendwie den Spagat geschafft haben, mir die nötigen Freiheiten zu lassen aber trotzdem an entscheiden Stellen für mich da zu sein.

Des Weiteren möchte ich mich bei Prof. Dr. rer. nat. Werner Manz bedanken. Vielen Dank nicht nur für die Zweitbetreuung, sondern auch für meine Rekrutierung an die Uni Koblenz und die vielen wichtigen Tipps zum „PostDoc“-Dasein, Antragschreiben und dem Arbeiten in großen Forschungsverbünden.

Prof. Dr. rer. nat. Karl-Heinrich Engesser möchte ich für seine Tätigkeit als Prüfungsvorsitzender danken. Außerdem möchte ich mich für die frischen Impulse während meines Qualifying Exams bedanken, welche diese Arbeit deutlich vorangebracht haben.

Mein ganz besonderer Dank geht an Dr. Sabine Gerbersdorf. Du hast mir so gut wie alles beigebracht, was für die Bearbeitung von Forschungsprojekten wichtig ist. Allen voran, natürlich Paper zu schreiben, aber auch wie wichtig Details und kleine, scheinbar unnötige Einzelheiten sind und dass man sich lieber vorher einen Plan machen sollte als zwischendrin improvisieren zu müssen.

Bei Moritz Thom möchte ich mich ebenfalls bedanken. Es war eine tolle Zusammenarbeit mit Dir und unsere Zigarettenpausen und Gespräche fehlen mir wirklich sehr.

Des Weiteren möchte ich allen Kooperationspartnern danken und allen die mir während meiner Doktorarbeit geholfen haben: Prof. rer. nat Ursula Obst, Dr. Sebastian Beherens, Dr. Lydia King, Dr. Michael Schweikert, Karin Stöger, Kerstin Matthies, Silke Kirchen und Caroline Thesenvitz.

Den Mitarbeitern des LWW und den Werkstätten möchte ich ebenfalls meinen Dank aussprechen. Es war eine wunderbare Zeit mit euch. An dieser Stelle ein besonderer Dank an des E-Team: für superminimalistische Soundtracks, gestochen scharfe Fotographien und Rat und Tat bei technischen und anderen Problemen.

Bei meiner Familie und Freunden möchte ich mich auch bedanken. Vielen Dank für den Rückhalt und die subtile, immer wiederkehrende Frage: „Und, wie läuft die Doktorarbeit?“

Zu guter Letzt gilt mein ganz besonderer Dank meiner Verlobten Veronika: durch deine moralische Unterstützung und die Gewissheit, dass du bedingungslos zu mir stehst bin ich auch in schweren Zeiten nie verzweifelt und konnte diese Arbeit beenden.

Content

Abbreviations ..... **V**

Summary ..... **VI**

Zusammenfassung ..... **VIII**

1. Introduction 12

1.1 Biofilms 12

1.2 Ecological relevance of biofilms 15

1.3 Microbial biostabilization 16

1.3.1 Background 16

1.3.2 Impact factors on microbial biostabilization 18

1.3.3 The role of extracellular polymeric substances (EPS) 19

1.3.4 EPS produced by bacteria and diatoms 20

1.3.5 The influence of EPS quality 23

1.3.6 Lotic systems 26

1.4 Focus of this thesis 28

1.5 Analytic approach 31

1.6 Structure of this thesis 32

2. Material and Methods 34

2.1 Experimental setup 34

2.2 Experiments and sampling 35

2.3 Analyses 38

2.3.1 Water chemistry, EPS and microbial biomass 38

2.3.2 Diatom community composition 38
2.3.3 Bacterial community composition 39
2.3.4 Surface adhesiveness 40
2.3.5 Statistical analysis 41
3. Results 42
3.1 Evaluation of the constructed straight flume mesocosm 42
3.1.1 Background 42
3.1.2 Water chemistry 43
3.1.3 Inter- and intra-flume comparison 43
3.1.4 Development of the biofilm during the experiment 44
3.2 The seasonal effect on biofilm development and microbial biostabilization 51
3.2.1 Background 51
3.2.2 Temporal development of the biofilm EPS matrix and microbial biomass 53
3.2.3 Temporal development of biofilm adhesiveness 53
3.2.4 Comparison of the different seasons 55
3.2.5 Interactions of different biofilm features 55
3.2.6 Microbial community 58
3.3 The effect of light intensity and shear stress on microbial biostabilization 61
3.3.1 Background 61
3.3.2 Comparison of the different boundary conditions 62
3.3.3 Temporal biofilm development 63
3.3.4 Detailed investigation of biofilm parameters 67

4. Discussion 80
4.1 Evaluation of the constructed straight flume mesocosm 80
4.1.1 Biofilm growth within the new mesocosm 80
4.1.2 Biomass and EPS 81
4.1.3 Microbial community 81
4.1.4 Biostabilization 83
4.1.5 Conclusions 84
4.2 The seasonal effect on biofilm development and microbial biostabilization 85
4.2.1 Seasonal effect upon biostabilization 85
4.2.2 Driving factors for biofilm growth and biostabilization 85
4.2.3 Conclusions 90
4.3 The effect of light intensity and shear stress on microbial biostabilization 90
4.3.1 Driving factors for development of the cultivated biofilms 90
4.3.2 Potential indicators for biofilm stability 95
4.3.3 The role of microorganisms for biofilm stability 96
4.3.4 The impact of the cultivation conditions in the used mesocosm setup 98
4.3.4 Conclusions 100
5. General Conclusion 101
6. Outlook 104
7. Appendix 109
Tables 117
Figures 118

References 121

Abbreviations

16S: 16 Svedberg

A. min.: *Achnanthidium minutissimum var. minutissimum*

BCC: bacterial cell counts

BCD: Bray-Curtis dissimilarity

BSS: bed shear stress

DCA: Detrended Correspondence Analysis

DGGE: denaturing gradient gel electrophoresis

DNA: Deoxyribonucleic acid

dNTPs: deoxyribonucleoside triphosphates

DW: dry weight

Dy: dynamics

E. min.: *Eolimna minima*

F. par.: *Fragilaria pararumpens*

Fo: functional organization

KWT: Kruskal-Wallis test

LB: Lysogeny broth

LI: light intensity

min: minute(s)

N. font.: *Nitzschia fonticola*

N. diss.: *Nitzschia dissipata var. dissipata*

PCR: polymerase chain reaction

Rr: range weighted richness

rRNA: ribosomal ribonucleic acid

SDS- PAGE: Sodium dodecyl sulfate - Polyacrylamide gel electrophoresis

S. sem.: *Sellaphora seminulum*

sec: second(s)

T3: adhesiveness threshold 3

Taq: *Thermus aquaticus*

UV: ultra violet

v/v: volume to volume

w/v: weight to volum

Summary

The microbial stabilization of fine sediments constitutes an essential ecosystem function with great ecological and economic implications e.g. in the context of reservoir and waterway management. Although this process is well researched in intertidal mudflats, there is still a major lack of knowledge for lotic systems.

To perform fundamental research in this field and to account for the associated very high level of complexity, expertise of natural and engineering science was combined in an interdisciplinary approach. A highly sophisticated mesocosm setup was designed and constructed to guarantee fully controllable and reproducible natural-like boundary conditions during biofilm formation. The overall aim of the performed studies in this doctoral thesis was a comprehensive investigation of all relevant parameters of the cultivated biofilms, such as the microbial biomass, the produced extracellular polymeric substances (EPS), and the composition of the microbial community as well as the stability of the biofilm. This extensive approach should allow the identification of functional key parameters of the biofilm as well as essential interactions and their impact on the overall biofilm ecosystem and resulting biostabilization. In a series of long-term experiments, different influencing factors on biofilm development and corresponding biostabilization were assessed. The first potential impact factor that was analyzed was the experimental setup itself. Furthermore, the influence of the seasonal changes of the microbial community in the utilized river water and the effects of different levels of bed shear stress and illumination intensity were assessed.

The results of these different experiments provided essential new insights into the process of biostabilization of lotic fine sediments. Firstly, the reliability of the used experimental setup could be proven, as no significant differences could be detected in biofilm formation and biostabilization comparing different mesocosm sections. The fact that very similar biofilms were developing when the boundary conditions were identical was a crucial prerequisite for any further investigations. In addition, the relevance of biostabilization in lotic systems, which was doubted for a long time, could be proven. However, freshwater and brackish habitat can be very different (e.g. in terms of nutrient availability). This was exemplarily indicated by significantly lower microbial biomass in the analyzed freshwater biofilms compared to biofilms from well-studied intertidal mudflats. Moreover, the very complex interplays between bacteria and diatoms in the biofilm matrix were underlined which led to a focus on this subject during further subsequent studies via an extensive genetic and microscopic profiling.

Secondly, the important role of EPS during biostabilization could be demonstrated, whereby the significance of extracellular proteins, such as adhesives produced by sessile diatoms, was suggested. This observation may extend the current EPS research which focusses on extracellular carbohydrates due to their high quantitative fraction in the EPS matrix. Furthermore, the interactions between the microbes, the extracellular matrix and the overall stability of the biofilm system appeared to be much more complex than formerly assumed.

Thirdly, the importance of the microbial community in the biofilm system could be elucidated. Even though a high correlation between mere microbial biomass and biostabilization could be detected, especially the seasonality experiments emphasized the impact of the life style of key players among the diatoms. These insights could be extended during the experiments analyzing the different levels of abiotic boundary conditions, where differently stable biofilms were clearly dominated by different assemblages of dominant bacteria. These observations constitute very important new insights into microbial biostabilization as a direct correlation between microbial ecology and the overall, actually measurable ecosystem function of the biofilm could be shown for the first time.

Concluding, the insights into the fundamental principles of biostabilization gathered during this thesis can be seen as important steps for further fundamental research. The construction of a reliable unique setup is complete, the reproducible biofilm cultivation in this setup is verified and first investigations of different driving factors during biostabilization were performed. These analyses paved the way for further studies to analyze currently hardly assessed boundary conditions and deeper assessments in order to generate a sound database for future modelling approaches of the dynamics of microbially stabilized lotic fine sediments.

Zusammenfassung

Die mikrobielle Stabilisierung von Feinsedimenten stellt eine wichtige Ökosystemfunktion dar, welche hohe Relevanz in ökologischen und ökonomischen Fragestellungen hat. So wird beispielsweise die Bewirtschaftung von Talsperren sowie von Wasserstraßen und Häfen durch die Dynamik von Feinsedimenten stark beeinflusst. Obwohl der Prozess dieser sogenannten Biostabilisierung bereits seit den 1990er Jahren in marinen und Brackwasserhabitaten untersucht wird, besteht vor allem im Bereich von Fließgewässern noch ein deutlicher Forschungsbedarf.

Um diesem überaus komplexen Forschungsgebiet, in dem Aspekte der Physik und Chemie sowie Mikrobiologie und Ökologie berücksichtigt werden müssen, ausreichend Rechnung tragen zu können wurde ein interdisziplinärer Forschungsansatz konzipiert, bei dem die Expertise von Naturwissenschaften und Ingenieurswissenschaften miteinander verknüpft werden kann. So wurde ein Versuchsaufbau in Form eines Mesokosmos entwickelt und umgesetzt, um die Vorteile von Feld- und Laborexperimenten vereinen zu können. Dabei lag die Gewährleistung sowohl naturnaher als auch kontrollier- und reproduzierbarer Randbedingungen während der Biofilmkultivierung im Fokus der Erwägungen. Das Hauptziel der in diesem Mesokosmos durchgeführten Versuchsreihen bestand in einer umfassenden Untersuchung aller im Prozess der Biostabilisierung beteiligter Biofilmbestandteilewie beispielsweise der mikrobiellen Biomasse, der Bakterien und Algen sowie deren sezernierter extrazellulärer polymeren Substanzen (EPS). Des Weiteren wurden die Zusammensetzung der mikrobiellen Gemeinschaft und die Adhäsivität (Oberflächenklebrigkeit) der Biofilme analysiert, um die Identifizierung möglicher funktioneller Schlüsselparameter sowie Schlüsselorganismen und deren Einfluss auf die resultierende übergeordnete Ökosystemfunktion der Biostabilisierung zu ermöglichen. Zu diesem Zweck wurden die Auswirkungen verschiedener möglicher Einflussfaktoren auf die Ausbildung und Entwicklung der Biofilme untersucht. Dabei war der erste mögliche Einflussfaktor der eigentliche Versuchsaufbau, da nicht ausgeschlossen werden konnte, dass dieser beispielsweise eine unterschiedliche Biofilmentwicklung in verschiedenen Teilabschnitten der Fließrinnen begünstigt, wodurch sich auch eine mögliche Beeinträchtigung der Biostabilisierung ableiten ließe. Außerdem wurde der Effekt der saisonal veränderlichen mikrobiellen Biozönose des Flusswassers, welches für die Experimente verwendet wurde, sowie die Auswirkungen unterschiedlicher

Beleuchtungsintensitäten und Sohlschubspannungen während des Biofilmaufwuchses getestet.

Aus den Ergebnissen der einzelnen Teilprojekte konnten essentielle neue Erkenntnisse bezüglich der Grundlagen der Biostabilisierung von Fließgewässerfeinsedimenten gewonnen werden. In einem ersten Schritt war es möglich, die Verlässlichkeit des Versuchsaufbaus zu bestätigen, da Biofilme aus verschiedenen Sektionen des Mesokosmos keinerlei statistisch signifikante Unterschiede hinsichtlich der Entwicklung und der daraus resultierenden stabilisierenden Wirkung auf das bewachsene Feinsediment zeigten. Dieser Nachweis, dass in dem verwendeten Versuchsaufbau bei identisch eingestellten Randbedingungen sehr vergleichbare Biofilme kultiviert werden können, stellte eine maßgebliche Voraussetzung für alle nachfolgenden Untersuchungen dar, da nur so eine Reproduzierbarkeit der Ergebnisse gewährleistet werden kann.

Darüber hinaus konnte die lange Zeit strittige Bedeutung der Biostabilisierung für Fließgewässersysteme gezeigt werden. Diese Zweifel wurden unter anderem mit dem Fehlen starker Ionischer Bindungen in der extrazellulären Matrix aufgrund geringer Ionenkonzentrationen im Süßwasser begründet. Dennoch zeigten sich deutliche Unterschiede zwischen marinen bzw. Brackwasserhabitaten und dem untersuchten limnischen System. So war die mikrobielle Biomasse in den untersuchten Süßwasserbiofilmen, möglicherweise als Folge von divergierenden Nährstoffverfügbarkeiten, deutlich geringer als beispielsweise in eingehend untersuchten Gezeitenzonen. Vor diesem Hintergrund ist es von großer Bedeutung, dass, wie in der vorliegenden Arbeit gezeigt werden konnte, bereits eine vergleichsweise dünne Biofilmschicht eine deutlich stabilisierende Wirkung auf das besiedelte Feinsedimente entwickeln kann. Darüber hinaus konnten hochgradig komplexer Wechselwirkungen zwischen Bakterien und Diatomeen im Biofilmgefüge nachgewiesen werden, die einen massiven Einfluss auf die Biostabilisierungsleistung entfalten. Daher sollte die mikrobielle Ökologie bei der weiteren Erforschung von mikrobieller Biostabilisierung durch Süßwasserbiofilme weiterhin als Schwerpunkt in vergleichbar umfangreichen genetischen sowie mikroskopischen Studien untersucht werden.

Des Weiteren konnte die wichtige Rolle der EPS während des Prozesses der Biostabilisierung beleuchtet werden. Hierbei war die große Bedeutung der extrazellulären Proteine auffällig, welche beispielsweise in Form von Adhäsionsproteinen durch Diatomeen

zur Anheftung an Sedimentkörner gebildet werden. Die durchwegs höhere ermittelte Korrelation zwischen dem Gehalt an EPS-Proteinen und der Biofilmstabilität als die zwischen dem Gehalt an EPS-Kohlenhydraten und der Biofilmstabilität unterstreicht die hohe Relevanz der extrazellulären Proteine für die Struktur und Funktionalität des Biofilms. Darüber hinaus veranschaulichen diese Ergebnisse die vergleichsweise fragile Natur der extrazellulären Kohlenhydrate, welche dadurch im Vergleich zu den extrazellularen Proteinen eine geringere strukturelle Bedeutung für die Biofilmmatrix aufweisen. Diese Beobachtungen erweitern unser aktuelles Wissen über die EPS das sich zurzeit noch hauptsächlich auf die extrazellulären Kohlenhydrate konzentriert, da diese den überwiegenden Teil der EPS darstellen. Generell deuten die Beobachtungen während des Biofilmaufwuchses auf ein Netzwerk weit komplexerer Wechselwirkungen zwischen Mikroben, deren produzierten EPS und der allgemeinen Biofilmstabilität hin als bisher in anderen Studien gezeigt werden konnte.

Neben anderen Einflussparametern zeigten sich die mikrobielle Gemeinschaft und deren Reaktion auf Umweltbedingungen von zentraler Bedeutung für die Entwicklung und Stabilität des Biofilms. Obwohl im Allgemeinen eine hohe Korrelation zwischen mikrobieller Biomasse und Biostabilisierungskapazität festgestellt werden konnte, demonstrierten vor allem die Untersuchungen zur jahreszeitlichen Veränderung der Mikrobengemeinschaft den massiven Einfluss von Schlüsselorganismen unter den Diatomeen. Dabei spielte die Lebensweise der dominanten Mikroalgen eine maßgebliche Rolle, da beispielsweise eine Dominanz sessiler Arten stets mit hoher Biofilmstabilität einherging, während hohe Abundanzen späterer, mobiler Sukzessionsstadien mit einer signifikant niedrigeren Biofilmstabilität zusammenfielen. Die Ergebnisse der Experimente zur Auswirkung unterschiedlicher Randbedingungen konnten diese Erkenntnisse zusätzlich um die Bedeutung der dominanten Bakterienarten erweitern. Denn auch hier zeigten sich klar unterschiedliche Dominanzmuster zwischen stabilen und fragilen Biofilmen. Ferner war es möglich, erste Gemeinsamkeiten in der Physiologie und Morphologie der bakteriellen Schlüsselorganismen herauszuarbeiten und diese mit der Auswirkung auf die Biofilmstruktur in Beziehung zu bringen. So erscheinen unbewegliche, koloniebildende Arten, die zu hoher EPS- Produktion fähig sind und somit als Pioniere für die spätere Absetzung der Diatomeen dienen können, stets in sehr stabilen Biofilmen vorzukommen. Im Gegensatz dazu dominierten hochgradig mobile Arten, welche bekanntermaßen hauptsächlich in Stresssituationen, wie beispielsweise Nährstoffknappheit, zur Biofilmbildung neigen und häufig zwischen mobilem und sessilem Lebensstil wechseln

vergleichsweise fragile Biofilme. Diese Beobachtungen stellen wichtige neue Ergebnisse dar, da sie zum ersten Mal eine direkte Verbindung zwischen mikrobieller Ökologie und der daraus resultierenden, übergeordneten, tatsächlich messbaren Ökosystemfunktion des Biofilms ermöglichen.

Insgesamt stellen die gewonnenen Ergebnisse wichtige Anknüpfungspunkte für weitergehende Studien dieser grundlegenden Prinzipien des Prozesses der mikrobiellen Biostabilisierung von Fließgewässerfeinsedimenten dar. Mit dem beschriebenen Mesokosmos steht ein Versuchsaufbau zur Verfügung, mit dem die Reproduzierbarkeit der Biofilmentwicklung eindeutig nachgewiesen werden konnte. Darüber hinaus wurden erste orientierende Versuchsreihen zu unterschiedlichen Einflussfaktoren durchgeführt. Diese Untersuchungen legen die Grundlage für weiterführende Untersuchungen, in denen beispielsweise bisher wenig betrachtete Randbedingungen bearbeitet oder detaillierte Einzeluntersuchungen durchgeführt werden können. So kann in Zukunft eine ausreichende Datengrundlage geschaffen werden, um dem Ziel einer Modellierbarkeit für die mikrobiell beeinflusste Dynamik von Fließgewässerfeinsedimenten näher zu kommen.

1. Introduction

1.1 Biofilms

On virtually every kind of interphase between the liquid and the solid phase, biofilms can develop and further, are able to proliferate. Thus, biofilms can be regarded as ubiquitous. They occur in all kinds of comparably moderate environments such as soil and the rhizosphere, or in aquatic ecosystems as growth-upon on aquatic plants and on the neuston on the water surfaces of water bodies. However, biofilms are also able to grow under extreme life conditions for example on the abyssal floor in close proximity to black smokers (Steen *et al.*, 2016), in salt lakes (Di Meglio *et al.*, 2014), or under the eternal ice of the poles and in alpine regions (Chrismas *et al.*, 2016; Mykytczuk *et al.*, 2016). Moreover, all kinds of animal epithelia can be colonized by different microbiomes. As a result, an extremely highly structured environment such as soil with various pores and differently shaped interphases between substrate, air and water can harbour a multitude of microbes and biochemical pathways (Trevors, 2010). In this context, the ability to form biofilms is regarded as one major evolutionary adaption and one reason for the success and flexibility of microbes – in combination with different forms of dormancy and a broad range for genetic transfer (Flemming *et al.*, 2016).

Being part of a biofilm ecosystem is such a successful lifestyle that most bacteria and many unicellular algae traverse a cycle of free living and biofilm developmental stages (Gerbersdorf & Wieprecht, 2015; Proctor & Hammes, 2015). The decisive advantages of a lifestyle in a microbial biofilm over a free-living existence lie in the structure of biofilms: Over 90% of the volume of a matured biofilm consists of a viscous matrix which is mainly composed of water with a low concentration of colloidal (water soluble) extracellular polymeric substances (EPS) – largely different carbohydrates and proteins as well as lower quantities of lipids and extracellular DNA- produced by microbes (Flemming *et al.*, 2000b; Flemming & Wingender, 2001; Flemming & Wingender, 2010). This is essential for the survival of biofilm microbes. Not only is the water content a protective layer against desiccation, but the extracellular biofilm matrix has a variety of important functions. Among these are a significant increase of the surface of the microbes which enhances diffusion, and the establishment of a confined reaction location which improves the effectiveness of extracellular metabolism. This enables e.g. bacteria to break down complex molecules via extracellular enzymes. Furthermore, the EPS can function as a nutrient storage or as

messenger molecules. The microbial cells embedded in the biofilm matrix additionally benefit from the close proximity to each other which facilitates the transfer of information e.g. the exchange of DNA or quorum sensing (Emge *et al.*, 2016). Thus, the biofilm community can rapidly adapt to different boundary conditions and changes in the environment via a large exoproteome (De Angelis *et al.*, 2015) which may provide a decisive advantage that can lead to the survival of the majority of the biofilm bacteria while a comparable number of separated planktonic cells might have already died.

However, it is very important to not only consider the single microbial cell, but the total biofilm biocoenosis. It is obvious that a biofilm matrix is characterized by various processes of competition e.g. for nutrients, light, oxygen or trace elements and that the different strategies to fight competing microbes (Rendueles & Ghigo, 2012) can result in the detachment or decay of microbes or even clusters of microorganisms. In natural, complex, mixed species and -taxa biofilms, predation also plays a very important role so that the abundance of certain species can be significantly reduced. Thus biofilms will be presently regarded as highly dynamic systems characterized by a continuous attachment and detachment of microbial cells. Furthermore, a repeated change between swarming or floating, and sessile forms in the life cycles of the biofilm microorganisms occurs (Rendueles & Ghigo, 2012) (also see ***Figure 1***). The resulting high temporal and structural heterogeneity of the biofilm system and the constantly changing conditions create a hotspot of microbial development and adaptation. Especially in emerging gradient zones of nutrients and metabolites, various niches can be established where different microbes can proliferate and new nutrient sources can be exploited (Lear *et al.*, 2008; Stewart & Franklin, 2008). Furthermore, this variety of different available nutrient sources can significantly increase the starvation resistance of microbes in a multispecies biofilm (Gao *et al.*, 2016).

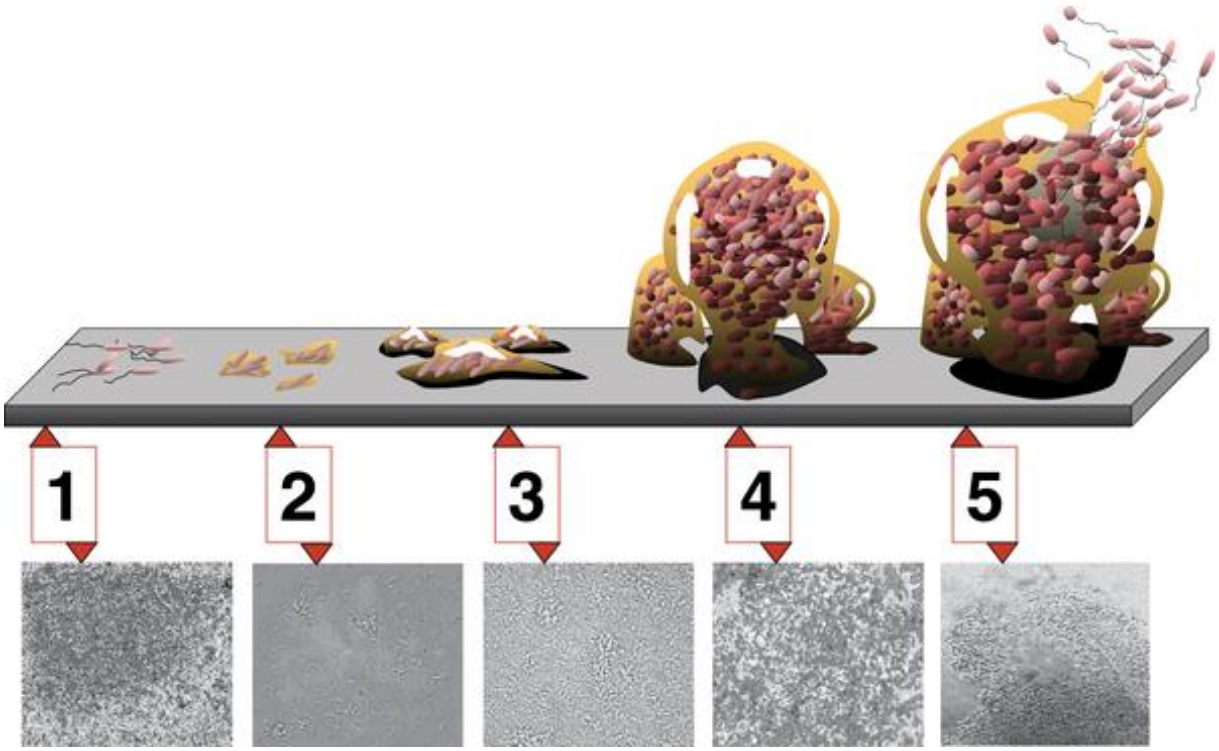


Figure 1*: Stages of biofilm development modified after Monroe (2007): 1. Initial attachment; 2. Irreversible attachment; 3. Early Maturation; 4. Late Maturation; 5. Dispersal.*

Especially in motile bacteria, the formation of a biofilm is often a stress response during starvation and has been reported for multispecies environmental bacteria isolates (Elhariry *et al.*, 2012). Specifically in *Pseudomonas aeruginosa,* this switching could be linked to phosphorus starvation and a complex regulatory system (Haddad *et al.*, 2009). During the process of settlement, specialized outer membrane lipoproteins such as NIpE in *E.coli* (Otto & Silhavy, 2002) sense the attachment to a solid surfaces and initiate genetic signalling pathways to synthesise adhesive proteins. These proteins can be produced relatively easily by modifying flagella proteins, e.g. flagellin which was demonstrated to be essential for bacterial attachment to abiotic surfaces (Nejidat, Saadi & Ronen, 2008; Zgair & Chhibber, 2013). In a nutrient rich environment, different bacterial cells were reported to react with decreasing attachment to biofilms and surfaces, exhibiting mostly the free living stages of cells (Rochex & Lebeault, 2007). In order to react on spatially and temporally very variable nutrient conditions in a complex natural environment both the free swimming- and the sessile form are required. Thus, some bacterial families such as *the Planctomycetaceae* reacted to this dilemma by multiplying in an anisomorphic reproduction cycle where a sessile mother cell produces motile swarmer cells that leave the biofilm system (Schlesner & Stackebrandt, 1986; Woese, 1987). Swarmer cells can exploit new habitats and are able to form new

biofilms. This avoids intraspecific competitions for nutrients while profiting from the benefits of a biofilm system.

1.2 Ecological relevance of biofilms

Due to their virtual omnipresence in different environments, biofilms play an essential ecological role in many different habitats. As a description of the huge variety of all different biofilm functions would go beyond the scope of this section, only the most important ecosystem services that are relevant in aquatic sediment systems will be stated.

Biofilms constitute the foundation of the benthic food webs and are indispensable as their metabolic pathways guarantee the aquatic nutrient cycling. As an example, microbial nitrogen fixation, e.g. by cyanobacteria, plays a very important role by making the atmospheric nitrogen compounds that are integrated into the microbial biomass available for other aquatic organisms such as algae or protozoa (Sveden *et al.*, 2016). The same holds true for phosphorus and sulphur (Hudek *et al.*, 2016; Mills, Antler & Turchyn, 2016). As biofilms develop on the benthos, the interphase of soluble chemicals in the water column and mineralized chemicals in the sediment, their metabolic turnover rates can be very high due to a combination of different extracellular enzymes (Sinsabaugh *et al.*, 1991). The benthic-pelagic feedback loop also provides essential nutrients to the water column and e.g. planktonic microbes. However, this microbial loop is not only vital for nutrient cycling, it also constitutes the first step in the capacity of self-purification in aquatic systems. Moreover, biofilms that consist e.g. of sulphate reducing and methanogenic bacteria and archaea have been demonstrated to play a crucial role during the process of bioremediation of contaminated aquifers and fine sediments e.g. after oil spills (Gieg, Fowler & Berdugo-Clavijo, 2014; McGenity, 2014). In this context, it is especially remarkable that even very toxic and complex molecules such as polycyclic aromatic carbohydrates or halogenated carbohydrates can be degraded to methane by a syntrophic mixed biofilm via a cascade of different fermentation processes.

Furthermore, natural biofilms can be seen as vast virtually unlimited biochemical libraries. Not only constitutes the microbial diversity in a biofilm system an important source of functional resilience, the immense richness in microbial species found in different habitats and the corresponding genetic diversity encodes a great variety of enzymes, metabolites, and other biochemical substances which up to now mostly stay unstudied. Their future

various utilizations as industrial substance, excipients or pharmaceutical substance cannot be predicted yet. This is the reason why research on biofilms is of huge importance.

1.3 Microbial biostabilization

1.3.1 Background

Another very important fact is that the EPS secreted by the different microbes during biofilm growth and development do not only attach the individual cells to the substrate but also glue the sediment particles to each other (see ***Figure 2***). Hereby, especially fine sediments (0.02 - 0.2 mm) are granted a higher resistance against erosive processes which was extensively described for intertidal mudflat habitats (Underwood & Paterson, 1993; Tolhurst, Gust & Paterson, 2002). This significant ecosystem service with broad economic and ecological implications is known as microbial biostabilization. From an engineering perspective, a deep understanding of the dynamics of fine sediments is essential for a broad range of applications such as the protection of coasts against erosion, or the maintenance of flood protections, waterways, dams or harbours.

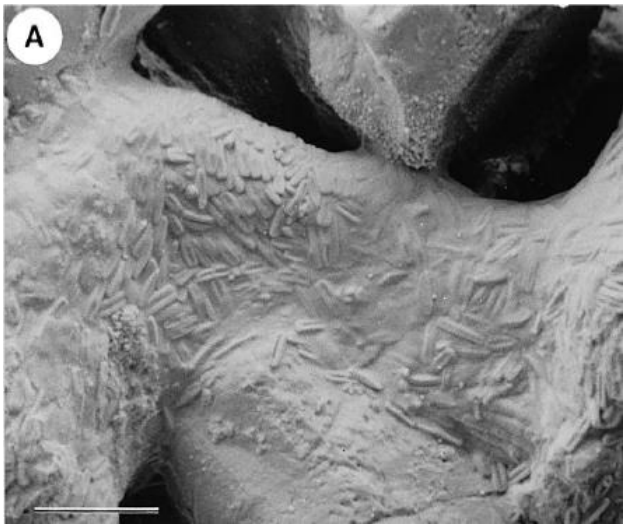


Figure 2: *ESEM image of a natural biofilm in an intertidal mudflat habitat (de Winder et al., 1999): the thick layer of EPS produced by the biofilm consisting of different diatoms has glued the sediment grains together so that no individual grains remain visible; scale bar: 250 µm.*

Moreover, microbial biostabilization is currently receiving increasing scientific interest as this process plays an essential role in many anthropogenic influenced systems. On one hand, a high quantity of macro- and micro pollutants such as dioxins or heavy metals can be

adsorbed to fine sediment grains and its organic matrix to be jointly deposited in river regions with lower flow velocities (Karickhoff, Brown & Scott, 1979; Audry *et al.*, 2004). In central Europe, different kinds of legacies, especially originated from the 60s, 70s and 80s, before environmental regulations were enacted, were immobilized and by now covered with new unpolluted fine sediments so that there is no acute danger for the overlying benthic and aquatic habitat. The permanent immobilization of pollutants within fine sediments largely depends on the substratum stability. Biofilms can delay or prevent the re-suspension of these legacies into the water body as well as their bioavailability, while microbial bioremediation pathways may initiate the slow degradation of those hazardous substances. As a result, microbial biostabilization can play an essential role for ensuring the quality of surface waters. Furthermore, as surface waters are connected to the groundwater aquifers via the hyporheic interstitial, this process may further be very important for the quality of drinking water.

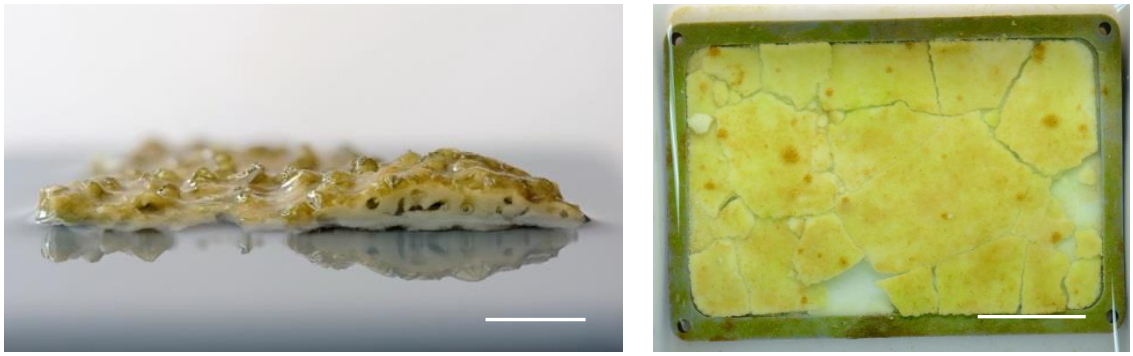


Figure 3: *Images of two exemplary, very distinct morphologies and physical properties of natural biofilms cultivated during the experiments of this thesis:* ***left:*** *very flexible, elastic biofilm/sediment mat;* ***right:*** *robust, compacted biofilm/ sediment sheet; scale bars: 1 cm.*

On the other hand, fine sediments that are considerably stabilized by biofilms can have adverse ecological effects on lotic river beds. During reservoir flushing, the downstream river sections are confronted with a sudden significantly increased load of suspended fine sediment originated from the bed of the dam (Burke, Jorde & Buffington, 2009) which can be much higher than during natural floods (Batalla & Vericat, 2009). As biofilms were developing on the fine sediments at the bed of the reservoir, the flushed sediment particles are colonized with biofilm as well. These fine sediment/ biofilm flocs are able to infiltrate into the coarser gravel bed of the downstream reach and the riverbed is continuously clogged (Brunke & Gonser, 1997) a process called colmation (Descloux *et al.*, 2010). As a consequence, the pores of the natural gravel sediment are gradually clogged with the

infiltrated fine sediment/ biofilm flocs which has great implications for the interstitial habitat. As one important example, the circulation of water and thereby oxygen can be limited resulting in low oxygen saturation within the spawning grounds of gravel spawning fish such as different salmonid species. Different pathways of microbial metabolism in the biofilms can decrease the remaining oxygen even further so that the survivability of affected fish eggs and even more of fish larvae can be significantly impacted (Heywood & Walling, 2007). This can even lead to such a low suitability as spawning ground that fish don't emerge on heavily colmated stretches of river beds (Sear *et al.*, 2008). In this process, biofilms may play an important role as well because their stabilizing impact upon the fine sediment in the pores of the coarser sediment might prevent a re-suspension and flushing of the pores during natural floods with increased discharges.

This ambivalence displays the impact of microbial biostabilization in the context of anthropogenic changed environments and demonstrates the complexity of this field of research and the necessity of sustainable approaches for log-term sediment management strategies. The first step for the implementation of these strategies lies in precise predictions of sediment dynamics which in turn rely on precise modelling approaches. However, traditional modelling approaches that rely exclusively on gravity fail to display the behaviour of fine sediments influenced by this form of significant biological impact that can result in very clear changes in the development of the bed morphology (Malarkey *et al.*, 2015). In addition, the strong gluing effect of biofilms and their produced EPS on fine sediments (Stal, 2003; Stal, 2010) can be able to drastically impact the characteristics of the colonized fine sediments (Tolhurst, Consalvey & Paterson, 2008). This clear change is even visible to the unaided eye, e.g. when the formally loose fine sediment grains are transformed into flexible biofilm/ sediment mats or robust sheets with significantly altered erosive behavior (see ***Figure 3***).

1.3.2 Impact factors on microbial biostabilization

Current studies were able to identify various driving factors for microbial biostabilization. The composition of the biofilm microbial community that reacts to a broad range of biotic and abiotic environmental parameters appears to constitute a fundamental and essential influencing factor. Lubarsky *et al.* (2010) could indicate the crucial role of a mixed-taxa symbiosis for biostabilization as biofilm assemblages consisting of bacteria as well as diatoms displayed higher stability than pure bacterial or axenic diatom biofilms. This fact that

diatoms as well as bacteria play this crucial role for biostabilzation was supported by further studies: Especially the EPS produced by diatoms was suggested to be very important for the structure and stability of the biofilm and underlying fine sediment (Mason *et al.*, 2003) as treatment with herbicides of natural biofilms in field experiments resulted in increased erosion rates of saltmarsh fine sediments. Moreover, Lubarsky *et al.* (2012) could show the importance of bacterial activity in the biofilm system and reported a significant impact of treatments with antibiotics on the bacterial biofilm community and the correlated biofilm stability.

As the exact functional principles e.g. inter-taxa molecular quorum sensing, communication and co-metabolic pathways of this important symbiosis network are very complex and still hardly addressed, research of many studies focused on the role of the EPS whose direct impact on microbial biostabilization could be clearly demonstrated. In this context, different diatom species were described to produce EPS with very distinct monomer composition which could be linked to varying viscoelastic characteristics of the EPS mucilage (Molino *et al.*, 2006). Thus, a potential influence on the biofilm matrix structure and stability can be assumed. Parallel to this observation, species-specific differences in the composition of produced EPS were also reported for a series of model bacteria such as *Pseudomonas aeruginosa* or *Bacillus subtilis* (Harimawan & Ting, 2016). Furthermore, these EPS compositions were described to be influenced by the developmental stage of the bacterial colony with the quality of polysaccharides displaying high importance for adhesive strength of the bacterial cells. Interestingly, the interactions of satellite bacteria and diatoms were very recently described to influence the EPS production of the involved diatoms (Windler *et al.*, 2015). As a reaction of satellite bacteria activity, diatoms of the species *Achnanthidium minutissimum* modified their EPS production to form a mucilage capsule and changed from a planktonic state to a lifestyle attached to the substrate. This reaction may have a direct impact on biostabilization because, as described above, a permanent attachment of microbes to sediment grains coupled with high EPS production can constitutes one important trigger for the stabilization of the colonized fine sediment.

1.3.3 The role of extracellular polymeric substances (EPS)

Detailed research into the role of EPS for the overall biofilm system revealed a broad range of different functions associated with these extracellular molecules. Firstly, as stated above, EPS constitute a source of nutrients for a variety of different microbes. In this context, it

could be shown that selected EPS components could be degraded very rapidly and were preferred over other sources of dissolved organic carbon in sediment habitats (Taylor *et al.*, 2013). Subsequently, EPS and EPS metabolites were reported to be incorporated into the cells of bacteria as well as diatoms. A variety of different metabolic pathways exists for this utilization of EPS which depends on the involved microorganism and the characteristics of the EPS (McKew *et al.*, 2013). Secondly, different EPS can be produced as a part of a reaction against environmental stressors. As an example, diatoms were described to produce a range of extracellular enzymes such as glucanases or proteases to impede the growth of bacteria that constitute competitors for nutrients (Buhmann *et al.*, 2016). Moreover, Verneuil et al. (2015) reported increased EPS protein production by diatoms that were treated with carbon nano tubes (CNT). The produced extracellular proteins could be shown to adhere to these stressors and a passage into the cell was prevented. While these two functions of EPS as nutrient source or protection exhibit a direct impact on biofilm development by engineering an optimal habitat and improved growth conditions for the present microbes, the effect on resulting biostabilization is rather vague.

However, specific EPS are also able to function as important structural elements and adhesives. As an example, the characteristics of cell adhesion molecules of the model diatom *Phaeodactylum tricornutum* have been investigated in a combined bioinformatics and molecular physiological study (Willis *et al.*, 2014) and a variety of adhesive proteins with high attachment capacity could be identified. In addition, a recent study (Arenas & Tommassen, 2017) could characterize a range of DNA/heparin-binding proteins that apparently are widely distributed among different microorganisms and that were stated to play an important role during the formation and for the structure of biofilms.

1.3.4 EPS produced by bacteria and diatoms

However, to understand the impact of EPS on the structure and stability of biofilms, it is essential to consider important physiological differences between bacteria and diatoms that directly influence the role of EPS for these microbes. As an example, single diatom cells can produce EPS for the purpose of active gliding migration over the sediment grains (Hay, Maitland & Paterson, 1993). In contrast to that, bacteria swim without constant contact to the surface using flagella whereas sessile bacteria produce EPS for stable attachment. In addition, the EPS matrix facilitates passive spreading e.g. by osmotic forces that allow the spread of the bacterial biofilm over a substrate surface to un-colonized areas (Seminara *et*

al., 2012). Although a single bacterial cell cannot actively control the direction of this osmotic biofilm distribution, EPS production by immotile bacterial cells may constitute a survival strategy that can exploit new sources of nutrients. This assumption was supported by Zhang *et al.* (2014) who described starting carbon starvation as trigger for the production of a thick EPS matrix in *Bacillus subtilis*.

Moreover, several studies investigated the role of the extracellular matrix for the adhesion and stability of bacterial biofilms: As an example, besides hydrophobicity and surface charge of the individual bacterial cell, the quantity of secreted EPS was stated to be a driving factor for the adhesion in *Pseudomonas aeruginosa* biofilms (Harimawan, Rajasekar & Ting, 2011). Volle *et al.* (2008) could correlate the adhesiveness and elasticity of bacterial biofilms to the composition of produced EPS and indicate the significance of long extracellular lipopolysaccharides for biofilm stability. Besides extracellular carbohydrates, EPS proteins could also play an essential role in the process of microbial biostabilization. In this context, extracellular proteins do not only constitute extracellular enzymes with a significant fraction of the metabolic activity in matured natural biofilms (Romani *et al.*, 2008) that promote the development and growth of the biofilms to influence biostabilization in an indirect way. Instead, bacteria can secrete specific adhesive proteins such as the well-studied Photorhabdus adhesion modification protein (PAM) that can per se serve as an adhesive and modify extracellular polysaccharide to form a robust matrix (Jones *et al.*, 2010). In addition, during the formation and maturation of biofilms, extracellular DNA (eDNA) can play an essential regulatory role (Mann *et al.*, 2009; Jermy, 2010; Sahu *et al.*, 2012). Parallel to extracellular proteins, extracellular DNA could also be of structural importance as indicated by Wang *et al.* (2015). When specific exopolysaccharides such as Psl (polysaccharide synthesis locus) integrate into eDNA, long rigid fibers can be formed that can function as structural element to increase the stability of bacterial biofilms.

This short highlighted summary indicates that fundamental molecular biology research into the formation and stability of bacterial biofilms was focused on studies of model biofilms of medical relevant bacteria such as *Pseudomonas aeruginosa.* Fundamental principles may be transferred to more complex, natural systems although the validity of results for the process of microbial biostabilization in benthic habitats may be limited.

In contrast to that, the influence of natural environmental factors for the production of algal EPS and the correlated consequences for the stabilization of fine sediments were intensively

studied in intertidal mudflats. One reason might be that diatom EPS production was reported to be significantly higher than bacterial EPS production and could clearly be correlated to sediment stability (Lundkvist *et al.*, 2007). Furthermore, the EPS produced by diatoms could be shown to significantly impact the community composition of heterotrophic bacteria in intertidal habitats (Bohorquez *et al.*, 2017). In addition, high resolution imaging techniques such as atomic force microscopy (AFM) approaches described by Pletikapic *et al.* (2011) could illustrate the production and release of EPS by diatoms indicating the expanded effect of these polymer strands and networks on the biofilm matrix, structure and stability (see ***Figure 4***).

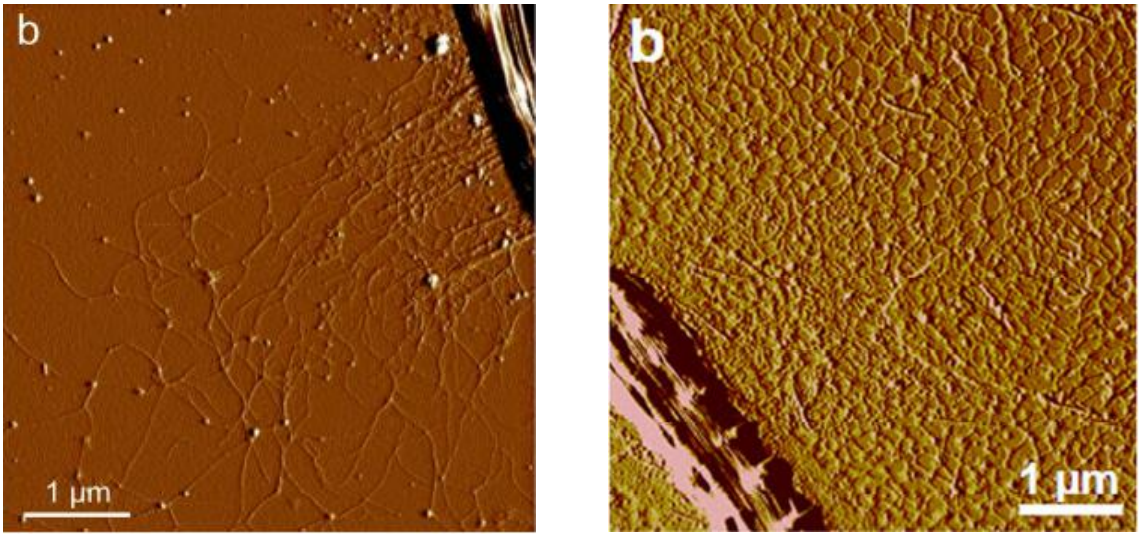


Figure 4: *AFM images of produced EPS network of the diatom Cylindrotheca closterium (Pletikapic et al., 2011);* ***left:*** *freshly produced polymers attached to the apex of the cell rostrum;* ***right:*** *self-aggregated EPS network around one cell of Cylindrotheca closterium.*

Thus, the reaction of diatoms on different environmental conditions such as nutrient availability, temperature and light intensity were studied in various habitats (Underwood, 2002; Apoya-Horton *et al.*, 2006). Further studies indicated that the EPS production and the stabilizing effect on fine sediment was significantly impacted by ultraviolet irradiation (Waring, Baker & Underwood, 2007) and seasonal changes in abiotic parameters e.g. in hydrodynamics, emersion time or pore water content (Pierre *et al.*, 2014; Passarelli *et al.*, 2015). Moreover, the EPS production could be correlated to the migration of diatoms as direct reaction to light conditions (Smith & Underwood, 1998; Perkins *et al.*, 2001). Furthermore, a high structural diversity in the intertidal mudflat habitat was suggested to significantly enhance the production of EPS and sediment stability (Passarelli *et al.*, 2012).

Summarizing, as benthic diatoms are supposed to dominate autotrophic primary production and correlated secretion of EPS during the process of microbial biostabilization, these microbes can be seen as essential functional key players in the biofilm system. A detailed investigation of these microbes is fundamental in order to understand their complex role for the stability of the biofilm matrix. Thus, this thesis aims to assess the impact of parameters that influence the metabolic productivity of the diatom community in the biofilm system. In addition, the composition of the diatom community is of major interest of this thesis because, as described above, species specific differences in produced EPS exist with potential implications for the stability of the biofilm matrix.

The first selective factor analyzed in this thesis is the seasonally changing natural microbial biocoenosis as it appears plausible that distinct successional stages exhibit different stabilization potentials. Possible reasons are significant differences between early and late successional stages concerning characteristics such as morphology, metabolic adaptability or migration behavior. In addition, the reaction of the diatom community to different environmental parameters may constitute a decisive factor for the spatial and temporal biofilm development and resulting stabilizing capability. Thus, another focus of this thesis was the assessment of the impact of light intensity upon the development of the biofilm. The investigation of biofilm formation and biostabilization under different intensities of ambient light was chosen as an experimental approach to manipulate photosynthesis driven EPS production without the necessity of the utilization of herbicides whose degradation products might also affect non-target organisms. If microbial biostabilization is in fact predominantly driven by diatoms and/ or their produced EPS, it can be assumed that the stabilizing capacity of the biofilms should increase with light intensity.

1.3.5 The influence of EPS quality

However, as addressed above, the quality of EPS might be even more important than EPS quantity as parallel to the various configurations of EPS proteins, different EPS carbohydrates may have very distinct chemical characteristics (such as three-dimensional structure and molecular binding sites that can significantly influence their stability). Generally, small monosaccharides that can be rapidly synthetized via diatom photosynthesis (Hay, Maitland & Paterson, 1993) can be transported into microbial cells very fast to be degraded very easily by a variety of different organisms (McKew *et al.*, 2013; Taylor *et al.*, 2013). Thus these small monosaccharides may only be of a minor structural

importance and are predominantly released during the lysis of dead microbial cells or as part of quorum sensing (Tseng *et al.*, 2016). In contrast to that, long secreted polysaccharides may play a decisive role as structural elements as their degradation requires specific extracellular enzymes to break these complex macromolecules into smaller oligo- or monosaccharide subunits. Furthermore, the suitability of exopolysaccharides as adhesive was demonstrated to be dependent on specific chemical features e.g. the amount of free amino or free aldehyde groups that can form covalent bindings with free amino groups of the substrate surface (Hoffmann *et al.*, 2009). In this context, the molecular composition of the exopolysaccharides may constitute a decisive factor: while homopolysaccharides that consist of only one type of sugar monomer (such as cellulose or dextran) are predominantly neutral, heteropolysaccharides (such as alginate or xanthan) with a variety of different monomers can be polycationic (Flemming *et al.*, 2000a) and possess these reactive free amino groups required for adhesion. This may be one explanation for the observations in different studies (Nichols *et al.*, 2009; Roy, Sun & Ji, 2014) demonstrating that different species of diatoms possess the capability to produce exopolysaccharides with very high gluing effect (with comparable or even higher gluing capacity than commercial/ industrial adhesives). Furthermore, there is evidence that the three-dimensional structure of these macromolecules may play an important role during the formation of the extracellular network. Flemming and Wingender (2001) suggested that unbranched polysaccharides such as alginate may contribute to the overall stability of a matured biofilm system. However, the degree of branching of the extracellular polymers may be of major importance for the formation of a complex extracellular three-dimensional network and the resulting physical biofilm stability as suggested by Pennisi (2002) and Wotton (2004).

Further evidence for the importance of EPS quality for microbial biostabilization can be found in the study of Spears et al. (2008) that described increasing sediment stability with salinity although microbial biomass and EPS concentrations were decreasing. These observations may have contributed to the focus of research into microbial biostabilization in brackish and marine habitats. In addition, these results support the current doctrine of the major ecological relevance of marine biostabilization due to the presence of ions in seawater that enable strong ionic bounds within the EPS as well as between EPS and the surface of fine sediments. The concentrations of different ions in the biofilm habitat were also described to have important regulatory effects on the microbial polysaccharide and protein synthesis (He *et al.*, 2016) and an effect on the EPS production was suggested. These observations may explain why rather few studies exist that address biostabilization in freshwater habitats. This

doctrine and concentration on marine and brackish habitats raise the question about the relevance of microbial biostabilization in freshwater habitats.

Thus, a central focus of this thesis was to investigate microbial biostabilization and the correlated ecological relevance of this process in a riverine system. On one hand, up to now no comprehensive knowledge about the stabilization capacity of riverine biofilms on fine sediments exists. The absence of high ion concentrations in the water column that results in a lack of strong ionic bounds in the biofilm matrix might limit the maximal magnitude of stabilization of riverine benthic microbes to an ecological insignificant level in contrast to the significant effects described in intertidal mudflat habitats. On the other hand, there are clear differences in environmental conditions between the well-studied intertidal and lotic habitats. These differences in selective factors for the biofilm microbes may be assumed to result in distinct adaptations of the microbial communities that shape the biofilm structure in different ways. As an example, mudflat microorganisms live in a rather stable habitat and have to adapt to the rhythmic tidal cycle and changing water levels as well as light intensities. In contrast to this, benthic microbes in riverine systems are affected by continuously changing boundary conditions such as the seasonal shifting in light, temperature and predation pressure. In addition, they are confronted with rather short term changes such as weather and precipitation conditions in the catchment area that influence the discharge of the river. As a result, these two very different habitats may not be absolutely comparable and knowledge gathered of the investigation of intertidal mudflat may not be transferable to lotic habitats in an unlimited way.

In addition, the stabilization potential of biofilms on fine riverine sediments is of great ecological and economic interests due to the high amounts of legacies of heavily contaminated sediments in great lowland rivers in Europe (e.g. the river Elbe or Weichsel). As the quantitative determination of EPS carbohydrate and protein contents constitutes a straight forward and cost efficient analytic tool, this thesis aims to evaluate if this investigation might be sufficient as a reliable proxy for biofilm activity and related stabilization capacity. Depending on the degree of correlation between EPS contents and microbial biostabilization, this determination might supplement in situ measurements of the erosion resistance of riverine fine sediments. Parallel to the determination of microbial biomass that is receiving increasing recognition during current assessments of the stability of riverine sediments, the assessment of EPS compounds may provide valuable additional information for a more comprehensive picture of the state of the investigated river region.

1.3.6 Lotic systems

While the majority of studies into microbial biostabilization focused on intertidal mudflat habitats, some investigations addressed the structure and development of lotic biofilms which constitutes one focus of the presented thesis as stated above. Although these studies may not have assessed the overall biofilm/ sediment stability, they provide an essential source of information about some important fundamental principles during the maturation of natural lotic biofilm communities.

Parallel to the observations in mudflat habitats, diatoms were described as important shaping factors for lotic biofilms (Besemer *et al.*, 2007) due to their high EPS production and metabolism turnover to directly influence the physio-chemical environment of the biofilm matrix and impact the development of the bacterial community composition. Moreover, besides the community composition of the biofilm bacteria, overall algal and bacterial biomass could be clearly demonstrated to be influenced by the hydrodynamic regime (Singer *et al.*, 2006). Subsequent studies were able to elucidate more detailed, essential information about the correlation between flow regime, biofilm structure and microbial community composition and indicated a very complex feedback loop of mutual impact between the biofilm system and the abiotic environment: Singer *et al.* (2010) demonstrated how the hydrodynamic regime and especially heterogeneities in flow characteristics such as near bed flow velocity impact the metabolic activity and the nutrient uptake of natural biofilms which could be correlated to a direct effect onto the diversity of the microbial community. As a reaction the hydrodynamic regime, different structural compartments of the biofilm develop such as long filaments that oscillate in the water column called “streamers” (Stoodley *et al.*, 2005) or dense biofilm mats with low profile. Interestingly, this structural differentiation that in turn affects the local small scale flow regime appears to be driven by the spatial development of the microbial community composition, as distinct structural elements were described to be dominated by significantly different assemblages of benthic bacteria (Besemer *et al.*, 2009).

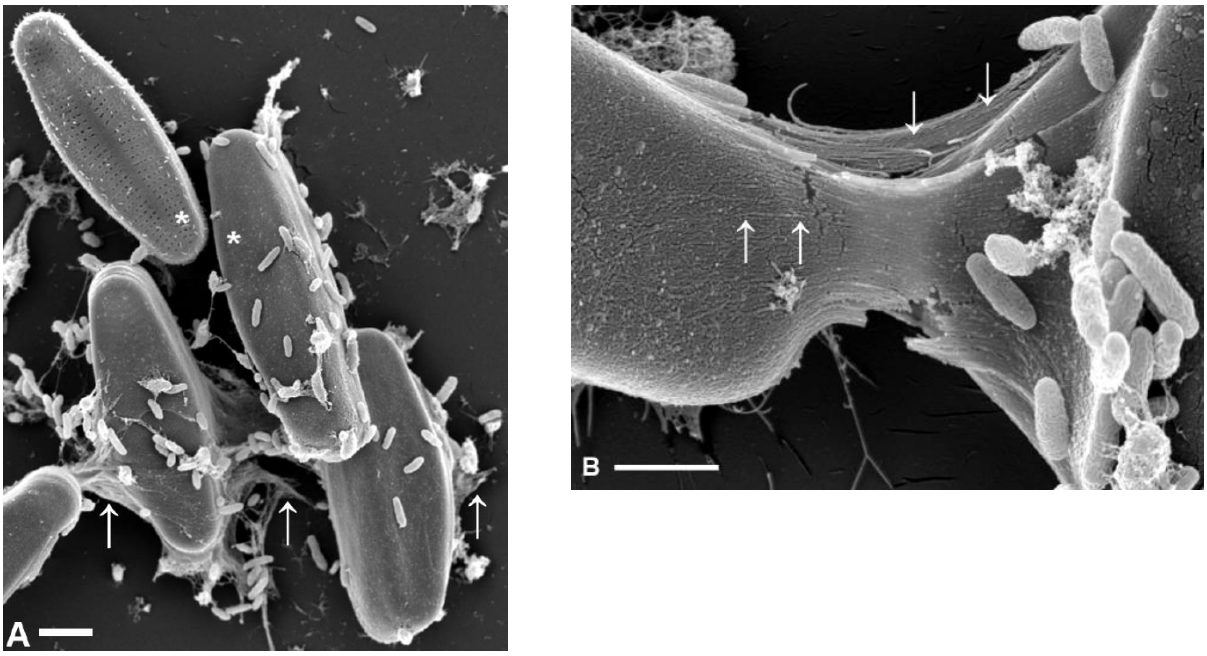


Figure 5: *ESEM image of the freshwater diatom Achnanthidium minutissimum and the secreted EPS capsule (Leinweber & Kroth, 2015);* ***left:*** *the EPS capsule is required for bacterial attachment and adhesion to the substrate (arrows indicate EPS attachment stalks); scale bar: 2 µm;* ***right:*** *detail image of produced EPS stalks required for attachment to the substratum; theses stalks exhibit high mechanical stability and longitudinal strength due to their composition of single parallel EPS fibrils (arrows); scale bar: 1 µm.*

Furthermore, the complex symbiotic interactions between lotic diatoms and different bacterial species significantly impact the development of the matrix of biofilms. As described above, diatoms of the species *Achnanthidium minutissimum* are able to modify their EPS production to form a mucilage capsule which induces the change to a lifestyle attached to the substrate as a reaction of satellite bacteria activity (Windler *et al.*, 2015). In turn, this formation of an EPS capsule was shown to be essential for bacterial attachment as well as diatom adhesion to the underling substrate as illustrated in ***Figure 5*** (Leinweber & Kroth, 2015).

These findings suggest two important aspects that are currently hardly addressed and therefore were studied in this thesis in order to gain a comprehensive understanding of the involved fundamental principles. Firstly, in contrast to other benthic habitats, the hydrodynamic regime appears to be of major driving factor for the composition of the biofilm community in lotic habitats as this direct physical force shapes the temporal, spatial and structural development of the whole biofilm system. This influence of flow velocity and bed shear stress was up to now very difficult to study as this aspect has very demanding

requirements for experimental design. The studies mentioned above addressed parts of this very complex subject and could indicate a differentiated reaction of the biofilm structure, the metabolic activity and the microbial community composition. However, these investigations did not include a determination of biofilm stability or microbial biostabilization which is of major interest in this thesis. One hypothesis evaluated in this thesis is that high flow velocity reduces the vertical expansion of biofilms into the running wave due to shearing off of exposed structures. As a result, compact biofilms with the orientation towards the sediment surface are developed with a potential high physical and biochemical interaction between sediment and biofilm matrix (e.g. due to EPS production) which may lead to higher stabilizing effects than under minimal flow velocity.

Secondly, while results of these studies indicate the role of distinct structural key players within the microbial community, no correlation could be established between the activity of certain dominant microbial species and resulting overall biofilm stability. It appears possible that the process of microbial biostabilization is dominated by diatoms but as different species exhibit distinct morphologies and lifestyles, their individual contribution to this process might vary. The same may be true concerning bacterial species and their complex interaction with the diatoms. However, it is also thinkable that the microbial community possesses such a high degree of functional redundancy that no effect of specific key player may became visible. This is the reason why this thesis aims to investigate the microbial community and its ecology in high detail in order to identify dominant diatom and bacterial species and unravel their impact on the overall biofilm stabilization potential.

1.4 Focus of this thesis

To summarize, up to now the development of different biofilms was investigated in three very specialized research approaches: the first field of research is the assessment of fundamental principles during the formation and maturation of bacterial biofilms focusing on molecular biology e.g. genomics, transcriptomics and/or proteomics. The second target of investigation is the process of microbial biostabilization of fine sediments concentrating on intertidal mudflats and the role of diatoms, their produced EPS and their adaption to different environmental conditions. The third field of study analyses the correlations between the maturing microbial community, flow of substances and structural elements as a result of different boundary conditions in lotic biofilms. While each of these individual disciplines were

able to unravel important pieces of information, there is still a lack of a comprehensive understanding concerning microbial biostabilization in riverine systems.

This current lack of knowledge constitutes the starting point of this thesis. The most important aim of this thesis was to elucidate the relevance of microbial stabilization of fine sediments in lotic systems. As stated above, the lack of high ion concentrations in the running water may lead to very low stabilization compared to intertidal habitats. However, the microbial community of the riverine benthos may traverse a very different adaption process than intertidal assemblages. As a result, the stability of the biofilm matrix and its corresponding stabilization capacity of fine sediments may be influenced in up to now unknown ways. Especially the microbial ecology appears to be a decisive influencing factor for the temporal and structural development of the biofilm system. Thus, this thesis aims to assess the community composition of the biofilm in high detail and to relate the state of the microbial community to the overall biofilm stability. In this context, the community composition of both, the apparently structural very significant diatoms as well as the metabolic very versatile bacteria was analyzed. This approach allows conclusions about potential interaction between these two different taxa to impact the biofilm habitat. Furthermore, by identifying dominant species among bacteria as well as diatoms, the relevance of possible functional key players can be contrasted with potential functional redundancies. This investigation constitutes unique fundamental research in the principles of riverine microbial biostabilization with a special focus on microbial ecology. The overall aim is to do the first steps in order to understand the importance of specific microbial species in this very complex process. Regarding a potential application of the gathered knowledge in future sediment management concepts, parameters are required that are more precise and easier to interpret than complex ecological characteristics. This is the reason why this thesis additionally aims to evaluate the significance of parameters for microbial biostabilization that are accessible via straight forward analytic approaches and with high cost and time-effectiveness. A detailed assessment of bacterial as well as algal biomass and the major two EPS compounds (carbohydrates and proteins) was performed in order to elucidate their suitability as proxies for biofilm stability and corresponding microbial biostabilization.

Furthermore, it may be assumed that parallel to the observations in intertidal mudflats, different boundary conditions might exhibit an important impact on the structural and functional development of the biofilms in a riverine system. As described in section 1.3.6,

some knowledge could be gained regarding lotic systems. However, studies on the development of riverine biofilms on fine sediments are currently relatively rare and there is still a significant lack of investigations that include microbial biostabilization in this habitat. Thus, this thesis aims to perform a comprehensive assessment of the impact of three major boundary conditions on the stabilization capacity of the biofilm system. Concerning abiotic environmental parameters, especially two abiotic boundary conditions appear to be of high importance. Firstly, light intensity shapes the autotrophic microbial community and influences its metabolic productivity with direct consequences for EPS production. Hence, light intensity possesses an essential relevance for the collocation and spatial organization of autotrophic microbes and the biochemical characteristics of the biofilm extracellular matrix. As a result, the physio-chemical features of the EPS and their potential gluing effect on the surrounding sediment grains may be found to be significantly influenced by the intensity of light available for autotrophic primary production. Secondly, different levels of bed shear stress can determine the degree and mode of microbial attachment to the underling substrate. During the formation of a biofilm, this physical forcing may constitute a major driving factor that shapes the spatial and structural arrangement of biofilm compartments as well as their three-dimensional appearance which in turn may be able to have a significant impact on the functionality and stability of the biofilm system and the underling fine sediment.

However, it is important to note that especially a microbial community in a riverine biofilm is not only subjected to direct abiotic conditions such as light intensity or flow velocity. In lotic systems, the benthic-pelagic feedback loop can be very pronounced e.g. due to the continuous water movement in combination with comparably low water depths in littoral zones. In this context, benthic biofilms constantly undergo a process of detachment of biofilm compartments as well as settling down and attachment of suspended particles and microbes. In addition, as the whole river can be seen as one great continuously changing ecosystem described in the river continuum concept by Barmuta and Lake (1982), these suspended particles can be transported to downstream river section to be deposited there and to influence local benthic communities. Furthermore, the whole biocoenosis of a river ecosystem is influenced significantly by seasonal changes (e.g. shading due to littoral vegetation, water temperature or entrainment of leafs). As a result, the microbial community in the running wave as well as benthic biofilms are subjected to a seasonal succession process. Although this factor may have a huge impact on the development of biofilms, its relevance in regards of microbial biostabilization of riverine fine sediments is up to now not

addressed. Thus, this thesis aims to elucidate the significance of the seasonal succession process in the microbial community for the corresponding stabilization capacity of the biofilm. This investigation is closely related to the aforementioned investigation of potential functional microbial key players.

1.5 Analytic approach

The overall motivation of this work was to gain deep insight into the fundamental principles of biostabilization in nascent and developing riverine biofilms. The central focus was the investigation of the biofilm ecology and how the microbial community influences the stability of the overall biofilm system under special consideration of allochton as well as autochton driving factors. However, as this subject is very complex, the main goal of a comprehensive understanding of the fundamental principles of this essential ecosystem function could only be achieved by dividing this topic in three smaller successive subprojects.

The first and crucial step was to assess the overall relevance of biostabilization in lotic systems. This was important as stated above, the current prevalent doctrine stated the importance of ionic bounds for the stabilizing effect of the biofilm that was regarded to favour marine and brackish biostabilization due to much higher ion concentrations in the surrounding water. For this purpose, the experimental setup had to guarantee natural-like boundary conditions which were constant, controllable and thereby reproducible. As natural conditions are constantly changing in a riverine system, the only option was to design and construct a mesocosm setup. Subsequently, the comparability of the biofilm formation and the development of microbial biostabilization under the same boundary conditions had to be thoroughly evaluated to exclude an impact of the experimental setup itself. This reproducible cultivation of biofilms constituted one central prerequisite for a detailed investigation into the temporal development of nascent biofilms and the resulting biostabilization under different boundary conditions.

The second step was to focus the analysis on seasonality, one key influencing factor which could not be controlled in the mesocosm setup. Due to long experiment durations and the requirement of a sufficient number of replicates, a series of experiments had to be performed over the course of two years throughout seasons and hence different vegetation periods. Thus, the inoculum for the biofilm formation in the experiments was subjected to the impact of seasonality as the suspended floating microbial biocoenosis was freshly retrieved with the water from a river. By keeping all boundary conditions constant, the influence of the

seasonal altered microbial communities upon the development of the biofilms and their stabilization capacity on the fine sediment could be investigated. The knowledge about specific biofilm characteristics gathered in these first two subprojects could provide the analytic base for the last and most complex subproject.

During the third subproject, the impact of abiotic boundary conditions could be assessed. Of general importance was the question whether a quantifiable impact of abiotic boundary conditions upon the formation of biofilms and resulting biostabilization could be detected. Furthermore, it was investigated whether extreme conditions might display a similar impact upon the formation of the biofilm and the development of biostabilization. The aim during this phase was to unravel how different biofilm components reacted to the environment, how they interacted, and in which way these interactions influenced the biofilm system and its overall functionality.

1.6 Structure of this thesis

The following chapters of this thesis reflect the aforementioned analytic approach of the performed experiments:

In chapter two, detailed information about all applied techniques is given. One central element is a detailed introduction of the straight flume mesocosm specially designed and constructed to meet the requirements of the planned experiments (Thom *et al.*, 2012b). An overview is given over the various sets of boundary conditions set during the individual experiments as well as the sampling strategy and the analyses of all investigated biofilm parameters. Hence, this section concentrates on a concise description of the different utilized methods used to gather the results presented in the subsequent chapters.

Chapter three focusses on the presentation of the results of the different analyses after a short description of the specific background of each of the included three sub-chapters. The focus of the observation in the first sub-chapter lies on the experimental setup itself. The straight flume mesocosm is subjected to a series of evaluations. There are two most important points in this section. The first point is a thorough evaluation of the suitability of the constructed experimental setup for reproducible biofilm cultivation. An extensive comparison of biofilm formation in various flume sections was performed to determine reproducibility and reliability of gathered results. In addition, the development of the biofilms was further analyzed to address the relevance of microbial biostabilization in a lotic system.

The main question of the second sub-chapter is: does the successional process in the diatom commodity have a significant impact on the stability of the biofilm system? The analytic focus of this section is laid on the diatom community composition due to their functional importance in the biofilm matrix. Thus, the short-term as well as the seasonal succession in the biofilms diatom community is investigated and correlated to different biofilm parameters and the resulting stability of the overall biofilm system. The last sub-chapter concentrates on an assessment of the impact of two important abiotic conditions on microbial biostabilization. For this purpose, the effect of light intensity and bed shear stress upon biofilm development were assessed. Considering the complex interaction between diatoms and bacteria in the biofilm system, this section additionally focuses on a detailed investigation of the bacterial community structure and the relevance of bacterial key players for biofilm stability and microbial biostabilization.

Chapter four follows the structure of chapter three and discusses the gathered results of each corresponding sub-chapter. Consequently, this chapter addresses the development of the biofilm as a reaction to the different boundary conditions. The central question is how the different biofilm components and their interaction influence the stabilizing capacity of the overall biofilm system. In this context, parallel to chapter three, the main focus of this discussion alternately targets the role of EPS, diatoms or bacteria in each sub-section which is summarized by a concise conclusion.

A general conclusion in chapter five summarizes the findings of the two chapters before and addresses the initial hypotheses and aims of this thesis.

This work is concluded by an outlook in chapter six where the relevance of this thesis is discussed in the context of challenges in reservoir and sediment management strategies. In addition, possible starting points for further studies and how the gathered insight of this thesis may be incorporated in sustainable sediment management approaches are suggested.

2. Material and Methods

2.1 Experimental setup

In order to cultivate biofilms under constant natural-like environmental conditions, a mesocosm consisting of six straight flumes, each with an individual, separate water circuit was used (Thom *et al.*, 2012a). The individual flumes (see ***Figure 2***; length x width x height: 3.00 m x 0.15 m x 0.15 m) were designed to allow a homogeneous flow field and constant shear stress across the biofilm cultivation section (length 1.32 m). This section contained 16 substratum cartridges (length x width x height: 0.08 m x 0.06 m x 0.02 m) that could be transferred outside the flume for further measurements.

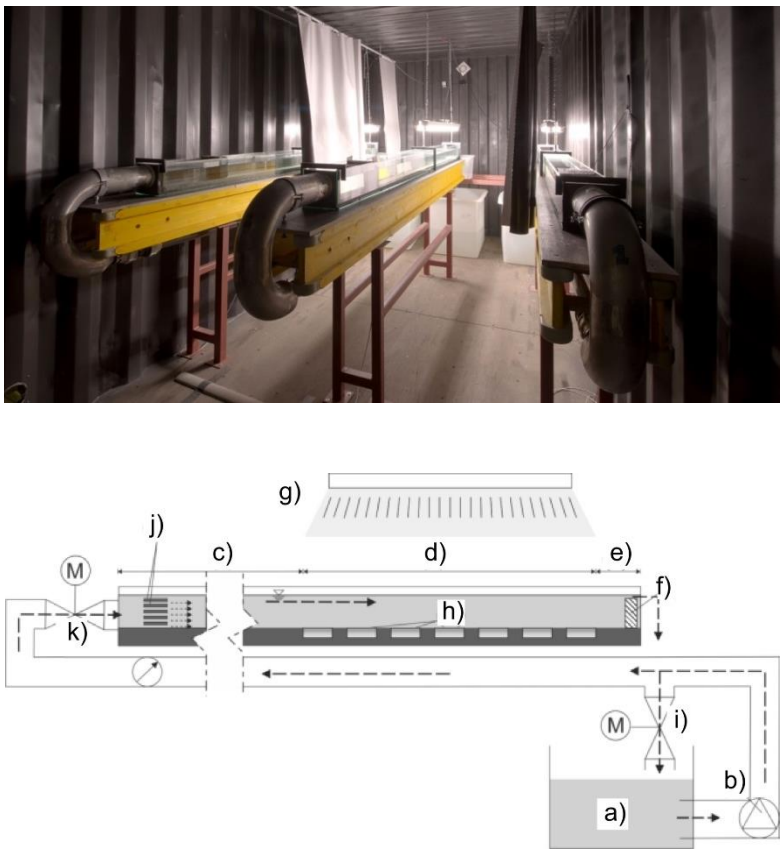


Figure 2: *Experimental setup. top: image of three equivalent straight flumes installed in one containers; bottom: schematic image of one straight flume (a) outflow tank, (b) pump, (c) inlet flow section with baffles, (d) biofilm cultivation section, (e) outlet flow section, (f) weir, (g) fluorescent tubes, (h) sediment cartridges, (i) bypass, (j) current abatement, (k) fine tuning valve (after Thom et al., 2012b).*

Cartridges were illuminated by two parallel fluorescent tubes (Osram Biolux; 480 – 665 nm). The distance of these light sources to the sediment surface was adjustable resulting in

different illumination intensities during an 8 /16 hours day/night cycle for the sediment and biofilm surface. Homogenous irradiation was confirmed by measurements of light intensity and wavelength irradiance of the photosynthetic active radiation (PAR) spectrum using a high resolution spectroradiometer (SR-9910, Macam Photometric Ltd., Livingston, Scotland) as described by Gerbersdorf and Schubert (Gerbersdorf & Schubert, 2011). Discharge and resulting flow velocity and bed shear stress affecting the surface of the sediment and biofilms could be regulated adjusting the by-pass and was continuous measured with an installed mini-flow meter (Bürkert 8030, Ingelfingen, Germany). On the first day of each experiment the inoculum for the biofilm cultivation consisting of fluvial water and swirled up fine river sediment was retrieved from the River Enz (Baden-Württemberg, Germany, 48°56'0.63''N 8°55'3.54''E). While adjusted to constant temperature (15.0 °C ± 0.3 °C) by a cooling water circuit, 200 L were circulated in each flume by a circulatory pump (BADU Eco Touch, Speck Pumpen, Neunkirchen am Sand, Germany) for the duration of the single experiments (overview given in the next section). Thus, indigenous microorganisms within the river water settled the cartridges filled with inert glass beads (diameter 100 - 200µm) eventually forming a biofilm.

2.2 Experiments and sampling

A total of six long term experiments was run (overview given in ***Table 1***). In doing so, the first experiment (during August 2012) was run with identical boundary conditions in all flumes to evaluate the reliability and reproducibility of the experimental setup (see section 3.1). During the experimental runs 2-6 the influence of abiotic boundary conditions - either underwater light intensity (LI) or bed shear stress (BSS) were thoroughly investigated (see section 3.3): While one condition was set at the same level for all flumes, the other was adjusted at three different levels, hereinafter described as "minimal", "medium" and maximal" (see ***Table 2***) always running two flumes with identical abiotic environment which constituted independent replicates. Furthermore, as two flumes were always operated with the same boundary condition, an additional investigation of seasonal aspects was facilitated (see section 3.2).

Table 1: Overview over the single experiments.

Experiment	Duration	Flume	BSS	LI	Sampling days
"August12"	01.08.-28.08.2012	1 2 3 4 5 6	minimum	medium	4, 7, 11, 14, 18, 21, 25, 28
"May"	30.04 – 07.06.2013	1 2 3 4 5 6	maximum medium minimum minimum medium maximum	medium	4, 7, 11, 14, 18, 21, 25, 28, 32, 35
"July"	28.06 – 06.08.2013	1 2 3 4 5 6	minimum	minimum medium maximum minimum medium maximum	4, 7, 11, 14, 18, 21, 25, 28, 32, 35
"August"	23.08 – 30.09.2013	1 2 3 4 5 6	minimum	maximum medium minimum maximum medium minimum	4, 7, 11, 14, 18, 21, 25, 28, 32, 35

"November"	22.11 – 20.12.2013	1	medium	medium	4, 7, 11, 14, 18, 21, 25, 28
		2	minimum		
		3	maximum		
		4	maximum		
		5	minimum		
		6	medium		
"March"	18.03. – 29.04.2014	1	minimum	medium	4, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39
		2		maximum	
		3		minimum	
		4		medium	
		5		maximum	
		6		minimum	

Table 2: Abiotic boundary conditions set in the experiments.

	Light intensity [µmol m^{-2} s^{-1}]	**Bed shear stress [N m^{-2}]**
"Minimum"	0	0.02
"Medium"	50	0.04
"Maximum"	100	0.08

The study (described in section 3.1.) performing detailed inter- and intra-flume comparisons during biofilm cultivation at identical boundary conditions (Schmidt *et al.*, 2015) displayed no significant differences in biofilm features, but showed that the well-known patchiness and heterogeneity of biofilms on the micro scale (Battin *et al.*, 2007) should be addressed. Consequently, at each sampling point half of the sediment surface of one cartridge was sampled withdrawing 15 biofilm samples with a cut-off 2 mL syringe (diameter 0.01m). The gathered material was pooled to obtain representative results and subsamples (0.5 or 1.0 cm^3 resp.) were transferred into Eppendorf tubes for further analyses. In addition, 1L of water was collected for chemical analyses.

2.3 Analyses

2.3.1 Water chemistry, EPS and microbial biomass

The concentration of ammonia and chloride ions in the water samples were analyzed according to DIN 38406-E5-1 and DIN EN ISO 10304, respectively. Nitrate, phosphate, fluoride and sulfate ions were measured spectrophotometrically (Hach Lange GmbH, Berlin, Germany) (detection limits: nitrate 1 mg L^{-1}, phosphate 0.1 mg L^{-1}, fluoride 0.1 mg L^{-1} sulfate 5.8 mg L^{-1}).

After extraction of the colloidal (water-extractable) EPS fractions of the biofilms according to Gerbersdorf et al. (2008), carbohydrates and protein contents were investigated by phenol assay and modified Lowry procedure in triplicates (Dubois *et al.*, 1956; Raunkjaer, Hvitvedjacobsen & Nielsen, 1994; Frolund *et al.*, 1996). The analysis of chlorophyll *a*/ pheophytin was performed in triplicates as given in the DIN 38 412/16.

For the determination of bacterial cell counts (BCC), samples were fixed with 4 % paraformaldehyde (final concentration), shaken horizontally for 1 min and treated (2 pulses @ 3 s at 10 % intensity) with a Sonopuls UW 3100 ultrasonic probe (Bandelin electronic, Berlin, Germany). After sedimentation of the glass beads for 1 min., subsamples of 99 µL were taken from the supernatant and stained with 1 µL SYTO 13 (500 µM) (Life Technologies, Carlsbad, CA, USA) for 15 min. Triplicates of the bacterial cell samples were counted at 488 nm excitation using a flow cytometer (FACScalibur, BD Bio Science, New Jersey, USA) after individual calibration with an undyed subsample of 100 µl for each sample. Results of EPS and biomass analyses where correlated to the dry weight (DW) of the corresponding samples.

2.3.2 Diatom community composition

Diatom samples fixed with Lugol's iodine (2%) were analyzed from each flume from day 11, 18 and 28 - reflecting the development phases of the algal community derived from *chlorophyll a* contents. The organic content of the samples was removed by boiling them in H_2O_2 (30%) followed by three washes with MilliQ water. The diatom frustules were embedded in Naphrax (Northern Biological Supplies, England) for species determination (Battarbee, 1986; Krammer & Lange-Bertalot, 1986-1991; Hofmann, Werum & Lange-Bertalot, 2011) at 1000x magnification using a Zeiss Axioscope (Carl Zeiss, Oberkochen, Germany) with differential interference contrast. 300 valves were identified and the relative

abundance of each taxon was recorded. Data were evaluated by calculation of Bray-Curtis dissimilarity (BCD) (Somerfield, 2008) and Shannon diversity index (Shannon & Weaver, 1963).

2.3.3 Bacterial community composition

Amplification of total DNA

Total DNA was isolated from the sediment samples with the Nucleospin Kit for Soil (Macherey and Nagel, Düren, Germany) according to the manufacturer's manual. Bacterial 16S rRNA genes were amplified via a PCR assay using the universal primers 27f (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 517r (5'-ATT ACC GCG GCT GCT GG-3') (Lane, 1990; Emtiazi *et al.*, 2004). For subsequent DGGE (Denaturing Gradient Gel Electrophoresis) a GC-clamp (5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCG CCC CCG CCC C-3') was attached to the primer 27f. Each PCR reaction (25 µL) amplifying 15 ng DNA consisted of: 16.38 µL sterile PCR water (Merck Millipore, Darmstadt, Germany), 2.5 µL 10x PCR buffer (QIAGEN, Venlo, Netherlands) provided by the enzyme manufacturer, 0.125 µL dNTPs (200 µM), 0.25 µL of each primer (40 µM) and 0.13 µL Taq DNA polymerase (HotStart™ Polymerase, 5 U/µL, QIAGEN, Venlo, Netherlands). Amplification was carried out in a GeneAmp PCR system 9700 (Applied Biosystems, Carlsbad, CA, USA) with the following specifications: 30 s at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 60 s at 72 °C. The final extension lasted 7 min at 72 °C. The PCR products were loaded onto 1% agarose gels in 1x TAE buffer (pH 8.0), stained with GelRed (GeneON, Ludwigshafen, Germany) and studied under UV illumination (600 nm).

DGGE analysis

DGGE was performed as described by Muyzer et al. (1993) using a Bio Rad DCode system (Bio-Rad Laboratories, Hercules, CA, USA): a 1.5 mm thick vertical gel containing 7.5 % (w/v) polyacrylamide (37.5:1 acrylamide: bisacrylamide) with a linear denaturing gradient of urea and formamide (40% - 70%) was loaded with similar sized PCR products. After electrophoresis in a 1x TAE buffer (pH 8.5) for 17 h at 70 V and 56 °C gels stained with GelRed (GeneON, Ludwigshafen, Germany) for 15 min were analyzed under a Lumi-Imager F1 Working Station (Roche Diagnostics, Mannheim, Germany). Images of each gel taken with a CCD camera system (The Imager, Appligene, Illkirch, France) and processed with the software Lumi Analyst 3.1 were the basis for DGGE banding patterns analysis. After

processing with the programs GelCompar II (6.0) and ImageJ (148 a) range-weighted richness (Rr), community dynamics (Dy) and functional organization (Fo) were calculated as described by Marzorati et al. (Marzorati *et al.*, 2008): the Rr value was derived from the number of DGGE bands and the stretch of the gel covered by the fingerprint reflecting the microbial diversity and capacity of the investigated system. While the Dy value reflects the "rates of change" in the microbial community derived from moving window analysis comparing consecutive DGGE bands, the Fo value was defined as the ratio between dominant and resilient microorganisms derived from Pareto–Lorenz evenness curves (Lorenz, 1905).

Clone libraries and sequencing of prominent bacteria

DGGE bands of interest were excised from GelRed stained gels and re-amplified via PCR (see above) using the same DGGE primers without GC clamp. 73 DNA fragments of appropriate length were purified with the Wizard ® Genomic DNA Purification Kit (Promega, Fitchburg, USA). Cloning of the purified DGGE bands was performed using the TOPO TA Cloning® kit (Invitrogen Inc. Carlsbad, CA) with the pCR® 4-TOPO® vector and One Shot Chemically Competent *E. coli* cells following the instructions of the manufacturer. Three to five clones per band were selected and grown overnight in 5 mL LB broth containing 100 µg/mL ampicillin. Plasmid purification and sequencing of individual DGGE inserts was done by GATC Biotech AG (Constance, Germany) using, the primers M13 forward and reverse. Obtained sequences were manually edited using the Chromas Lite software package (Technelysium, South Brisbane, Australia), checked against the BLAST database (NCBI, Bethesda; USA) (see ***Table 17***) and subsequently aligned using the SINA aligner of the ARB software package (v 5.2) and the corresponding SILVA SSU Ref 102 database (Pruesse *et al.*, 2007).

2.3.4 Surface adhesiveness

Measurements of biofilm stabilization capacity were performed using a modified Magnetic Particle Induction (MagPI) system (Larson *et al.*, 2009) including a highly magnetisable µ metal core electromagnet with low remanence. The electromagnetic inductor was positioned vertically in the defined distance of 0.4 cm to the measurement spots on the biofilm surface (in total 36 replicates per sampling point) on which ferromagnetic particles were distributed with a pipette after suspension of the particles in water. The adhesion force of the analyzed surface is equivalent to the strength of the magnetic field required to retrieve the

ferromagnetic particles and can be deduced via calibration of the used electromagnet from the applied electric current (amperage). Thus, the measured amperage can be used as a proxy for the biofilm stability hereafter denoted as “adhesiveness”. The determination of the biofilm adhesiveness described by Larson et al. (2009) used four different thresholds to describe the varying behaviour of the ferromagnetic particles during increasing current. The adhesiveness threshold 3 (T3), shown as the amperage when half of the particles are attracted, is considered as the most objective and reproducible value for biofilm adhesiveness. In addition, an automated MagPI method was set up (Thom *et al.*, 2015b).

2.3.5 Statistical analysis

Statistical analysis was performed using the software Analyze-it225 (1.0.5.0.): firstly, Shapiro-Wilks tests (confidence interval 95%) were applied to check for normal distribution of the data sets. In order to compare the different groups a one-way ANOVA (confidence interval 95%; chi -square approximation; Tukey error protection) was performed if data were normally distributed. Otherwise, a Kruskal-Wallis test (KWT) (chi -square approximation; Bernoulli correction for ties) was conducted. By calculating Spearman's rank correlation coefficients, correlation of different parameters was tested. A Detrended Correspondence Analysis (DCA) for the diatom communities was carried out. It included the abundances of all species from two replicate samples collected on day 11, 18 and 28 of the five experiments, twice during spring and summer and once during autumn, using the program CANOCO (ter Braak, 1987-1992; ter Braak & Smilauer, 1998).

3. Results

3.1 Evaluation of the constructed straight flume mesocosm

3.1.1 Background

Knowledge about biostabilization of fine sediments is steadily increasing and new techniques to measure adhesiveness of biofilms in high resolution have been established (Cuadrado, Carmona & Bournod, 2011; Larson *et al.*, 2009; Lubarsky, 2011). Moreover, the influence of various environmental factors on the formation of biofilms has been investigated; mostly by experimental procedures (e.g. (Chavant *et al.*, 2002; Shrout *et al.*, 2006)). In the laboratory, parameters can be adjusted and reproduced relatively easy while insights into interspecies relations and larger scaled ecosystem developments are limited; especially since most studies concentrate on mono-species biofilm (e.g. (Rogers *et al.*, 1994; Lemon, Higgins & Kolter, 2007)). Other projects focused on field observations that illustrate diverse ecological phenomena (Artigas *et al.*, 2012; McKew *et al.*, 2011). Nevertheless, the environmental conditions are barely controllable and it is difficult to unravel specific links between species abundances, community composition, physiology and ecosystem functionality.

The presented setup maximizes the advantages of laboratory (controlled reproducible settings) and field investigations (natural relevance) while combining engineering and biological expertise. Several studies investigated biofilm formation in microcosms (Battin *et al.*, 2003; Singer *et al.*, 2006; Besemer *et al.*, 2009). Here, a continuous flow system is used consisting of six straight flumes run with natural river water. Detailed information about the construction, the hydraulic regimes and flow velocities of the straight flumes are given in Thom et al. (2012a). The new flow channel system is unique because: 1) the flume dimensions guarantee fully developed turbulence, uniform water flow and constant discharge as important requirements in hydraulic research, 2) the inoculation and development of biofilm from natural water on natural-like substratum minimizes behavioural artefacts of the microorganisms as a response to a more artificial physical environment and 3) microbial growth and development can be linked to biofilm functionality: here biostabilization as one important ecosystem service. Thereby, well-established methods from engineering science meet protocols in microbial and chemical analysis as well as molecular approaches to gain insights into the process of biostabilization with its various complex interactions. However, the main focus of this section is the evaluation of the newly

designed straight flume setup with respect to the following question: is biofilm growth and development within and between the individual flumes comparable under controlled boundary conditions despite the well-known heterogeneity of natural waters? This is an essential prerequisite for further research into the phenomenon biostabilization at different habitats and environmental scenarios in order to reliably relate the manipulated boundary conditions to the observed effects on biofilm functionality. A four-week experiment was conducted in which biofilm growth was evaluated intra-flume and inter-flume wise. The analytical focus of this section was on biochemical and molecular biological parameters of the developing biofilm and on biofilm adhesiveness as a proxy for substratum stability.

3.1.2 Water chemistry

Nutrient concentrations of the water samples were constant over the experimental time and except for nitrate, at the detection limit (according to LAWA (Länderarbeitsgemeinschaft Wasser, 1998)): phosphate < 0.2 $mg*L^{-1}$, ammonium and nitrate approx. 0.04 ± 0.03 and 2.9 ± 0.1 $mg*L^{-1}$, respectively and sulphate with 48.1 ± 0.4 $mg*L^{-1}$. Concentrations of fluoride and chloride were below 0.2 and around 58.7 ± 0.7 $mg*L^{-1}$, respectively.

3.1.3 Inter- and intra-flume comparison

Comparison of the data on biochemical analysis, microbial biomass and surface adhesiveness showed no significant difference neither between the different regions within one flume nor between the different flumes (***Table 3-5***).

***Table 3*:** Intra flume comparison: mean values of EPS (carbohydrates and protein) and chlorophyll *a* contents (n=144), bacterial cell counts (n=24) and surface adhesiveness (n=162) during the experiment and 6 flumes (with STDev).

Flume region	Carbohydrates ($\mu g*gDW^{-1}$)	Proteins ($\mu g*gDW^{-1}$)	Chlorophyll *a* ($\mu g*gDW^{-1}$)	Bacterial cells (10^7*gDW^{-1})	Biofilm adhesiveness (mA)
Front	27.9 ± 12.5	2.4 ± 1.6	1.3 ± 1.6	1.5 ± 0.8	618 ± 99
Middle	27.3 ± 14.7	2.9 ± 2.2	1.4 ± 1.7	1.6 ± 0.9	603 ± 115
Back	27.0 ± 13.3	2.9 ± 2.1	1.4 ± 1.8	1.7 ± 1.0	599 ± 104

***Table 4*:** Inter flume comparison: mean values of EPS (carbohydrates and protein) and chlorophyll *a* contents (n=120), bacterial cell counts (n=40) and surface adhesiveness (n=135) during the experiment and 5 flumes (with STDev).

Flume	Carbohydrates (µg*gDW^{-1})	Proteins (µg*gDW^{-1})	Chlorophyll *a* (µg*gDW^{-1})	Bacterial cells (10^7*gDW^{-1})	Biofilm adhesiveness (mA)
1	23.1 ± 9.9	2.0 ± 1.7	1.1 ± 1.0	2.2 ± 1.7	630 ± 107
2	26.5 ± 11.2	2.7 ± 1.2	1.0 ± 1.0	1.9 ± 1.7	600 ± 96
3	25.5 ± 8.2	3.1 ± 1.6	1.4 ± 1.5	1.9 ± 1.0	558 ± 91
4	31.0 ± 16.5	2.5 ± 2.0	1.6 ± 2.1	2.0 ± 1.4	623 ± 132
5	32.7 ± 18.0	3.4 ± 3.0	2.1 ± 2.5	1.5 ± 0.9	623 ± 88

***Table 5*:** Results of the Kruskall- Wallis tests: intra-flume and inter-flume comparisons of the measured data.

Comparison	Carbohydrates	Proteins	Chlorophyll *a*	Bacterial cells	Biofilm adhesiveness
Intra- Flume	p=0.8203 (n=144[1])	p=0.5865 (n=144)	p=0.9492 (n=144)	p=0.8540 (n=24[2])	p=0.7670 (n=162[3])
Inter-Flume	p=0.3364 (n=120[4])	p=0.1223 (n=120)	p=0.5432 (n=120)	p=0.9522 (n=40[5])	p=0.0631 (n=135[6])

*[1]: 3 regions * 6 flumes * 8 sampling points; [2]: 3 regions * 1 flume * 8 sampling points; [3]: 3 regions * 6 flumes * 9 sampling points (incl. blanks); [4]: 3 regions * 5 flumes * 8 sampling points; [5]: 1 region (middle) * 5 flumes * 8 sampling points; [6]: 3 regions * 5 flumes * 9 sampling points*

3.1.4 Development of the biofilm during the experiment

EPS matrix

Generally, contents of colloidal EPS carbohydrates and proteins exhibited an overall increase throughout the experiment (***Figure 7***), e.g. mean carbohydrate contents increased circa four-fold from 11.6 ± 3.5 µg gDW^{-1} (day 2) to 42.8 ± 13.4 µg gDW^{-1} (day 26) while mean protein values increased circa five-fold from 1.0 ± 0.8 µg gDW^{-1} to 5.2 ± 3.0 µg gDW^{-1}. However, the increase between two subsequent sampling points was only significant between the days 11 and 14; for EPS carbohydrates (KWT; n=120; p=0.0162) as well as for EPS proteins (KWT; n=120; p<0.0001). Nevertheless, the mean values measured from day 14 onwards were significantly higher than those

determined until day 7 (for both, EPS carbohydrates and proteins: KWT; n=120; p<0.0001). Overall, the contents of EPS carbohydrates and proteins showed a strong positive correlation during the experiment (Spearman; rs=0.70; n=120; p<0.0001).

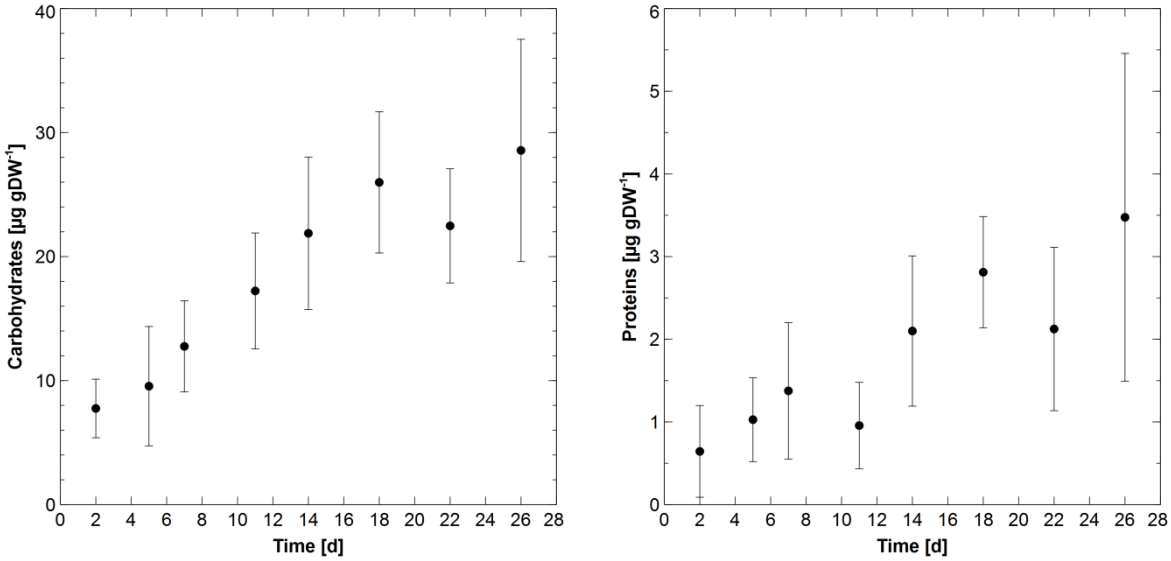


Figure 7: *Development of the colloidal EPS of the biofilms. Left: mean carbohydrate contents; right: mean protein contents (for both n=15; with corresponding STDev).*

Microbial biomass

Chlorophyll *a* contents and bacterial cell counts (BCC) increased during the experiment (***Figure 8***). For instance, mean chlorophyll *a* contents increased from 0.1 ± 0.1 µg gDW^{-1} (day 2) to 3.7 ± 2.1 µg gDW^{-1} (day 26). Meanwhile, mean BCC increased tenfold from 4.6 ± 1.1 $x10^{6}$*gDW^{-1} to 4.3 ± 1.4 $x10^{7}$*gDW^{-1}. Thus, highly significant differences were detected for both parameters between earlier biofilm stages (until day 7) and matured biofilms (from day 22 onwards) (KWT; $p<0.0001$; n= 120 for chlorophyll *a*; n= 40 for BCC).

However, while BCC showed significant increases from day 5 to day 7 and from day 7 to day 11 (KWT; n=40; p=0.0471 and p=0.0074, respectively), the values dropped significantly from day 11 to day 14 (KWT; n=40; p=0.0009). In contrast, chlorophyll *a* content increased significantly from day 11 to day 14 (KWT; n=120; p=0.0210), with an even more pronounced microalgal growth between day 14 to day 18 (KWT; n=120; p=0.0053). Over the total experimental time, BCC and chlorophyll *a* as a proxy for algal biomass were positively related (Spearman; rs=0.69; n=40; $p<0.0001$). Still, between day 11 and 14, the relation was negative although not significant (Spearman; rs=-0.56; n=10; p=0.089). In addition, chlorophyll *a* values were positively related to EPS carbohydrates (Spearman; rs=0.75; n= 120; $p<0.0001$) as well as EPS proteins (Spearman; rs=0.60; n= 120; $p<0.0001$) during the entire experiment.

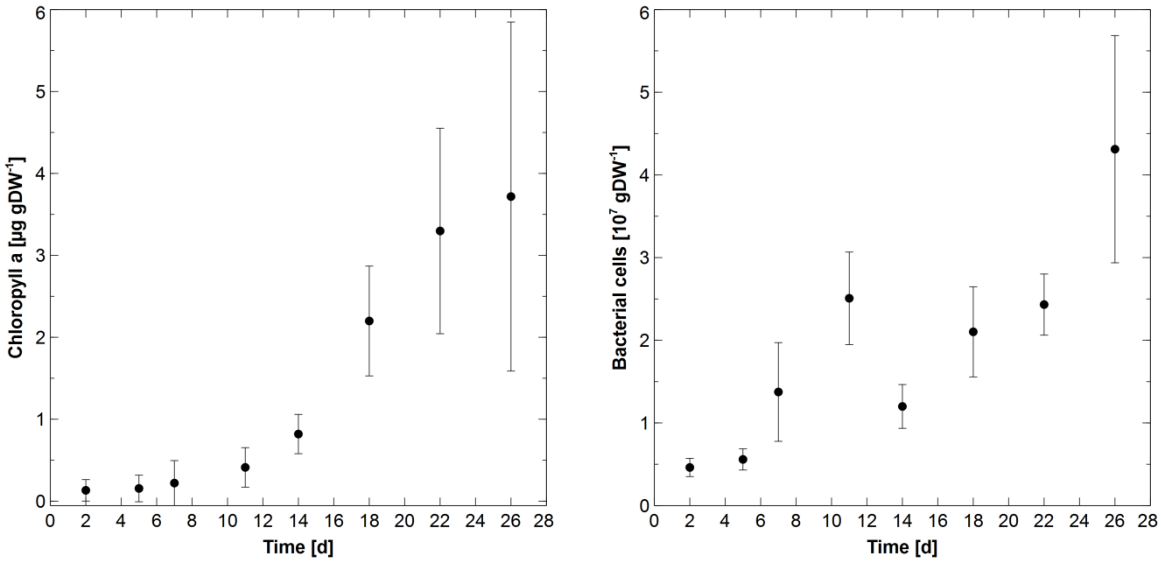


Figure 8: *Development of the microbial biomass in the biofilms. Left: mean chlorophyll a contents (n = 15; with STDev); right: mean bacterial cell counts (n = 5; with STDev).*

Microalgae community

Intra-flume comparisons of the diatom community displayed a high Proportional Similarity Index (PSI) from the beginning onwards that even increased over the experiment: PSI 0.71 (day 7), 0.76 ± 0.09 (day 14) and 0.86 ± 0.03 (day 22). The PSI of the inter-flume comparison was similar (e.g. 0.72 ± 0.05 at day 22). While most algal species were determined sporadically in single flumes (listed in ***Table 6***); three diatoms occurred ubiquitously in all flumes: *Nitzschia fonticola*, *Nitzschia paleacea* and *Surirella brebissonii var. kuetzingii*. Apparently, *Nitzschia paleacea* was the dominant species throughout all flumes while the other two diatoms showed varying abundances in the different flumes (*Nitzschia fonticola* dominant in flume 2 and *Surirella brebissonii* dominant in flume 4). Besides the generally prevailing diatoms, the green algae *Scenedesmus sensu lato* was detected within the biofilms from day 7 onwards.

***Table 6*:** Algae species of the mature biofilms (22nd day): displayed as proportional abundances; species with a relative abundance of less than 2.0% were summarised as "others".

	Flume #				
	1	**2**	**3**	**4**	**5**
Cyclotella menighiana		3.8		3.0	
Fragilaria construens f. venter			3.0		
Myamaea atomus var. permitis			3.6		
Navicula capitatoradiata		3.5			
Navicula reichardtiana		2.0	2.1		
Nitzschia abbreviata		2.6			
Nitzschia acicularis	2.1				
Nitzschia fonticola	11.2	21.9	8.9	4.5	6.1
Nitzschia fonticola - romana form *	2.9	10.5	5.6		3.4
Nitzschia palea var. debilis	6.6				
Nitzschia palea var. palea	4.1	2.0		2.7	
Nitzschia paleacea	48.5	37.0	61.4	54.5	69.2
Stephanodiscus sp.	3.7				
Surirella brebissonii var. kuetzingii	2.9	3.5	3.0	26.9	9.8
others	17.8	13.1	12.5	8.4	11.6

*: (SWF Vol. 2/2, Tafel 75, Nr. 7/8)

Bacterial ecology

The bacterial range weighted richness (Rr) showed no significant inter-flume variations over time (1-way ANOVA; n=20; p=0.9740), but indicated two different stages during biofilm growth. During the first two weeks, the initially high (mean) RR of the biofilms (day 5: 41.4 ± 9.8) decreased to a medium (day 7: 19.6 ± 6.0) and virtually low level (day 14: 10.7 ± 4.5). In contrast, a strong increase could be detected in RR over the last two weeks (day 22: 51.4 ± 14.1). Generally, high dynamics was observed (mean rate of weekly change 31.5 ± 8.0 %), indicating severe changes within the bacterial community. Over time, mean dynamics (rate of change) within the bacterial community increased from day 5 to day 7 (from 25.0 ± 10.7 % to 30.8 ± 13.7 %), followed by a decrease until day 11 (to 25.4 ± 10.5 %, ***Figure 9***). Until day 14, mean dynamics reached a new maximum (42.9 ± 7.5 %) and decreased again subsequently (to 25.0 ± 8.3 % until day 26). The inter-flume comparison of the dynamics development pattern showed no significant differences (1-way ANOVA; n=35; p=0.506).

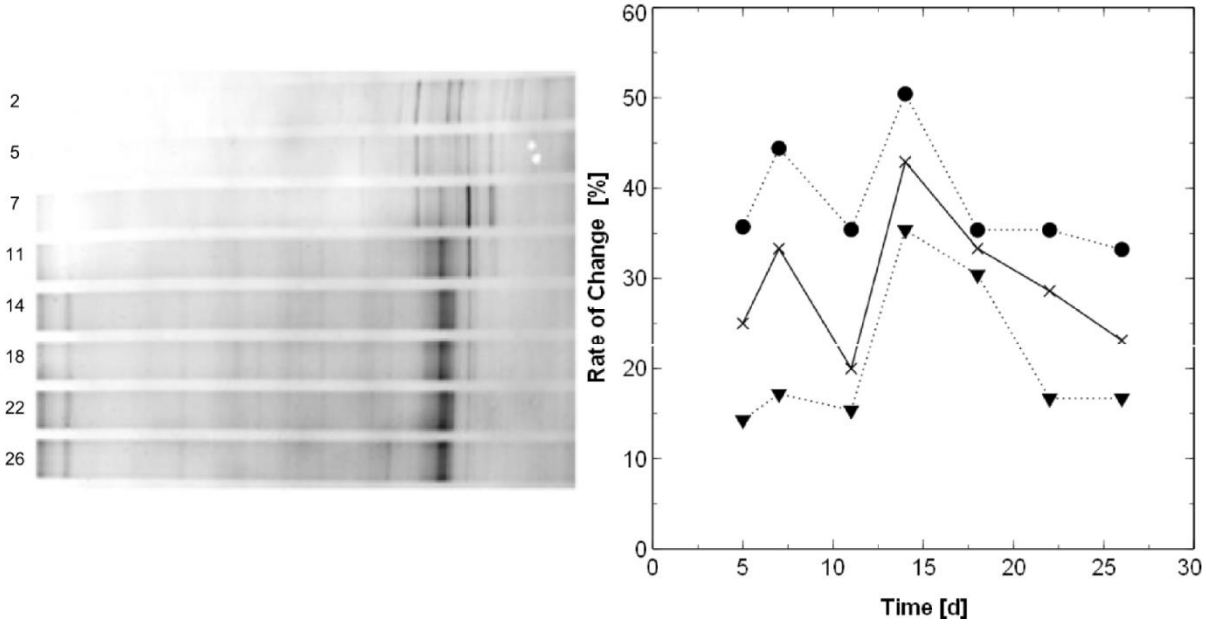


Figure 9*: Dynamics within the bacterial community. Left: DGGE band patterns of 16S rDNA gene diversity of biofilms exemplified shown for flume 1 (stained with ethidium bromide; inverted picture; numbers represent days of growth); right: moving window analysis of the bacterial community's dynamics in the same flume (crosses) based on densitometry similarity matrices. Additionally, shown are the maximal (round dots) and minimal (triangular) change rates over the residual flumes.*

Over the experiment, the functional organization of the bacterial community in the biofilms increased steadily (***Figure 10***) and showed no significant inter-flume difference (1-way

ANOVA; n=20; p=0.3910): on day 5 20.0 % of the bacterial DGGE bands corresponded to a mean proportion of 52.9 ± 7.2 % of the cumulative band abundance indicating a medium degree of functional organization. This value slightly increased until day 7 (59.8 ± 4.7 %) and subsequently reached a high level at 73.8 ± 10.8 % on day 14. Until day 22 and for the rest of the experiment this increase was mitigated, but still detectable (77.2 ± 5.2 % on day 22).

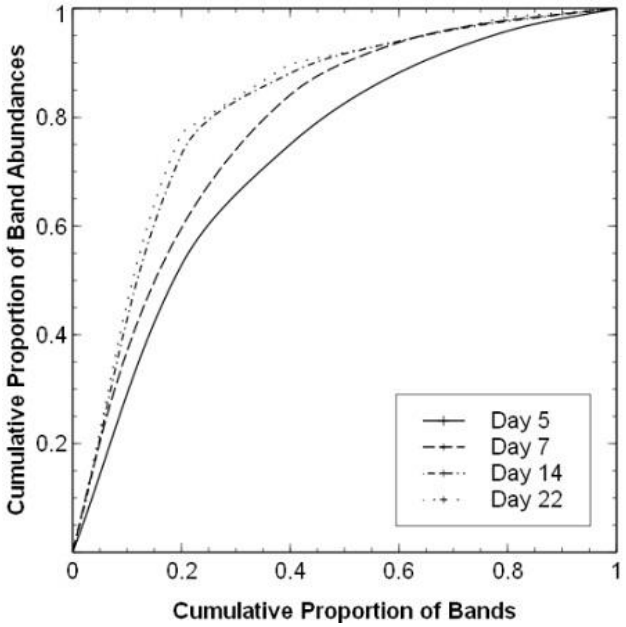


Figure 10: *Functional organization of bacterial community expressed as Parento-Lorenz curves obtained via densitometry and normalization of DGGE peak patterns.*

Biofilm adhesiveness

The developing biofilms established a surface adhesiveness which was up to four times higher than the abiotic sediment (***Figure 11***). This increase from 94.9 ± 44.9 mA at the start of the experiment to 272.3 ± 89.7 mA at day 5 was highly significant (KWT; n=135; p<0.000, days 1 - 2; p=0.0131, days 2 - 5). After a decline between day 5 and 7, new maximum values up to 427.1 mA were measured on day 11, with a mean value of 304.4 ± 118.8 mA. Subsequently, adhesiveness slightly decreased and stagnated at a level of ca. 227.4 ± 89.3 mA for the rest of the experiment.

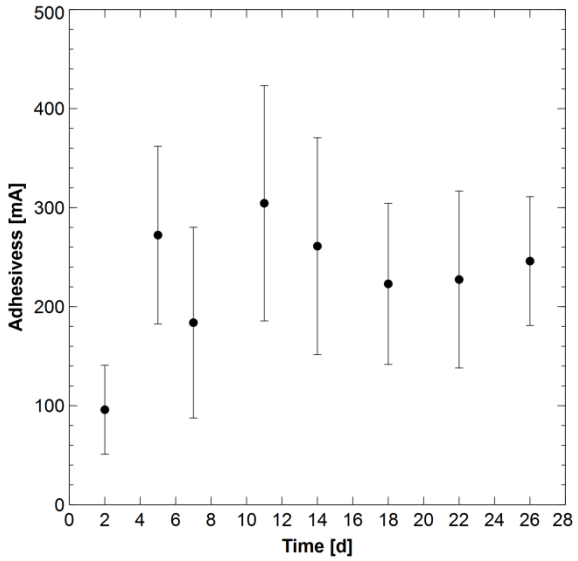


Figure 11: *Temporal development of the adhesiveness of the biofilms displayed as mean determined current [mA] (n = 15; with STDev).*

3.2 The seasonal effect on biofilm development and microbial biostabilization

3.2.1 Background

In lotic waters, the dynamics of fine sediments are significantly influenced by a strong feedback between the benthos and the water column, the ETDC-cycle (Erosion, Transport, Deposition and Consolidation) (e.g. (Paterson & Black, 2000; Noack *et al.*, 2015). As the majority of nutrients binds to the grains of fine sediments (Mortimer, 1941; Sfriso, Pavoni & Marcomini, 1995) this interaction not only affects essential features of sediment particles floating in the free water (Droppo, 2001; Droppo, 2004), but also the primary production of benthic microalgae (Schreiber & Pennock, 1995). Thus, the ETDC-cycle is a driving factor for nutrient cycling within aquatic ecosystems. Especially in shallow reaches, the sediment surface harbors biofilms adapted to different small-scale environmental niches. Various microbes forming and inhabiting these biofilms secrete extracellular polymeric substances (EPS) such as adhesive proteins, which allow diatoms to attach to sediment grains (Dugdale *et al.*, 2005; Willis *et al.*, 2013), or carbohydrate monomers described to be essential for bacterial adhesion (Li *et al.*, 2005). In doing so, sediment grains also can be glued together resulting in a phenomenon called biostabilization, where the resistance of fine sediments towards erosion can be significantly increased (Black *et al.*, 2002; Gerbersdorf, Manz & Paterson, 2008; Lubarsky *et al.*, 2010). Besides providing ecological services e.g. retention of contaminated sediment (Förstner, 2004), biostabilization represents a major scientific challenge in the context of sediment management strategies (e.g. concerning maintenance of reservoirs). The huge economic importance of understanding sediment dynamics is evidenced by the constantly evolving standards and regulations concerning modelling of sediment dynamics and different sediment management strategies (Gölz *et al.*, 2010; Aberle *et al.*, 2013; Kopmann *et al.*, 2014). Though appropriate modelling solutions for the dynamic of sediments of grain sizes down to fine sand exist and are commonly used (Wu, Wang & Jia, 2000; Wang & Wu, 2004), only a few semi-empirical approaches (Righetti & Lucarelli, 2007) have been developed to assess cohesive sediments under the influence of biological activity. New mathematical approaches like the fractal-based one described by Xu et al. (2014) address the various interactions between different sized sediment aggregates. However, due to a significant lack of understanding on how biofilms impact physical features of fine sediments (Grabowski, Droppo & Wharton, 2011), no universal modelling solution or erosion prediction model currently exists for sediments of grain sizes smaller than 63 µm. One possible reason for this absence might be the ever-changing environmental conditions

of riverine systems: Over the course of a year, constantly shifting abiotic environmental parameters such as temperature, flow velocity or underwater light intensity have a significant impact on the biocoenosis in the river resulting in clearly visible succession processes within the benthic biofilm (Garnier, Billen & Coste, 1995; Sekar *et al.*, 2004; Lyautey *et al.*, 2005). While knowledge of this seasonal effect upon benthic and biofilm organisms is steadily increasing (Coma *et al.*, 2000; Olapade & Leff, 2004; Moss *et al.*, 2006) and some implications for biofilm functionality (e.g. on aquatic food webs) have been studied (Yoshioka, Wada & Hayashi, 1994; Power, Parker & Dietrich, 2008), the link to biostabilization of fine sediments is still to be examined.

This section presents the first approach combining biological and engineering science to analyze the fundamental processes shaping the seasonal dynamics in the stabilization capacity of lotic biofilms. While Thom *et al.* (2015a) showed the seasonal shifting erosion resistance of fine lotic sediments, this paper aims to examine the underlying biological processes; the overall objective is to relate the development of different biological and biochemical parameters to the resulting erosion resistance of biofilm-covered fine lotic sediments. For this purpose, the experimental set-up described in section 2.2 was used while the biofilm inoculum was collected during different seasons from a nearby river. As a consequence, besides seasonal changes in water quality, seasonal shifting microbial species composition in the river constitutes the only varying parameter in this experimental design. Furthermore, the importance of the microbial community for biostabilization - the overall biofilm ecosystem function – can be elucidated.

3.2.2 Temporal development of the biofilm EPS matrix and microbial biomass

Generally, mean EPS carbohydrate contents displayed fluctuating values. While increases over time (not significant) were detected in each experiment during spring and summer, autumn biofilms showed approximately stable EPS carbohydrate content. In contrast, a significant increase in EPS protein content (KWT: p= 0.0041; n=45) from 2.9 ± 0.6 µg gDW^{-1} on day two to 27.6 ± 7.4 µg gDW^{-1} on day 28 was detected during spring, but neither summer nor autumn showed a significant rise (***Figure 12***). Biofilm chlorophyll *a* content increased over time in each season; this was highly significant for spring and summer (KWT: spring: p= 0.0010; n=45; summer: p< 0.0001; n=44) and a strong trend was observed in autumn (KWT: p= 0.0488; n=16). So, on day 28 mean chlorophyll *a* content reached 2.3 ± 0.3 µg gDW^{-1} during spring, 2.6 ± 0.7 µg gDW^{-1} during summer and 1.4 ± 0.3 µg gDW^{-1} during autumn. Bacterial cell counts (BCC) also increased significantly during spring and summer (KWT: spring p=0.0233; n=20; summer p=0.0156; n=20) and showed a similar trend during autumn (KWT: p= 0.0611; n=16) (data not shown). This resulted in mean BCC value of 2.3 ± 1.2 x 10^7 gDW^{-1} during spring, 4.3 ± 1.6 x 10^7 gDW^{-1} during summer and 1.8 ± 0.8 x 10^7 gDW^{-1} during autumn on day 28.

3.2.3 Temporal development of biofilm adhesiveness

In accordance with the chlorophyll *a* contents, biofilm adhesiveness showed two distinctly different developmental stages manifesting in a significant difference between early (until day 18) and later biofilm developmental stages (from day 21 onwards; ***Figure 12***). Adhesiveness T3 showed a highly significant increase during spring and summer (KWT: spring: p< 0.0001; n=45; summer: p< 0.0001; n=44) and a similar trend in autumn (KWT: p= 0.0547; n=16). However, maximal mean values were clearly different: in spring mean T3 was 543.3 ± 102.3 mA on day 28, while it was 263.9 ± 49.8 mA in summer and 78.4 ± 18.8 mA in autumn (***Figure 12***).

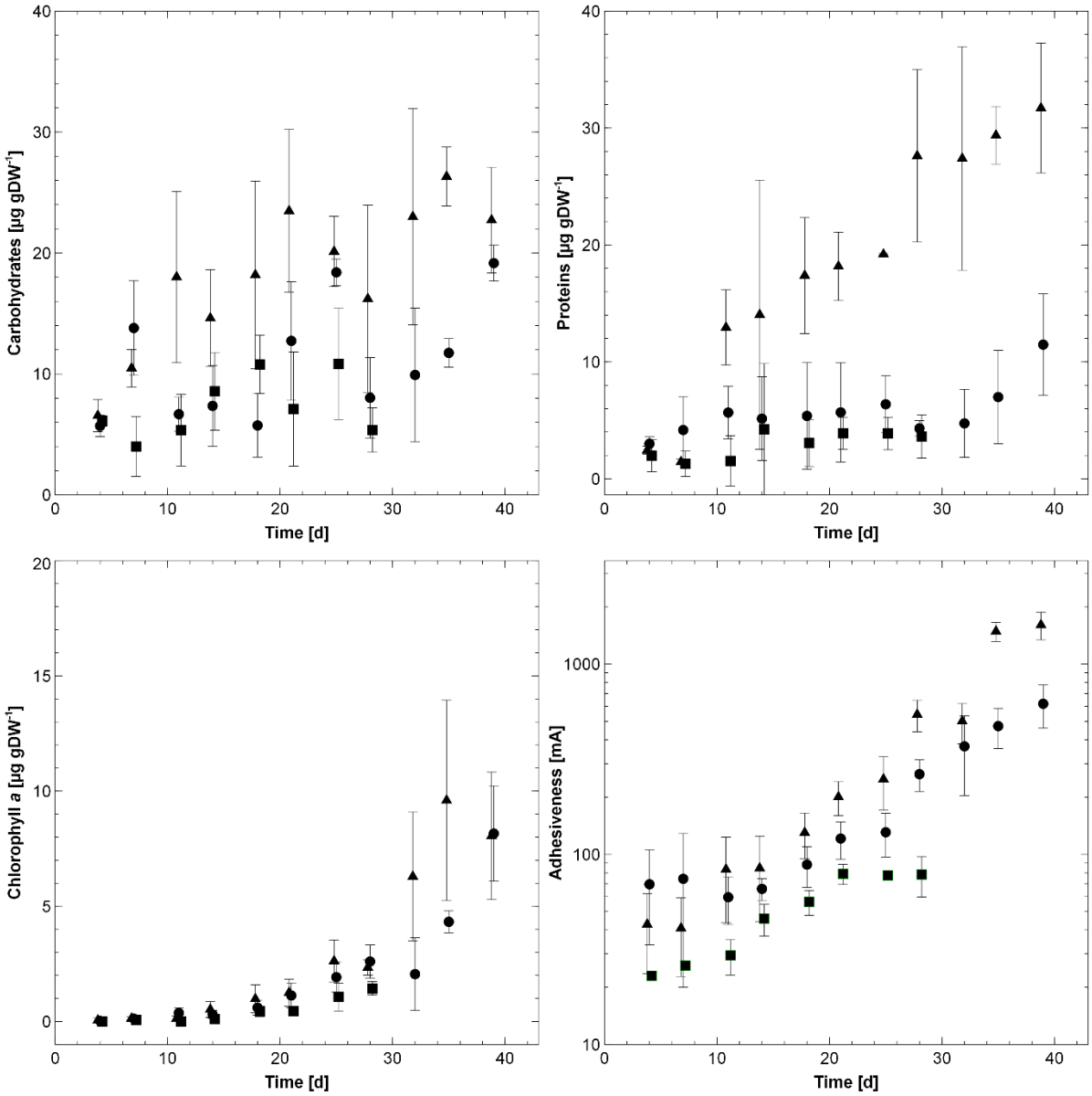


Figure 12: *Temporal development of selected biofilm features (mean values with corresponding STDev): spring (▲) as well as summer (●) n=4; autumn (■) n=2. Upper left: EPS carbohydrate contents - per g dry weight of the sediment (DW); upper right: EPS protein contents; lower left: chlorophyll a contents; lower right: biofilm adhesiveness (T3) – with logarithmic ordinate.*

3.2.4 Comparison of the different seasons

To investigate seasonal variation in biofilm development, results from day one to 28 of all experiments were compared. The overview of the analyzed biofilm parameters in ***Table 7*** reflects the two distinct biofilm developmental stages – early, transparent and bacteria dominated until day 18 and the later greenish, brownish and diatom dominated from day 21 onwards. Although no significant differences were detected in biomass - chlorophyll *a* content and bacterial cell counts (BCC), spring biofilms clearly differed from biofilms of the remaining seasons: Biofilm adhesiveness T3 and EPS contents were significantly higher in spring (KWT; $p=0.0013$; $n=80$ for T3; $p< 0.0001$; $n=64$ for both carbohydrates and proteins). However, diatom diversity on day 28 (mean Shannon index 1.7 ± 0.8 compared to 2.0 ± 0.8 in summer and 2.5 ± 0.3 in autumn) and the dynamics within the bacterial community were lowest during spring (KWT; $p< 0.0001$; $n=68$ for dynamics).

3.2.5 Interactions of different biofilm features

As biostabilization was highest during spring, the investigation of interactions between different biofilm features and biofilm stability focused on this season. EPS protein content, biofilm chlorophyll *a* content and BCC showed high correlations to biofilm adhesiveness T3 (spearman; proteins-T3: $rs=0.73$; $p<0.0001$; $n=45$; chlorophyll *a*-T3: $rs=0.71$; $p<0.0001$; $n=45$; BCC-T3: $rs=0.75$; $p=0.0001$; $n=45$; ***Table 8***). Furthermore, high correlations between EPS protein content and chlorophyll *a* as well as BCC was detected (spearman; proteins-chlorophyll: $rs=0.75$; $p<0.0001$; $n=45$; proteins-BCC: $rs=0.72$; $p=0.0004$; $n=20$). Evidence for the importance of the development within the bacterial community was suggested as its functional organization (Fo) showed high correlations to chlorophyll *a* content and biofilm adhesiveness T3 (spearman; Fo-chlorophyll *a*: $rs=0.82$; $p<0.0001$; $n=42$; Fo-T3: $rs=0.78$; $p<0.0001$; $n=42$; ***Table 8***).

Table 7: Seasonal comparison of biofilm productivity, microbial community and functionality (mean values and corresponding StDEV).

Biofilm parameter		Spring		Summer		Autumn		Difference between seasons
		Early Day 0-18	**Late Day 21-28***	**Early Day 0-18**	**Late Day 21-28***	**Early Day 0-18**	**Late Day 21-28**	
EPS	**Carbohydrates [µg gDW^{-1}]**	15.9 ± 8.1	19.3 ± 5.7	10.2 ± 9.2	11.8 ± 8.4	6.0 ± 2.6	8.5 ± 3.7	S
	Proteins [µg gDW^{-1}]	8.1 ± 8.9	10.2 ± 10.4	4.2 ± 3.1	5.5 ± 3.6	2.3 ± 2.7	3.6 ± 1.3	S
Biomass	**Chlorophyll *a* [µg gDW^{-1}]**	0.2 ± 0.3	2.4 ± 1.5	0.2 ± 0.2	1.9 ± 0.9	0.1 ± 0.1	0.7 ± 0.5	N
	Bacterial cells [*10^7 gDW^{-1}]	1.2 ± 1.8	3.0 ± 2.2	0.9 ± 0.7	4.2 ± 2.4	0.7 ± 0.3	1.5 ± 0.9	N
Bacterial Community	**Range weighted richness**	18.7 ± 3.1	26.2 ± 13.9	13.7 ± 5.2	12.9 ± 8.1	13.9 ± 5.4	27.9 ± 7.7	N
	Functional organization	45.3 ± 7.3	61.1 ± 7.9	50.8 ± 11.1	49.9 ± 10.1	51.2 ± 2.7	39.8 ± 3.8	N
	Dynamics	10.2 ± 6.1	10.8 ± 5.7	13.7 ± 6.0	16.5 ± 10.5	22.0 ± 2.1	34.9 ± 2.2	S
Diatom community	**Shannon Index**	2.9 ± 0.1	1.7 ± 0.8	2.8 ± 0.2	2.0 ± 0.8	3.1 ± 0.1	2.5 ± 0.3	N
	Evenness	0.7 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	N
Biofilm Stability	**Adhesiveness [mA]**	64.2 ± 34.6	294.4 ± 210.8	63.2 ± 33.4	171.8 ± 76.3	31.1 ± 10.3	72.8 ± 13.5	S

: data given in **Figure 12 for spring and summer exceed this observation period until day 28.*

N: no significant difference detectable; S: significant different observed

***Table 8*:** Correlations of biofilm parameters during spring.

		EPS		Biomass		Bacterial community			Biofilm adhesiveness
		Carb	**Prot**	**Chloro**	**BCC**	**Rr**	**Fo**	**Dy**	**T3**
EPS	**Carb**	1							
	Prot	0.55	1						
Biomass	**Chloro**	0.18	**0.75**	1					
	BCC	0.54	**0.72**	0.55	1				
Bacterial community	**Rr**	0.15	-0.42	-0.10	-0.06	1			
	Fo	0.21	**0.70**	**0.82**	0.65	0.08	1		
	Dy	-0.09	0.38	0.24	0.40	-0.48	0.18	1	
Biofilm adhesiveness	**T3**	0.40	**0.73**	**0.71**	**0.75**	0.01	**0.78**	-0.02	1

3.2.6 Microbial community

Bacterial community

For the bacterial community the temporal development of the three ecology indices “range weighted richness” (Rr), “dynamics” (Dy) and “functional organization” (Fo) showed distinct differences during the different seasons. No significant changes were observed in any index for the summer biofilm. The only season with a significantly changing Rr was autumn, which displayed a significant increase over time (KWT; p=0.0430; n=14). Here, a trend towards increasing dynamics (KWT; p=0.0514; n=12) was also detected. Spring was the only season showing a highly significantly growing Fo (KWT; p= 0.0005; n=42) as initial mean Fo approximately doubled until the end of the experiments (from 40.02 ± 5.75 to 79.75 ± 7.07) (see ***Figure 13***). 16S rRNA gene based identification of bacterial key players can be found in section 3.3.2.

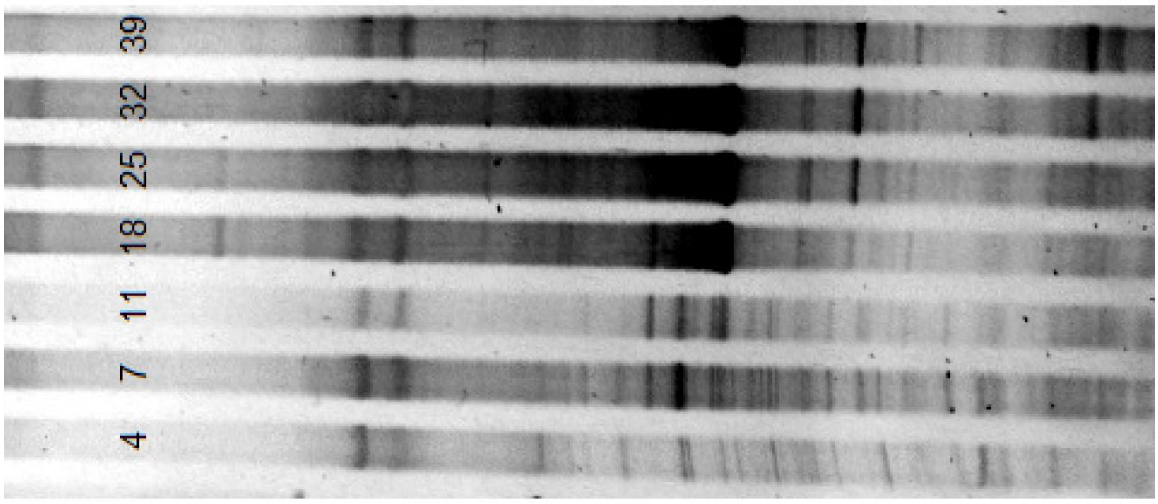


Figure 13*: Temporal development of the bacterial community. Inverted image of DGGE band patterns taken during spring from the experiment “May”. The indicated numbers represent days of growth. The developing specialization of the bacterial community and dominance of single bands became very plain.*

Diatom community

Generally, biofilms were dominated by a variety of different diatoms (see ***Table 9*** in the appendix), while only few green algae such as *Scenedesmus sensu lato* were detected in low abundances. Hence, the analysis is focused on the diatom community: Statistical analysis of the complete data set for Bray-Curtis dissimilarity (BCD) indices of the diatom communities displayed no significant differences between the single flumes of one single experiment or between the two corresponding experiments representing one season (KWT: p=0.934, p=0.3928 resp.; for both n=132). However, significantly increasing BCD indices were detected comparing the diatom communities of day 11 with day 18 and day 28 (KWT: day 11 vs. day 18: p= 0.0054; day 18 vs. day 28 and day 11 vs. day 28: p<0.0001; for all n=132). On day 28, BCD indices obtained from the comparison of the diatom communities of spring and autumn were significantly higher than the ones between summer and autumn or between spring and summer (ANOVA: p=0.0185; n=44) reflecting the annual succession in riverine waters. The DCA including all diatom samples (***Figure 14***) confirms this seasonal succession as it indicated three distinct clusters for the different seasons, where spring and summer are divided along the ordinate, while summer and autumn as well as autumn and spring are separated along the abscissa.

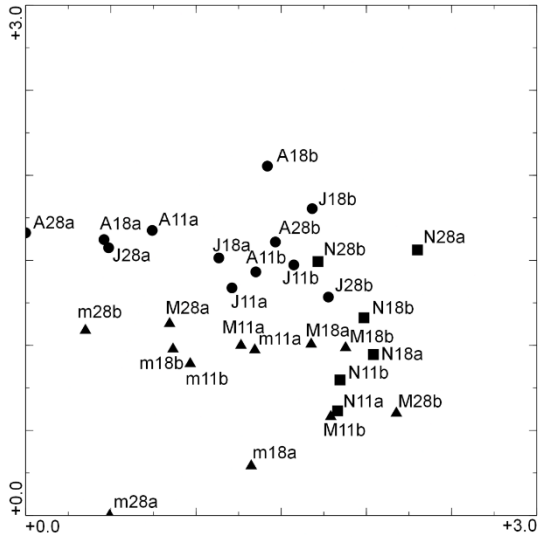


Figure 14: *DCA of the diatom communities. Triangles: spring; circles: summer; squares: autumn. Numbers represent sampling days. A: "August", J: "July", M: "May", m: "March", N: "November". The additional "a" and "b" represent the two different flumes or replicates run during one single sediment.*

Late successional biofilms (day 28) were always dominated by a combination of the four diatom species *Achnanthidium minutissimum var. minutissimum* (*A. min*.), *Sellaphora*

seminulum (*S. sem.*), *Nitzschia fonticola* (*N. font.*) and *Nitzschia dissipata var. dissipata* (*N. diss.*) with abundances changing during the course of the year (***Figure 15***). During spring *A. min.* and *S. sem.* dominated late successional biofilms while other diatom species such as *Mayamaea atomus var. permitis* or *Eolimna minima* (*E. min.*) were subdominant. In summer biofilms, *S. sem.* developed very high abundances while the fractions of other diatom species such as *Nitzschia paleacea* and *Eolimna subminuscula* were lower. In contrast to spring and summer, autumn diatom communities were dominated by *N. font.* and *N. diss.* representing almost half of the diatoms in the late biofilms.

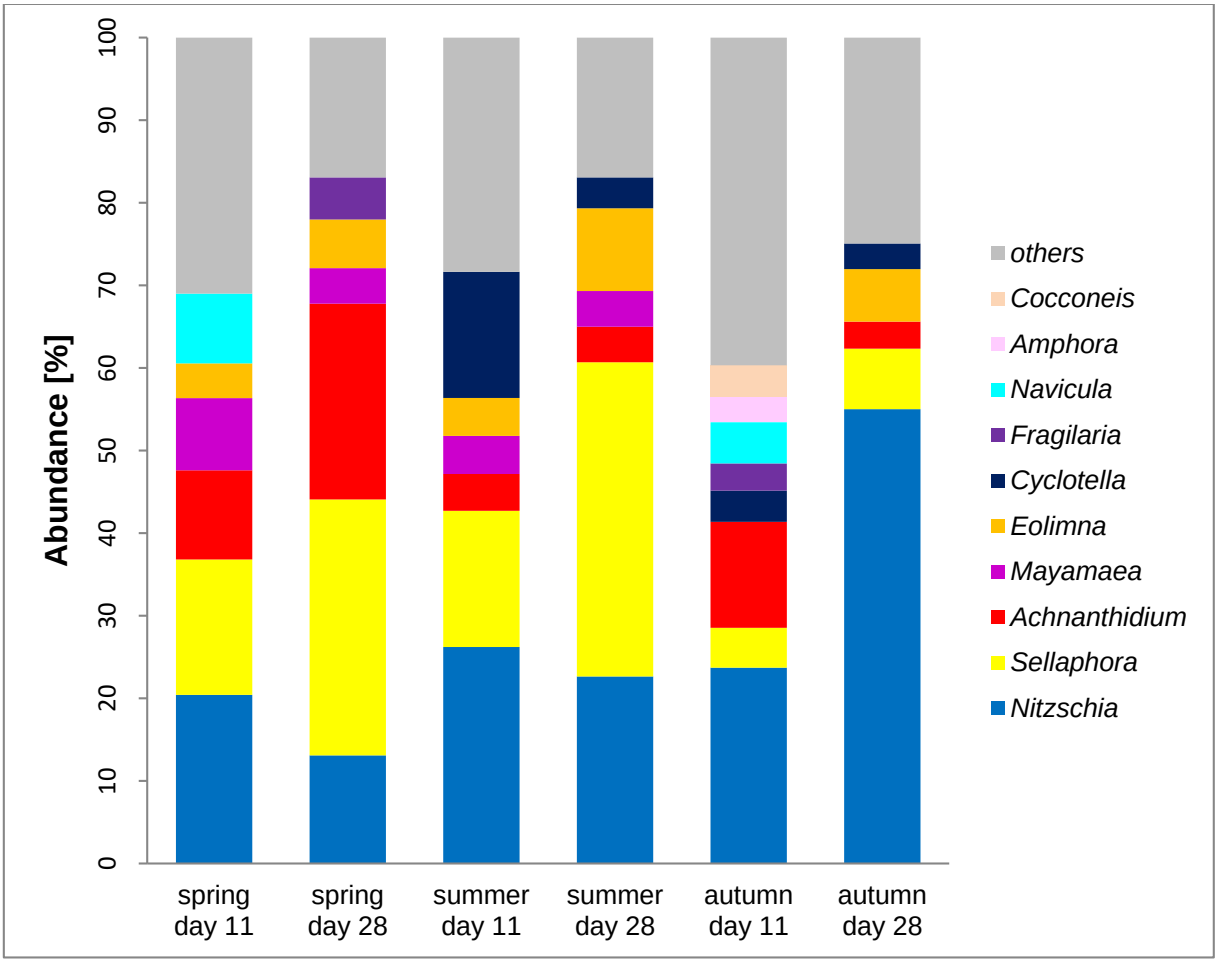


Figure 15: *Relative abundances of diatom genera [%] of early and late successional biofilm stages during all seasons. Genera with a relative abundance of less than 3.0 % were added up and summarized as 'others'.*

3.3 The effect of light intensity and shear stress on microbial biostabilization

3.3.1 Background

In recent years, biofilms are recognized as increasingly important subjects of research. One obvious reason for this development can be found in the high economic importance of biofilms – independent of the point of view whether they are regarded to be beneficial e.g. in the context of bioremediation (Kwon *et al.*, 2016; Lai *et al.*, 2016) or adverse e.g. during biofouling (Mueller-Steinhagen, Malayeri & Watkinson, 2011; Trueba *et al.*, 2015; Moreira *et al.*, 2016) or colmation of riverine sediments (Mueller *et al.*, 2013). Depending on the research focus, these and various other studies concentrate on one of two fundamental perspectives: either the impact of environmental conditions upon the development and characteristics of the biofilms is investigated or the "performance" e.g. the rate of nutrient cycling or contaminant retention of the biofilm system is of major interest. Furthermore, numerous studies investigated the influence of different environmental factors on the development of different biofilms, the main focus was either the investigations of the resulting biofilm structure (Blenkinsopp & Lock, 1994), the affected metabolism pathways (Romani *et al.*, 2004; Marcarelli *et al.*, 2009; Kendrick & Huryn, 2015) or the analysis of the microbial community (Lawrence *et al.*, 2004; Wagner *et al.*, 2015). As a result, knowledge about the impact of important environmental conditions such as light or nutrient availability upon biofilm systems is constantly increasing. Moreover, the different feedback loops between biofilm and environment are unravelled.

However, the process of biostabilization of fine sediments is still poorly understood, even if recent studies focused on this essential ecosystem function. Many recent studies concentrated on the role of different components in the EPS matrix (Taylor, Paterson & Mehlert, 1999; Tolhurst, Gust & Paterson, 2002) as these substances are generally considered as driving factors for biostabilization. On the one hand, results of these investigations highlighted the important role off algal EPS production (Yallop, Paterson & Wellsbury, 2000), on the other hand the essential role of bacteria was also indicated (Gerbersdorf *et al.*, 2009). These findings indicate how complex this field of research is and that the stabilizing potential of biofilms is affected by various abiotic and biotic parameters. As these boundary conditions are constantly changing during field experiments, fundamental research on this subject appears only feasible via the usage of mesocosm experiments (with a high amount of replicates) where every environmental parameter could

be controlled and biofilms could be cultivated under constant, reproducible natural-like boundary conditions. Using this corresponding experimental setup whose design and reliability were presented in section 3.1, the impact of environmental conditions on biostabilization could be assessed (Thom *et al.*, 2015a), albeit without including detailed information about the composition of the microbial community and the role of potential microbial key players in the process of biostabilization. Thus, the central focus of this section was to gather a comprehensive insight into the impact of two major abiotic environmental factors (flow velocity and illumination intensity) upon the biofilm development with special consideration of the microbial community composition.

The investigated boundary conditions were set in three natural-like levels (see ***Table 2***). In this context, the applied three levels of bed shear stress (BSS) ranged from a situation with very low flow velocity such as in shallow reaches of abandoned meanders, up to a level of physical stress closed to the point of erosion of the used fine sediment. The three set light intensities (LI) reflected situation of virtually no illumination penetrating the water column and reaching the river bed e.g. in very turbid and/ or heavily shaded rivers up to very high levels of irradiation as found in very shallow reaches illuminated by bright sunlight during the midday hours.

Essential prerequisite for a deep understanding of biostabilization as the overall functionality of the biofilm system was the assessment of various other biofilm characteristics which were demonstrated to impact biofilm adhesiveness. Thus, this investigation included the physiology of the biofilms such as algal and bacterial biomass and produced EPS in the biofilm matrix as well as the community structure of bacteria and algae in order to identify potential key players during the process of biostabilization.

3.3.2 Comparison of the different boundary conditions

Comparing the three different levels of bed shear stress (BSS), significant differences became plain in the parameters EPS protein content (KWT; n= 175; p=0.0178), biofilm adhesiveness (KWT; n= 173; p=0.0398), Rr (KWT; n= 172; p=0.0001), Dy (KWT; n= 140; p=0.0047) and Fo (KWT; n= 175; p=0.0213) (***Table 10***). In this context, adhesiveness and Rr of biofilms grown under high shear stress were comparably low, while Dy and Fo of these biofilms were very high. Furthermore, EPS protein contents displayed maximal mean values in biofilms grown under medium bed shear stress. The comparison of the three different levels of light intensity (LI) displayed significant differences between all analysed parameters

(***Table 10***). Amongst others, biofilms cultivated under minimal illumination intensity showed highly significantly minimal mean values for adhesiveness (KWT; $p<0.0001$; $n=229$), Fo (KWT; $p=0.0005$; $n=212$) and Rr (KWT; $p<0.0001$; $n=226$).

3.3.3 Temporal biofilm development

The cultivation under different boundary conditions clearly influenced the temporal development of the analysed biofilm parameters. In this context, the three levels of BSS displayed various impacts: while the contents of EPS carbohydrates showed fluctuating values without significant changes, EPS protein contents significantly increased under minimal and maximal BSS (see ***Table 11***). Furthermore, algal biomass significantly increased in all biofilms but BCC only displayed a significant growth over time under minimal bed shear stress (KWT; $p<0.0001$; $n=48$). The bacterial community displayed very variable reactions to the different levels of BSS: a significant increase in Fo could be detected in all biofilms, but Dy only increased significantly under medium and maximal BSS. Moreover, the only case of significantly growing Rr was observed in biofilms grown under minimal BSS. In addition to the observed significant minimum in adhesiveness of biofilms under maximal BSS, this parameter did not show a significant increase (KWT; $p=0.0643$; $n=28$) – in contrast to the adhesiveness of biofilms cultivated under minimal or medium BSS.

Concerning the temporal development of the biofilms cultivated under different levels of light intensity (LI) (summarized in ***Table 12***), it became plain that under minimal LI only BCC (KWT; $p=0.0023$; $n=30$) and biofilm adhesiveness (KWT; $p=0.0395$; $n= 61$) increased significantly over time. In contrast, biofilms cultivated under maximal illumination displayed various significantly increasing parameters including mean EPS protein contents, algal and bacterial biomass and biofilm adhesiveness. In addition, this is the only treatment where a significant increase in mean EPS carbohydrate content could be detected.

Table 10: Summarized comparison of the different boundary conditions.

Biofilm parameter		Bed shear stress		Difference between conditions	Light intensity		Difference between conditions
		n	p		n	p	
EPS	**Carbohydrates**	175	p = 0.7474	N	235	p = 0.0004	S
	Proteins	175	p = 0.0178	S	235	p = 0.0002	S
Biomass	**Chlorophyll *a***	177	p = 0.1805	N	235	p < 0.0001	S
	Bacterial cells	80	p = 0.8905	N	121	p = 0.0013	S
Bacterial community	**Range weighted richness**	172	p = 0.0001	S	226	p < 0.0001	S
	Functional organization	162	p = 0.0213	S	212	p = 0.0005	S
	Dynamics	140	p = 0.0047	S	174	p = 0.0123	S
Biofilm stability	**Adhesiveness**	173	p = 0.0398	S	229	p < 0.0001	S

N: no significant difference detectable; S: significant different observed

***Table 11*:** Temporal biofilm development under different levels of bed shear stress.

Biofilm parameter		Minimum BSS		Medium BSS		Maximum BSS	
		n	**p**	**n**	**p**	**n**	**p**
EPS	**Carbohydrates**	105	p = 0.0576	40	p = 0.1981	30	p = 0.4511
	Proteins	105	p = 0.0002	40	p = 0.0833	30	p = 0.0424
Biomass	**Chlorophyll *a***	105	p < 0.0001	40	p = 0.0005	32	p = 0.0026
	Bacterial cells	48	p < 0.0001	13	p = 0.0853	12	p = 0.0883
Bacterial community	**Range weighted richness**	102	p = 0.3665	40	p = 0.0550	30	p = 0.0721
	Functional organization	96	p < 0.0001	38	p = 0.0138	28	p = 0.0183
	Dynamics	80	p = 0.7847	32	p = 0.0240	22	p = 0.0347
Biofilm Stability	**Adhesiveness**	106	p < 0.0001	40	p = 0.0009	28	p = 0.0643

***Table 12*:** Temporal biofilm development under different levels of light intensity.

Biofilm parameter		Minimum LI		Medium LI		Maximum LI	
		n	**p**	**n**	**p**	**n**	**P**
EPS	**Carbohydrates**	65	p = 0.4069	105	p = 0.0576	65	p = 0.0199
	Proteins	65	p = 0.2770	105	p = 0.0002	65	p = 0.0024
Biomass	**Chlorophyll *a***	65	p = 0.9523	105	p < 0.0001	65	p < 0.0001
	Bacterial cells	30	p = 0.0023	48	p < 0.0001	25	p = 0.0143
Bacterial community	**Range weighted richness**	62	p = 0.8215	102	p = 0.3665	62	p = 0.6016
	Functional organization	54	p = 0.8196	96	p < 0.0001	48	p = 0.0473
	Dynamics	44	p = 0.1643	80	p = 0.7847	62	p = 0.0074
Biofilm Stability	**Adhesiveness**	61	p = 0.0395	106	p < 0.0001	62	p < 0.0001

3.3.4 Detailed investigation of biofilm parameters

As illustrated in section 3.2, seasonality has a strong influence for the biofilm growth with a clear maximum in biostabilization detected during spring. Thus, a more detailed examination of the biofilm development during this season constitutes an essential source of deeper information about the reaction of the biofilm community to the different environmental conditions and resulting biostabilzation. In this context, it is important to take into account the aforementioned two different successional development stages of the biofilm (nascent bacteria dominated in the first two weeks versus matured algae dominated from week two on). Although matured biofilms cultivated under medium BSS displayed maximal mean values for chlorophyll *a* (5.9 ± 3.6 µg gDW^{-1}) and bacterial cell counts (6.1 ± 2.7* 10^7 gDW^{-1}), the different levels of bed shear stress (BSS) did not have a statistically significant impact on the bacterial or algal biomass of the biofilms (KWT; BCC: p=0.2329; n=36; Chlorophyll *a*: p=0.1948; n=85). However, the bacterial communities were clearly influenced by the different flow velocities: On the one hand; range weighted richness (Rr) was significantly the lowest under maximal BSS (KWT; p<0.0001; n=84). On the other hand, dynamics in the community were significantly the lowest under minimal BSS and the highest under maximal BSS (KWT; p<0.0001; n=66). Furthermore, the influence of BSS upon biofilm development and biofilm adhesiveness became plain by a strong trend indicating decreasing biofilm stability with increasing flow velocity (KWT; p=0.05124; n=85). Detailed information about the influence of the different applied levels of BSS during biofilm cultivation upon all assessed biofilm parameters can be found in ***Table 13***. Biofilms cultivated under different levels of light intensity (LI) displayed significant differences in many investigated parameters (see ***Table 14***): mean contents of carbohydrates as well as proteins in the EPS matrix were significantly the lowest in matured biofilms grown under minimal LI (KWT; EPS carbohydrates: p=0.0006; n=87; EPS proteins: p=0.0082; n=87). Besides their significantly lowest algal biomass (KWT; p<0.0001; n=87), the biofilms developing under minimal LI displayed very low bacterial Rr (KWT; p<0.0001; n=84) but constantly high Dynamics (KWT; p=0.0158; n=68). Furthermore, the functional organization of the bacterial community under minimal LI stagnated throughout the experiments, while the bacterial communities under medium and maximal LI increased in their specialization leading to significantly higher mean values in matured biofilms (KWT; p=0.0026; n=82).

Table 13: Comparison of biofilm grown under different levels of bed shear stress.

		Minimum BSS		Medium BSS		Maximum BSS		
		Early Day 0-18	**Late Day 21-35**	**Early Day 0-18**	**Late Day 21-35**	**Early Day 0-18**	**Late Day 21-35**	**Difference between treatments**
EPS	**Carbohydrates [µg gDW^{-1}]**	12.9 ± 6.1	21.6 ± 8.5	13.6 ± 6.7	26.3 ± 8.1	7.1 ± 5.0	23.6 ± 10.9	N
	Proteins [µg gDW^{-1}]	10.2 ± 9.6	33.1 ± 10.2	13.4 ± 6.3	31.5 ± 13.2	4.5 ± 3.2	29.8 ± 8.0	S
Biomass	**Chlorophyll *a* [µg gDW^{-1}]**	0.3 ± 0.3	6.9 ± 6.5	0.5 ± 0.5	5.9 ± 3.6	0.2 ± 0.2	3.6 ± 3.0	N
	Bacterial cells [*10^7 gDW^{-1}]	1.8 ± 2.3	4.7 ± 3.7	1.6 ± 1.9	6.1 ± 2.7	0.6 ± 0.6	4.6 ± 1.7	N
Bacterial community	**Range weighted richness**	6.1 ± 1.8	9.5 ± 6.2	8.2 ± 2.2	10.9 ± 5.0	4.7 ± 1.2	4.8 ± 2.3	S
	Functional organization	41.6 ± 7.0	68.0 ± 11.4	59.3 ± 7.0	67.9 ± 7.3	59.7 ± 5.1	61.4 ± 12.0	S
	Dynamics	10.0 ± 5.1	17.3 ± 3.3	18.3 ± 9.2	21.2 ± 9.1	26.6 ± 18.8	32.9 ± 15.0	S
Diatom community	**Shannon Index**	1.8 ± 0.1	1.7 ± 0.8	3.1 ± 0.2	2.8 ± 0.8	2.6 ± 0.1	2.1 ± 0.3	N
	Evenness	0.5 ± 0.1	0.5 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	N
Biofilm Stability	**Adhesiveness [mA]**	72.9 ± 47.3	810.3 ± 1044.6	73.5 ± 50.4	435.0 ± 316.7	84.8 ± 55.4	340.6 ± 270.8	N

N: no significant difference detectable; S: significant different observed

Table 14: Comparison of biofilms cultivated under different levels of illumination intensity.

		Minimum LI		Medium LI		Maximum LI		
		Early Day 0-18	**Late Day 21-35**	**Early Day 0-18**	**Late Day 21-35**	**Early Day 0-18**	**Late Day 21-35**	**Difference between treatments**
EPS	**Carbohydrates [µg gDW^{-1}]**	15.7 ± 7.1	10.3 ± 4.3	18.8 ± 9.1	23.6 ± 7.6	10.7 ± 5.8	20.3 ± 13.4	S
	Proteins [µg gDW^{-1}]	4.0 ± 2.1	6.1 ± 0.9	2.7 ± 1.5	8.4 ± 3.1	3.9 ± 3.5	10.2 ± 6.1	S
Biomass	**Chlorophyll *a* [µg gDW^{-1}]**	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	2.4 ± 2.9	0.3 ± 0.2	3.7 ± 4.4	S
	Bacterial cells [*10^{7} gDW^{-1}]	0.8 ± 0.3	1.3 ± 0.7	0.4 ± 0.3	3.0 ± 0.9	1.5 ± 1.0	4.3 ± 4.0	S
Bacterial community	**Range weighted richness**	5.4 ± 0.9	4.3 ± 2.8	31.2 ± 2.9	38.6 ± 11.1	32.4 ± 1.0	39.5 ± 9.0	S
	Functional organization	50.6 ± 2.9	50.9 ± 4.1	50.2 ± 4.5	56.8 ± 5.5	52.2 ± 2.2	64.9 ± 4.6	S
	Dynamics	16.0 ± 5.2	15.6 ± 3.4	10.3 ± 6.1	4.9 ± 2.6	8.7 ± 1.4	14.7 ± 9.4	S
Diatom community	**Shannon Index**	/*	/	3.3 ± 0.2	1.0 ± 0.1	2.7 ± 0.2	1.4 ± 0.2	N
	Evenness	/	/	0.9 ± 0.1	0.4 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	N
Biofilm Stability	**Adhesiveness [mA]**	71.2 ± 11.0	73.6 ± 28.4	55.5 ± 12.7	675.9 ± 837.8	58.0 ± 17.3	1001.7 ± 994.1	S

**: under minimal illumination, no significant algal development could be observed*

N: no significant difference detectable; S: significant different observed

Biofilm adhesiveness

While initial levels of adhesiveness were very similar in all early biofilm stages, the highest mean adhesiveness in late biofilm stages could be detected in biofilms grown under maximal LI (1001.7 ± 994.1 mA). With a value of 810.2 ± 1044.6 mA, the mean adhesiveness of biofilms cultivated under minimal BSS was insignificantly lower. Furthermore, minimal LI was the only boundary condition where no significant increase in biofilm adhesiveness could be detected (see ***Figure 16***).

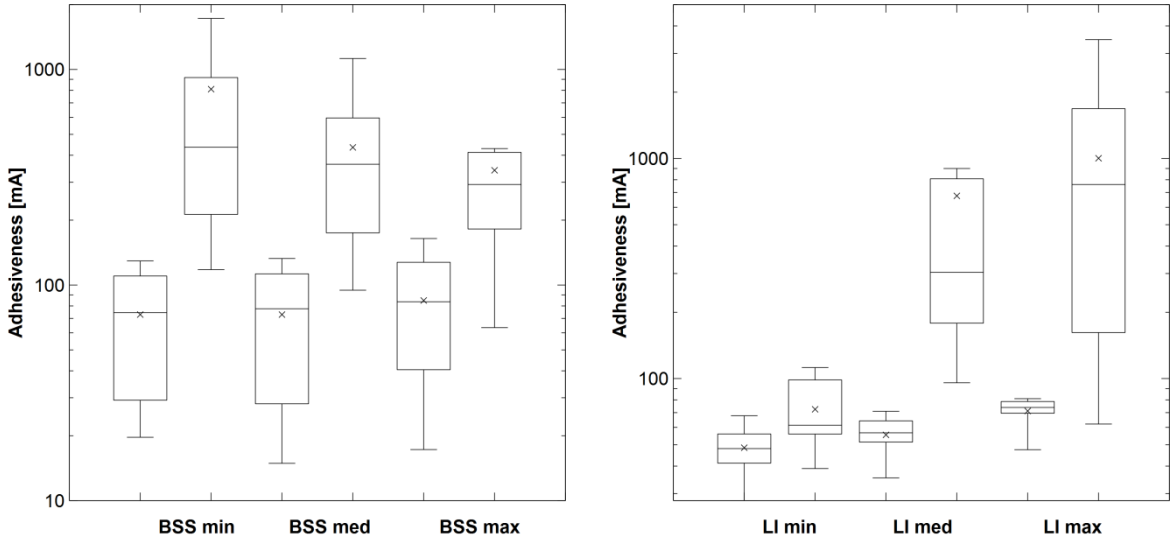


Figure 16*: Adhesiveness of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI (with logarithmic ordinate).*

EPS matrix

Comparing the different levels of BSS, it became plain that early biofilm stages under maximal flow velocity displayed lower mean EPS carbohydrate and protein contents. However, this differences were mitigated in late biofilms. The reverse development could be detected comparing the different levels of LI: although all early biofilms had a similar content of carbohydrates and proteins in the colloidal EPS, late biofilms cultivated under minimal LI had the significantly lowest mean values of EPS carbohydrates and proteins contents (***Figure 17***).

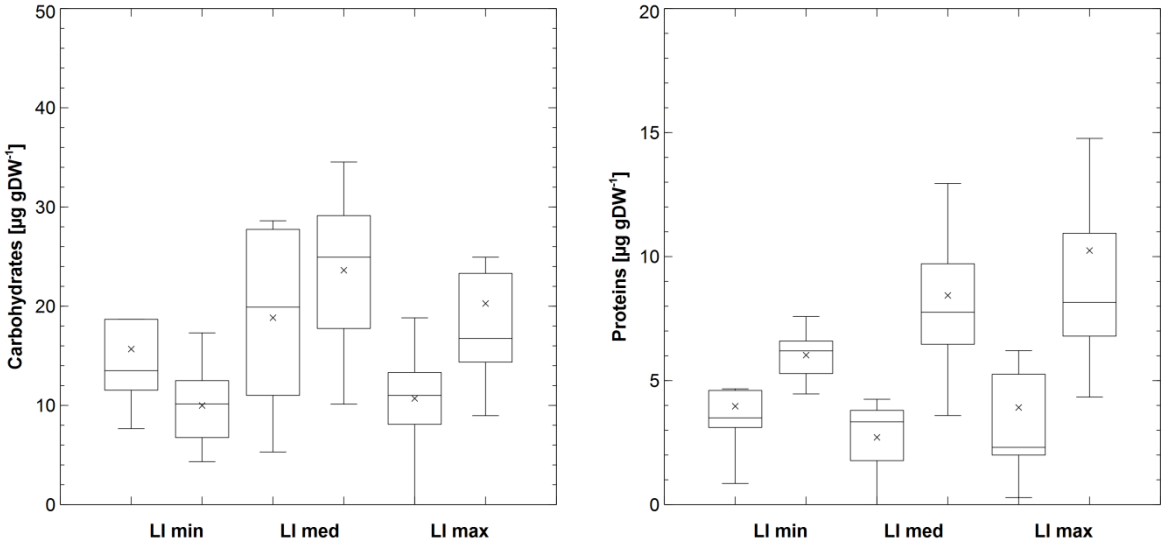


Figure 17: *Content of EPS components of early and late biofilm stages under different levels of LI; left: EPS carbohydrates; right: EPS proteins.*

Microbial biomass

Under the different levels of BSS, the development of the bacterial and algal biomass was very similar to the observed changes in EPS components: after early biofilm stages developing under maximal flow velocity displayed lower mean values than biofilm under minimal or medium bed shear stress, the levels of bacterial and algal biomass were more similar in late biofilms. However, biofilms under maximal BSS mitigated the difference in BCC more than the differences in chlorophyll *a* content (see ***Figure 18***). In contrast to this, especially matured stages of biofilms cultivated under medium or maximal LI showed significantly higher bacterial and algal biomass than biofilms grown under minimal illumination intensity.

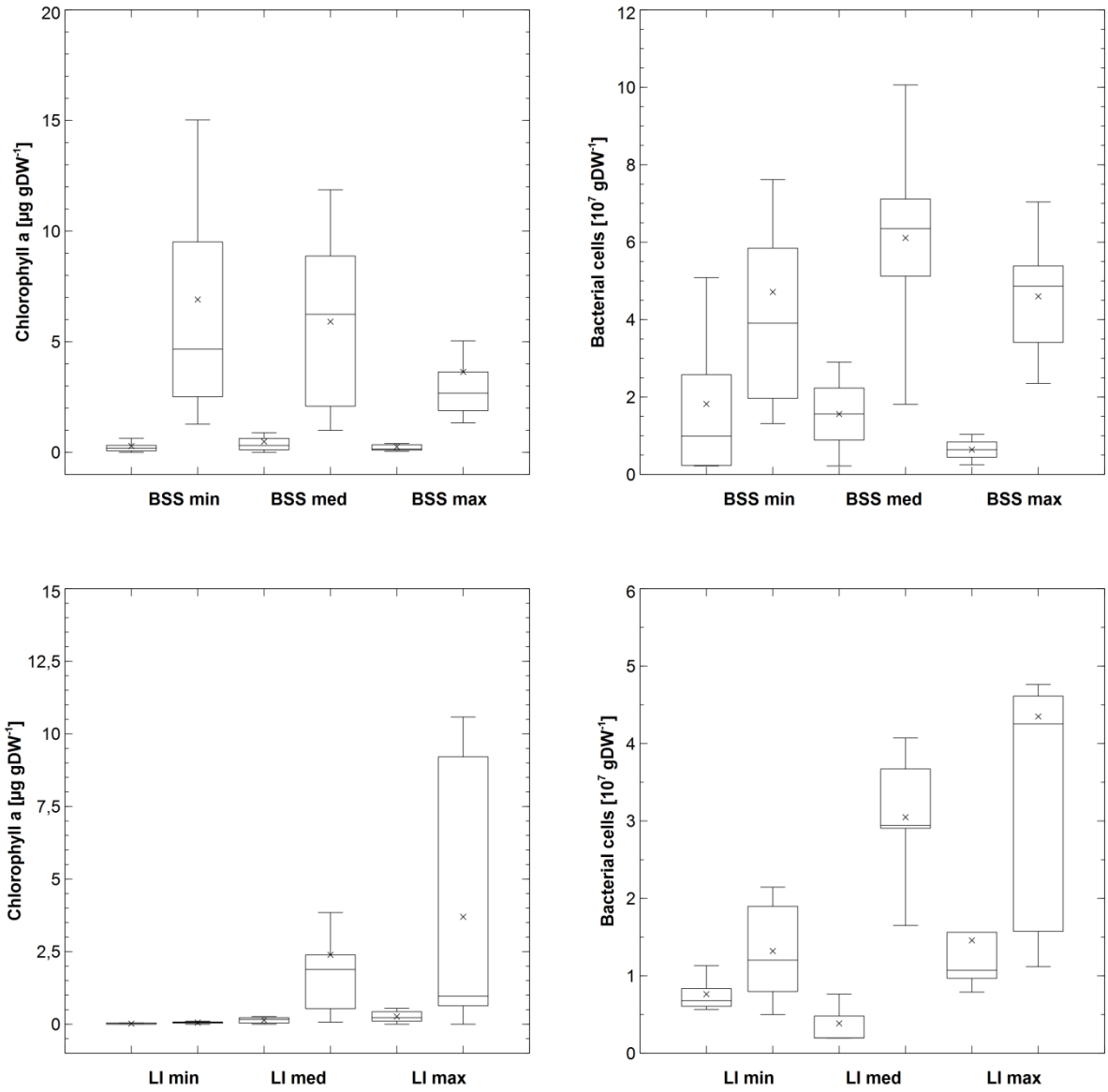


Figure 18: *Microbial biomass of early and late biofilm stages. Upper left: Algal biomass under different levels of bed shear stress; upper right: Bacterial biomass under different levels of bed shear stress; lower left: algal biomass under different levels of light intensity; lower right: Bacterial biomass under different levels of light intensity.*

Bacterial ecology

The Range weighted richness (Rr) of biofilms grown under minimal and medium BSS displayed increasing mean values, while the mean Rr of biofilms cultivated under maximal BSS stagnated at a significantly lower level (KWT; p<0.0001; n=84). Parallel to this development, the mean Rr of biofilms developing under medium and maximal LI increased over time, while the mean values of biofilms grown under minimal LI were significantly lower (KWT; p<0.0001; n=84) and decreasing over time (see ***Figure 19***).

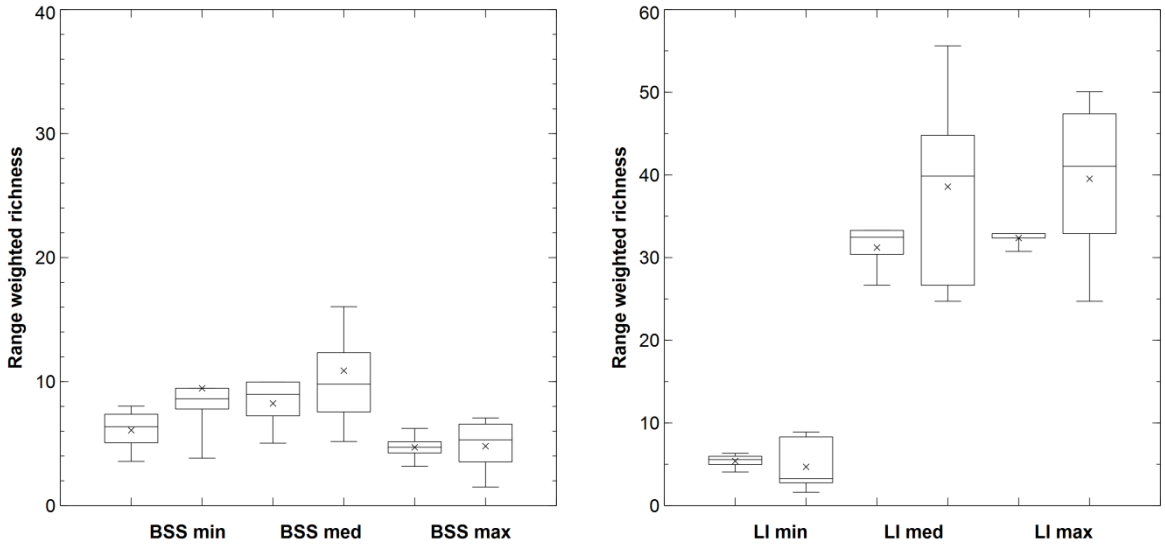


Figure 19*: Range weighted richness (Rr) of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.*

The mean functional organization (Fo) in early biofilms was significantly lower under minimal BSS than under medium or maximal BSS (KWT; p=0,0014; n=78). However, this mean value significantly increased over time (KWT; p<0.001; n=96), so that no significant difference in mean Fo could be detected in late biofilms under the different levels of flow velocity (KWT; p=0.1879; n=84). Minimal LI resulted in stagnating Fo, while medium and maximal illumination led to an increase in Fo over time. As a consequence, significant maximal mean Fo could be detected in late biofilms under maximal LI (KWT; p<0.0001; n=114) see ***Figure 20***.

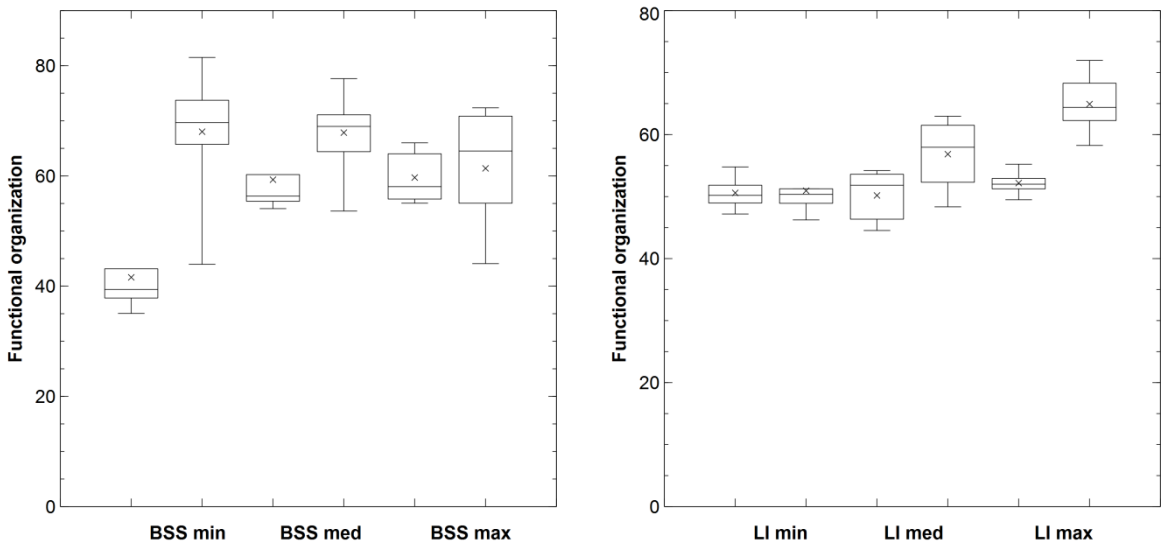


Figure 20: *Functional organization (Fo) of the bacterial community of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.*

While no clear trend was observed in biofilms under different levels of LI (stagnating dynamics under minimal LI, decreasing dynamics under medium LI and increasing dynamics under maximal LI), a clear correlation was indicated under the different levels of flow velocity (***Figure 21***): mean dynamics significantly raised with increasing BSS (KWT; $p<0.0001$; $n=66$).

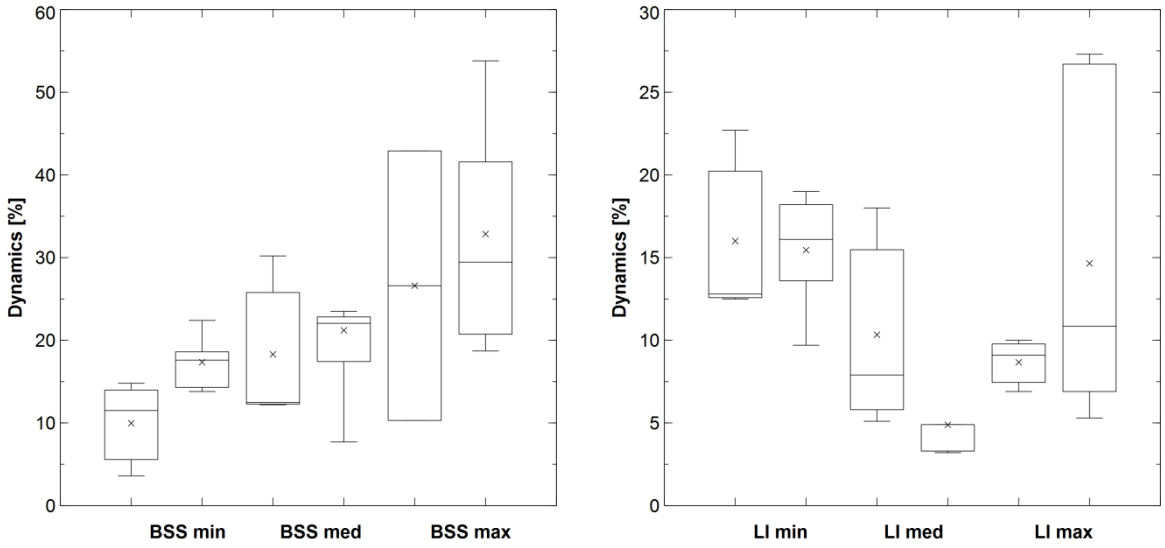


Figure 21: *Dynamics within the bacterial community of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.*

Interactions of the different biofilm parameters

Generally, the correlations of the different investigated biofilm parameters (***Table 15***) displayed many similarities to the results of the assessment of the impact of seasonality on biofilm development (section 3.2.; ***Table 8***): A high correlation between algal biomass and bacterial cell counts could be detected (spearman; rs= 0.7; p< 0.0001; n=150). Furthermore, the content of proteins in the EPS matrix displayed high correlations to algal biomass and bacterial cell counts (spearman; chlorophyll a- EPS proteins: rs= 0.58; p<0.0001; n=305; BCC-EPS proteins: rs= 0.56; p< 0.0001; n=150). In addition, a similar negative correlation between range weighted richness and dynamics in the bacterial community could be observed (spearman; rs= -0.24; p<0.000144; n=228). Concerning the biostabilization capacity of the biofilms, the microbial biomass (chlorophyll *a* as well as BCC) and the EPS protein content correlated significantly to biofilm adhesiveness (spearman; chlorophyll a- biofilm adhesiveness: rs= 0.81; p< 0.0001; n=295; BCC-biofilm adhesiveness: rs= 0.74; p<0.0001; n=147). Similar to the observations in section 3.2, the functional organization of the bacterial community had the highest correlation to biofilm adhesiveness (spearman; rs= 0.48; p=<0.0001; n=271) – even if it was lower than during the seasonality study.

Table 15: Comprehensive overview over correlations of biofilm parameters.

		EPS		Biomass		Bacterial community			Biofilm adhesiveness
		Carb	**Prot**	**Chloro**	**BCC**	**Rr**	**Fo**	**Dy**	**T3**
EPS	**Carb**	1							
	Prot	0.51	1						
Biomass	**Chloro**	0.47	**0.58**	1					
	BCC	0.42	**0.56**	**0.70**	1				
Bacterial community	**Rr**	-0.09	-0.14	0.17	0.04	1			
	Fo	0.38	0.43	0.44	0.43	-0.43	1		
	Dy	0.10	0.14	0.19	0.14	-0.24	0.21	1	
Biofilm adhesiveness	**T3**	0.42	**0.56**	**0.81**	**0.74**	0.13	**0.48**	0.07	1

Carb: carbohydrates; Prot: proteins; Chloro: chlorophyll a; BCC: bacterial cell counts; T3: biofilm adhesiveness threshold 3

Microbial community

Bacterial species

Analysis of the 211 prominent bacterial DGGE bands/ sequences yielded 77 different bacterial species (see ***Table 17*** in the appendix for detailed information about the sequence of every investigated DGGE band). As described in section 3.2 and by Schmidt et al. (2016), the influence of seasonality is of great importance for the composition of the microbial community and resulting biofilm stability. Thus, the species distribution among the different seasons is assessed in a first step. While 16 species could be identified during more than one season e.g. members of the genus *Aquabacterium*, *Brevundimonas* or *Gemmatimonas*, the number of bacteria exclusively detected in only one season varied to a great extend: 51 during spring experiments versus 7 during summer experiments and 3 during winter. Furthermore, in various experiments and under different boundary conditions, microbial organisms generally considered as typical generalists and widely spread were detected. Examples for these bacteria of typical freshwater biofilm were *Aquabacterium sp*. (Kalmbach *et al.*, 1999), *Brevundimonas diminuta* (Vancanneyt *et al.*, 2009) or *Gemmatimonas phototrophica* (Zeng *et al.*, 2015; Zeng *et al.*, 2016). Besides these mentioned micro-organisms, several bacterial species were identified independent of illumination intensity. However, four species dominated biofilms grown under high light intensity: *Rubrivivax gelatinosus* (Wawrousek *et al.*, 2014) and *Rhodoferax saidenbachensis* (Kaden *et al.*, 2014) in early development stages, *Neosynechococcus sphagnicola* (Dvorak *et al.*, 2014) and Leptolyngbya sp. (Kanellopoulos *et al.*, 2015) in matured biofilms. In comparison to this, the different levels of shear stress displayed no significant influence upon the bacterial community composition - with *Pseudomonas taeanensis* constituting the only exception with a stronger appearance in biofilms cultivated under high flow velocity.

Diatom community

In total, 13 different diatom genera were present in relative abundances greater than 3% in the investigated biofilms. Among these, 4 different diatom genera – *Fragillaria, Sellaphora, Nitzschia* and *Achnanthidium* appeared to dominate the biofilms in variable abundance ratios. Biofilms cultivated under minimal BSS were clearly dominated by *Achnanthidium minutissimum var. minutissimum* (*A. min.*) (mean relative abundance of 52.0 ± 3.6 % in early biofilms and 59.4 ± 4.2 % in late biofilms). Under increased BSS *A. min.* became less dominant and diatoms of the genus *Nitzschia* -mainly *Nitzschia fonticola, Nitzschia*

abbreviata and *Nitzschia dissipata*- increased in dominance in matured biofilms as a comparison of their cumulative mean relative abundances clearly showed: 9.9 ± 0.2% in biofilms cultivated under minimal BSS, 24.9 ± 3.3% in biofilms grown under medium BSS and 40.8 ± 3.5% in biofilms developing under maximal BSS). Besides this, *Sellaphora seminulum* (*S. sem*.) reached a mean relative abundance of 22.1 ± 3.3% which was similar to the members of the genus *Nitzschia* in matured biofilms under medium BSS (see ***Figure 22***). The high dominance of *A. min* in late biofilms cultivated under minimal BSS was also reflected by minimal mean diversity and evenness (see ***Table 13***).

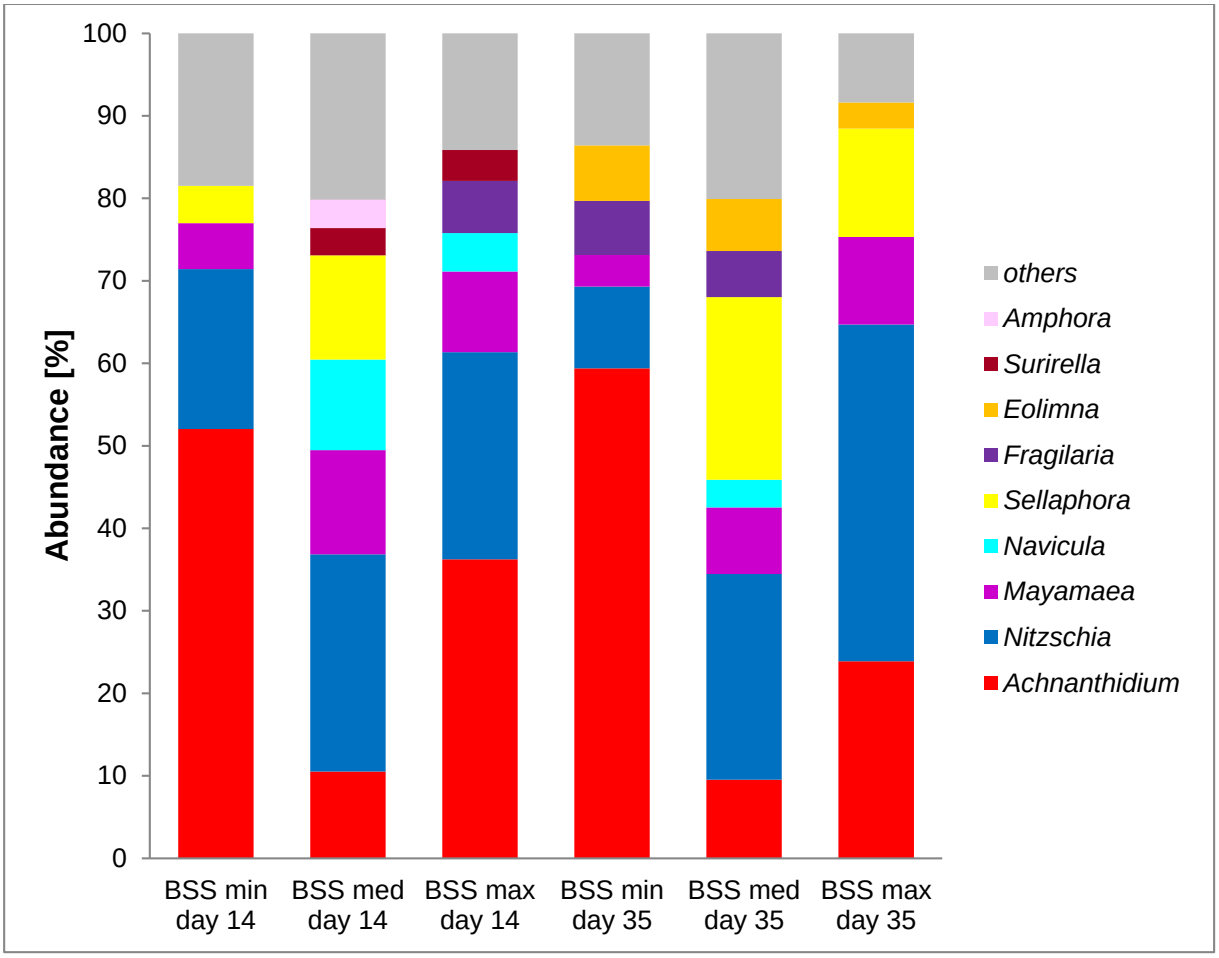


Figure 22*: Composition of early and late diatom communities under different levels of bed shear stress. Genera with a relative abundance less than 3% were summarized as "others".*

The most apparent impact of the applied different levels of light intensity (LI) was that no clear algal development could be detected under minimal LI. Furthermore, in contrast to the biofilms cultivated under different levels of BSS, members of the genus *Nitschia* were less dominant in the algal communities (see ***Figure 23***). Late biofilm stages were always dominated by a variable combination of *A. min*., *S. sem.* and members of the genus

Fragilaria - mainly *Fragilaria construens* and *Fragilaria pararumpens* (*F.par.*): *A. min.* clearly dominated biofilms grown under medium LI (mean relative abundance of 65.2 ± 5.1%) while *S.sem.* had a mean relative abundance of 24.7 ± 3.3%. In late biofilms which grew under maximal LI, *A. min.* and *F.par.* developed to similar dominant fractions of the diatom community (44.6 ± 2.7% and 37.2 ± 3.3%). The development of dominance by a few species was reflected by decreasing diversity (Shannon index) and evenness of the diatom communities under medium and the highest LI (see ***Table 14***).

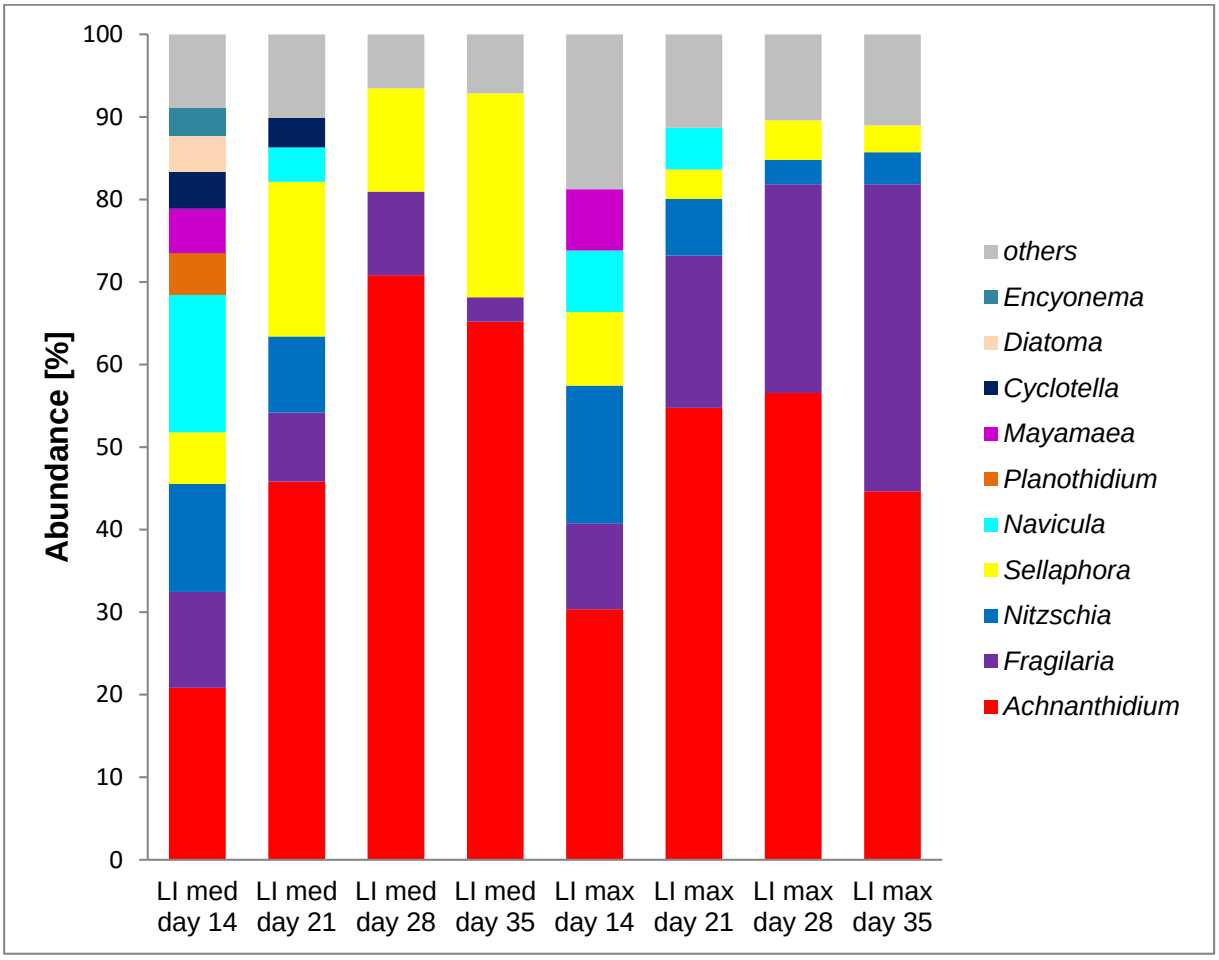


Figure 23: *Temporal development of the diatom community under different light intensities: no algal growth was detectable under minimal illumination. Genera with a relative abundance less than 3% were summarized as "others".*

4. Discussion

4.1 Evaluation of the constructed straight flume mesocosm

4.1.1 Biofilm growth within the new mesocosm

The main purpose of the present study was to test this new design of straight flumes in terms of nature-like biofilm settlement and –cultivation. Despite the absolutely identical set-up of the six flumes, biofilm development could still differ due to smallest, possibly undetected variations in e.g. the flow field. Moreover, biofilm growth is *per se* characterized by spatial heterogeneity. Thus, for the new flume design, it remained to be shown whether deviations in biofilm growth increase strongly along the test sections of one flume or between the individual flumes. This is very important since the comparability of biofilm growth and composition in the different straight flumes as well as within different flume regions is an essential prerequisite for further studies on the impact of environmental parameters on biofilm ecology and functionality.

The high spatial heterogeneity in biofilms and their complex mutual interactions with the environment have been described manifold (Donlan, 2002; Wimpenny, Manz & Szewzyk, 2000; Bernard *et al.*, 2012). Biofilms have even been described as microbial landscapes that are shaped in their spatial configuration by multiple physico-chemical factors but also alter their environment in considerable dimensions due to their growth (Battin *et al.*, 2007). Thus, not surprisingly, biofilms settling in the flumes exhibited high small-scale heterogeneity (on a single cartridge), but these pattern were clearly similar in all flume regions and flumes. This reflects the exact same settings of temperature, illumination and most importantly, hydrodynamics in all flumes to provide the same settling and growth conditions for biofilms. Most common, an integral approach (Reynolds) is applied to account for the flow conditions within a flume as a precisely controlled hydrodynamic regime is either not necessary or not practicable. However, an exact determination of near-bed turbulences and bed shear stress directly at the sites of interest is essential for this study because various boundary effects such as eddy developments along the walls of a flume may influence hydrodynamics and near-bed turbulence significantly. Consequently, this might impact the erosive forces acting on biofilms as well as their nutrient replenishment to affect biofilm morphology and -activity. Hence, in this study the turbulence distribution was determined via high resolution Laser Doppler Anemometry (LDA) before this experiment and later on checked with Acoustic Doppler Velocimetry (ADV) (Thom *et al.*, 2012a). In addition, the long inlet flow sections as

well as the design of the flumes (with a sufficient distance of the biofilm cartridges to the flume walls) ensure a homogenous turbulence distribution over the biofilm growth section. Another essential fact to keep in mind is that bed shear stress is an exponential function of the flow velocity emphasising the importance of constant discharges within the flumes. This is why high resolution discharge measurements in all flumes were performed throughout the experiment. These data gave evidence on the constant and similar discharges in all six flumes and thus, on the identical bed shear stress levels over all biofilm growth sections in the presented experiment. This is one important prerequisite for the high reproducibility of biofilm cultivation that could be shown for this new experimental set-up in this study.

4.1.2 Biomass and EPS

Several studies investigating intertidal biofilms suggested a complex non-linear relationship between microbial biomass and EPS production (Yallop, Paterson & Wellsbury, 2000; Smith & Underwood, 2000; Hanlon *et al.*, 2006). The occasionally fast shifting nutritional and physiological states of the biofilm microbes might be of major importance for EPS production and secretion. This was underpinned by Underwood *et al*. (2004) who described that diatom EPS production was, among others, dependent on the nutritional condition of the cells. The natural riverine water used in the flumes contained moderate nutrient concentrations comparable to oligotrophic habitats that remained stable over the experiment. Besemer *et al*. (2007) and Artigas *et al*. (2012) reported microbial biomass similar to the values determined in the present experiment under comparable conditions of nutrient availability (e.g. bacterial cell densities up to 1.13 $x10^8$cells*cm^{-2} or chlorophyll *a* concentrations of 0.03 - 6.28 µg cm^{-2}). In contrast, biofilms in a eutrophic river (Neckar) were characterized by high microbial biomass and EPS production (Gerbersdorf *et al.*, 2008) e.g. an up to 16 fold higher chlorophyll *a* content than in this study emphasizing the effects of different water qualities upon biofilm development, corresponding metabolic rates and possibly, functionality. Low supply of nutrients may have restricted EPS production during biofilm development in the present experiment; still, the biostabilization effect (as discussed later) was impressive.

4.1.3 Microbial community

As Marzorati *et al*. (2008) stated, range weighted richness (Rr), dynamics and functional organization calculated from the DGGE fingerprints should be seen as qualitative indicators, not as absolute measures due to the known drawbacks of molecular fingerprinting techniques. Nevertheless, they are an important tool to describe, compare and interpret

different DGGE band patterns in order to obtain a higher level of information about ecological processes in biofilms. Generally, observing the microbial community of biofilms in this study revealed two distinct stages during development. The first phase could be described as an initial colonization of the abiotic substrate by planktonic bacteria. The findings of Beier *et al*. (2008) or Crump and Hobbie (2005) e.g. 40 bacterial TGGE bands in comparable riverine water support the initially high bacterial Rr in this experiment. During the subsequent establishment of a bacterial biofilm within the first 11 days, competition and specialization of the bacterial community became visible by decreasing Rr and increasing functional organization. Manz *et al*. (1999) and Araya *et al*. (2003) described similar shifts within the bacterial community composition during the formation of lotic biofilms. In the period between day 11 and 14 severe changes within the bacterial community could be observed. Along with significantly decreasing cell counts and Rr, increasing dynamics and functional organization of the bacteria might be due to bacterial biofilm detachment, the initial algal colonization or settlement of grazing ciliates as described by Wey *et al*. (2012) - or a combination of these processes.

Besemer *et al*. (2007) highlighted the role of diatoms as key-players in river biofilms as they shape the bacterial community in a combination of physical and biological processes. The importance of diatoms in this experiment became obvious since parallel to the development of the diatom community (from day 14 on), a steady increase of vertical and horizontal heterogeneity of the biofilms could be visually observed. As described by Besemer *et al*. (2009) this spatial small-scale heterogeneity was a major driver affecting the development of the microbial community. Thus, diatoms apparently influenced the bacterial community in different ways. On one hand their photosynthetic activity and EPS production may have supported the growth of associated bacteria as indicated by high increase rates of the total cell counts after day 14 of the experiment; a phenomenon already described by Espeland *et al*. (2001). On the other hand, the pronounced three-dimensional structures of their colonies may have allowed the formation of various physical and biochemical transition/gradient zones e.g. O_2 gradients as described by Glud *et al*. (1999) or Fenchel and Glud (2000). Due to this formation of many microenvironments, accumulation of different metabolic products of the diatoms as well as their associated bacteria in the biofilm matrix and several metabolic cascades, various niches for a broad range of bacteria with different physiologies can develop (Stewart & Franklin, 2008). In this context, specialized bacteria may have found optimal conditions in the matured biofilms as reported by Manz *et al*. (1999). Summarizing, the diatom development apparently led to dominance of a few

bacteria species but may also have led to the establishment of various niches where diverse bacterial species might occur in low abundances, indicated by steadily decreasing dynamics within the bacterial community and parallel increasing bacterial Rr.

4.1.4 Biostabilization

Due to its high economic and ecological importance, sediment dynamics in lotic systems plays a major role in hydraulic engineering. Different modelling approaches approximated the highly complex sediment-water system. Briefly: the Shields model (1936) is commonly applied to determine the stability of sediments versus erosional forces by defining characteristic diameters and the density of the sediment grains. However, this approach is not applicable for sediments with strong biological influence. Righetti and Lucarelli (2007) described the first model based on the Shields equation introducing adhesiveness/adhesion as a new parameter in order to incorporate the influence of biofilms on sediment stability. But only after the development of the MagPI device that has been applied successfully in the marine habitat (Larson *et al.*, 2009; Lubarsky, 2011; Anderson *et al.*, 2011), the determination of biological-induced adhesiveness in high temporal and spatial resolution could be achieved. While most studies focused on brackish/intertidal areas (Yallop, Paterson & Wellsbury, 2000; Austen, Andersen & Edelvang, 1999; Gerbersdorf *et al.*, 2009)), up to now biostabilization of lotic fine sediments is virtually unaddressed. Spears *et al*. (2008) suggested the major importance of biostabilization in marine/brackish habitats supporting the current doctrine that high quantities of strong ionic bounds significantly strengthen the EPS matrix. Nevertheless, despite observing significantly lower EPS values as well as microbial biomass (as compared to brackish/marine biofilms), a significant biostabilization effect was detected. This emphasized the importance of biofilm-induced stabilization of fine sediments in lotic waters (and contradicted the current doctrine).

Furthermore, this study gave first insights in the development of various geochemical and (micro-) biological parameters during biofilm growth affecting this important ecosystem function. In this context, the content of colloidal EPS compounds could be seen as an approximate marker for biostabilization capacity. Further investigation of the quality of these polymers could lead to the identification of single carbohydrate or protein moieties with high gluing and stabilizing capability. While biofilm stability appeared to be related to total cell counts in nascent biofilm stages, the development of the bacterial community composition turned out to be even more crucial. Thereby, short and long-term shifts within the bacterial

and algal community occurred simultaneously to significantly changing biofilm stability. This emphasizes the importance of the molecular biology tools used in this study to address the biofilm composition and diversity. Identification of bacterial key players in biostabilization and their interactions with algae could be the next step to further elucidate the contribution of microbiology to sediment stability. Future studies should also consider the role of the protozoa community in shaping microbial biofilm community and functionality, here biostabilization.

4.1.5 Conclusions

The stabilizing effect of biofilms upon lotic fine sediment is currently unaddressed despite its broad range of economic and ecological implications. To investigate the complex interactions between the biofilm and its environment a sophisticated and unique set-up was designed combining biological and engineering expertise.

The evaluation of biofilm growth in these new flumes presented in this section demonstrates that comparable biofilms could be cultivated while exposed to the same abiotic environment. Furthermore, this first biofilm cultivation under strictly controlled boundary conditions demonstrated the importance of biostabilization (known to be substantial in intertidal mudflats) regarding lotic fine sediments. In doing so, first insight into various ecological processes which shape the microbial community and impact the overall biofilm functionality could be gained; for instance, the change of a bacteria dominated nascent biofilm to a diatom dominated matured development stage resulted in stable biofilms which constantly stabilized the underlying sediment significantly.

Summarizing, regarding their biological and biochemical features, representative biofilms can be grown in this novel system- an essential prerequisite for further research into natural biofilm colonization and development. In particular, the mutual interactions of various environmental parameters impacting biofilms can now be addressed and reliably related to each other. With the link to biofilm functionality, the significance of biostabilization can thus be investigated for different niches in freshwater habitats.

4.2 The seasonal effect on biofilm development and microbial biostabilization

4.2.1 Seasonal effect upon biostabilization

The comparison of biofilm adhesiveness among seasons revealed a maximum of biostabilization capacity during spring. This has been verified by Thom *et al.* (2015a) reporting maximal stability for biofilm-covered fine sediments during spring using a standardized erosion flume (Witt & Westrich, 2003). Maximal biofilm stability during spring might be an evolutionary adaptation in rivers of mid-to-high latitude temperate regions: during spring, snow melting in higher regions of the catchment regularly leads to rising discharge resulting in elevated flow velocity and bed shear stress, which can dislodge benthic biofilms. Possible adaptation strategies of biofilm microbes facing this situation could be either to resist the increased mechanical stress by attaching tightly to the sediment surface or to colonize uncovered sediment very quickly. Thus, the annual repetition of a stressful hydraulic regime during spring could favour rapidly growing biofilms (i.e. early succession stages of the diatom community). In the epipotamal (downstream) region of a river, the combination of high illumination intensities (lacking riparian vegetation) and high nutrient availability (lacking macrophyte concurrence) may additionally enhance diatom growth, their EPS production and the resulting biostabilization capacity. In this context, the annual successional process not only shapes the benthic community but also the functionality of the overall biofilm system, in this case biostabilization. Although the used experimental set-up constitutes a great simplification of the natural system, it was necessary to guarantee that the only variable parameter influencing biostabilization was the investigated seasonal changing composition of the microbial community. Furthermore, field studies (Widdows *et al.*, 2000; Amos *et al.*, 2004; Tena, Vericat & Batalla, 2014) confirm results of this study as they demonstrated the seasonality of biostabilization in estuary and intertidal mudflats as well as in a riverine system.

4.2.2 Driving factors for biofilm growth and biostabilization

EPS matrix

Extracellular carbohydrates are considered a driving factor for biofilm formation and stability (Sutherland, Grant & Amos, 1998; Underwood & Paterson, 2003; Tolhurst *et al.*, 2006); hence, the composition of diatom EPS carbohydrates and its influence upon the viscosity of the cell coating mucilage and biofilm structure have been studied intensively (Staats *et al.*,

1999; Khandeparker & Bhosle, 2001; Sutherland, 2001; Higgins *et al.*, 2002; Magaletti *et al.*, 2004). Furthermore, Svetlicic et al. (2013) highlighted the importance of the EPS carbohydrate network and its role during colony formation of diatoms. The present study confirmed the reported high correlation between biofilm stability and EPS carbohydrate content (Yallop, Paterson & Wellsbury, 2000) with the highest values for both parameters detected during spring. However, it also indicated that EPS carbohydrates are not the only important extracellular components influencing biostabilization since biofilm stability was also strongly correlated to EPS protein content. Becker et al. (1996) noted that a high EPS carbohydrate content does not necessarily result in stronger diatom adhesion to a surface and showed that the relation between EPS and biofilm stability is much more complex, as the production of extracellular carbohydrates has a number of functions for microbes that are not necessarily linked to biofilm stability. For example different carbohydrates from the group of acyl-homeserine lactones are used by a broad range of different bacteria for intra and inter species quorum sensing (Waters & Bassler, 2005). Additionally, diatoms can produce EPS carbohydrates to avoid photo-oxidative stress due to high illumination intensities (Fogg, 1983). They also produce relatively fragile extracellular carbohydrates for their locomotion through the sediment (Smith & Underwood, 1998). In general, the production and composition of EPS carbohydrates has been found to reflect the general condition of diatom colonies, such as their physiological and nutritional state, the developmental stage of the colonies and the level of environmental stress (Smith & Underwood, 2000; Underwood *et al.*, 2004; Abdullahi, Underwood & Gretz, 2006). These influencing factors may cause clear shifts in content and composition of extracellular carbohydrates. These fluctuations were hinted at during this study but apparently had no direct effect on biofilm stability (Fig. 3). On the other hand, the detection of strongly correlating biofilm adhesiveness and EPS protein content during spring indicated the importance of extracellular proteins. Extracellular proteins also have been identified as important structural elements in bacteria e.g. linking together the strands of the extracellular matrix (Lind *et al.*, 1997; Chiovitti *et al.*, 2003; Chiovitti, Dugdale & Wetherbee, 2006) and as specific adhesive proteins produced by diatoms in order to attach to a surface (Dugdale *et al.*, 2006; Dugdale, Willis & Wetherbee, 2006). This indicates the important role of extracellular proteins for the mechanical strength of the EPS matrix which greatly influences the stability of the biofilm and its biostabilization capacity. Furthermore, the results of this study suggest a much more complex relationship between the microbes in the biofilm and biofilm EPS content than the reported correlation between microbial biomass and EPS

production (Yallop, Paterson & Wellsbury, 2000; Underwood & Paterson, 2003): on one hand, EPS protein contents showed high correlation with microbial biomass during spring, while on the other hand, the highest EPS contents were not reflected in microbial biomass when comparing among seasons. This emphasizes the great importance of analyzing the microbial community to derive a comprehensive understanding of the driving factors influencing biostabilitzation.

Microbial community

Bacterial community

Moreover, the complexity of biostabilization was demonstrated by the fact that the seasonal changing biofilm stability was not mirrored by seasonal differences in microbial biomass. Furthermore, the observed Rr values indicate similar “carrying capacities” of the biofilms during all seasons, which are comparable to the findings of Lubarsky et al. (2012). These medium levels of Rr values (Marzorati *et al.*, 2008) indicate that all biofilms in the relatively unpolluted water of the River Enz could harbor a moderate diversity of bacteria. This finding emphasizes the importance of a comprehensive observation of the temporal development within the microbial community. Especially functional organization and dynamics appeared to be of importance as maximal biostabilization during spring was reflected by higher Fo and especially significantly lower Dy compared to autumn with very low stabilization capacity. The comparable higher Fo values and relatively low Dy during spring might indicate that the system could have been more stable and that a more specialized bacterial community could evolve. The high correlation between chlorophyll *a* content and Fo during spring might suggest an adaptation process of the bacterial community to the developing diatom dominance known to shape the biochemical and physical micro environment of biofilms (Besemer *et al.*, 2007). The fact that diatoms have a range of specific satellite bacteria mainly associated with them was outlined by Amin et al. (2012). Furthermore, bacteria and their secreted substances were shown to influence diatom growth and EPS production (Bruckner *et al.*, 2011). Other studies stated the important role of bacteria during diatom attachment (Gawne *et al.*, 1998) and during the formation of diatom aggregates (Gaerdes *et al.*, 2011). Furthermore, Lubarsky et al. (2010) demonstrated that this symbiosis between diatoms and bacteria had a direct impact on the overall biofilm network and was an essential driving factor for biofilm stability and biostabilization. Considering both the maximum in biofilm stability during spring and the high correlation between Fo and T3, this study might

be the first to report a specialized bacterial community with high stabilization potential. The trigger for this specialization might be the influence of the diatoms. However, parallel to the diatom community, the bacterial community in the inoculum is probably even more essential because its composition is also significantly influenced by seasonality (Karrasch *et al.*, 2001; Yannarell & Triplett, 2005). In addition, it directly impacts species composition in “river snow” (Böckelmann *et al.*, 2000) as well as in biofilms (Brümmer, Fehr & Wagner-Döbler, 2000; Brümmer, Felske & Wagner-Döbler, 2003; Lyautey *et al.*, 2005).

Diatom community

The diatom community showed typical representatives of European freshwater habitats (Battarbee, 1986; Krammer & Lange-Bertalot, 1986-1991; Hofmann, Werum & Lange-Bertalot, 2011) with *A. min.*, *S. sem.* and *E. min.* being considered typical pioneer species that are primary colonizers of fresh uncovered sediment (Peterson, 1996). During the course of the year, clear evidence for the seasonal succession process within the diatom community was detected. Considering the effect of the diatom community upon biostabilization, the morphology and life forms of the different diatom species in the biofilm appears to be an essential influencing factor. In this context, many studies have examined the process of diatom locomotion through the biofilm and sediment via EPS secretion (Paterson, 1989; Smith & Underwood, 1998; Wang *et al.*, 2013), as well as its importance as a survival mechanism in a dynamic habitat e.g. shifting sediment surfaces, water depths or underwater light intensities (Stal, 2003; Underwood & Paterson, 2003; Apoya-Horton *et al.*, 2006). However, while knowledge on the physiological and molecular biological reaction of selected diatoms to their environment and stress such as nutrient limitation or trace elements is constantly growing (McKay, Geider & LaRoche, 1997; Kudo *et al.*, 2000; Dyhrman *et al.*, 2012) the various effects of their movement upon the biofilm matrix and biostabilization are currently barely addressed. Nevertheless, parallel to the observations that macrozoobenthos migration can disturb biofilm stability (de Deckere, Tolhurst & de Brouwer, 2001) it can be assumed that the movement of large motile diatoms may impact the overall biofilm structure. Besides a potential positive effect upon the growth of heterotroph microbes degrading the mucilage trails this microbioturbation can increase the microroughness of the biofilm surface resulting in higher turbulence and erosive forces affecting the biofilm. This might destabilize the biofilm as stated by Gerbersdorf and Wieprecht (2015). To investigate possible effects of the diatom community compositions during the different seasons upon the biofilm structure, surface area and volume of the detected diatoms were calculated

(Hillebrand *et al.*, 1999; Sun & Liu, 2003). It became clear that *N. font.* and *N. diss.* dominated autumn biofilms, with up to eightfold higher biovolume and up to sevenfold higher surface area than smaller diatoms (e.g. *A. min.*, *S. sem.* or *E. min.*) which were more abundant during spring and summer. In contrast to *A. min* which represented a big fraction of the microphytobenthos during spring and which is known to be immotile and to attach permanently to the sediment via short EPS stalks (Potapova & Hamilton, 2007), different *Niztschia* species such as *N. font.* and *N. diss.* were described to be highly mobile (Lange et al. 2011). Different studies could show that there are major differences in the composition of sugar monomers and the overall structure between adhesive stalks, the mucilage capsule and EPS strands involved in locomotion (Wustman, Gretz & Hoagland, 1997; Wustman *et al.*, 1998; Wang *et al.*, 2000). There are strong indications that the different forms of attachment induced either by immotile diatoms which permanently glue to sediment grains or by constantly migrating specimens may be represented in EPS composition. Although the complex relationship between EPS composition and biofilm stability is still not fully understood, this might constitute one possible explanation for the observed significantly different adhesive capacities and biostabilization of biofilms. Evidence for this might be obtained in the results of recent studies: Higgins et al. (2003) described clear differences in EPS coating of different species of diatoms: while some species known to be persistent fouling organisms such as *Crasepdostauros australis* E. J. Cox showed the whole cell surface covered in a thick compressible layer of EPS, the apparently more mobile *Nitzschia navis-varingica* Lundholm et Moestrup displayed only few EPS strands near the raphe region. It was suggested that this arrangement of EPS may play an important role for the biofilm network on a bigger scale and connection of structural elements in the extracellular matrix – especially in a range very near to the cell surface. Other studies (de Brouwer & Stal, 2002; de Brouwer *et al.*, 2005; Molino *et al.*, 2006) demonstrated that the features of produced EPS are greatly influenced by the physiology and species of diatoms i.e. distinct types of diatoms produced different quantities and compositions of EPS with diverse viscoelastic properties and adhesive features which can affect the structural stability of the whole biofilm. Even if these results were gathered via lab experiments with some model diatoms and may not cover all processes in a natural system, these findings are essential as they indicate a link between species composition, EPS production and overall biofilm stability.

4.2.3 Conclusions

To sum up, the presented experiments could evidently demonstrate the clear seasonal changing biofilm stability with a maximum during spring and a minimum during autumn/winter. Interestingly, this result was clearly reflected in seasonal changes in the community composition of the biofilm diatoms and their corresponding lifestyles. Moreover, this unique comprehensive assessment could unravel various complex interactions of the EPS in the biofilm matrix, the responsible microbial producers and the overall effect on the biofilm and sediment stability.

These observations have great implications for further investigations into biostabilization as the detected seasonality clearly affects comparability of results and experimental setups and data analysis have to be adapted to the significantly different development of biofilm during the different seasons. Moreover, only further analyses such as a combination of mass spectrometric and transciptomic approaches may elucidate e.g. which adhesive proteins are produced when, how and by which microbes. Furthermore, deeper investigations into the bacterial key players via clone libraries and sequencing were found to be necessary to increase the understanding of driving factors influencing the overall biofilm functionality and biostabilization.

4.3 The effect of light intensity and shear stress on microbial biostabilization

4.3.1 Driving factors for development of the cultivated biofilms

The decisive factor for biofilm development and resulting biofilm stability was the reaction of the microbial community to the present boundary conditions. Three of these parameter - illumination, temperature and hydrodynamics - were relatively easy to set up, keep constant and control. However, the development of the microbial community is influenced by various complex interactions between abiotic and biotic conditions e.g. the competition of different taxa and species for nutrients and light as energy source. Furthermore, the microbes influence their environment e.g. due to the steadily changing biofilm morphology (Besemer *et al.*, 2007). This is the reason for the extended analysis of the community of biofilm bacteria and algae as well as the identification of functional key players. However, the question how the experimental setup and the arrangement of all applied experimental conditions – besides the investigated effects of different levels of shear stress and illumination intensity- influenced the resulting biostabilization is of great interest.

Hydrodynamic regime

The influence of the hydrodynamic regime was one of the two investigated essential abiotic boundary conditions. Due to the high importance of a fully developed, homogenous turbulence over the test section of the flumes the experimental setup was designed and constructed with knowhow of engineering science (see sections 2.1 and 3.1.3). Generally, hydrodynamics or flow velocity respectively can have a major, very visible and graspable influence upon benthic microbial communities. As an example, different structures in rivers that influence shear stress were demonstrated to have massive impact on the microbial composition of biofilms, e.g. recessed areas were shown to promote the growth of long filamentous chlorophytes, which were not abundant in exposed areas (DeNicola & McIntire, 1990). This example demonstrates the great potential of the hydrodynamic boundary conditions to shape the living conditions of biofilm microorganisms: one the one hand, the biofilm is diffusion limited in its uptake of nutrients and an increased turbulence can reduce the diffusive boundary layer. This can accelerate the mass transfer from the overlaying water column (Horn & Hempel, 1998; Holtappels & Lorke, 2011) - especially mass transfer rates into and within biofilm voids (Rasmussen & Lewandowski, 1998). This diffusion limitation may be one reason why very thick biofilm (Rasmussen & Lewandowski, 1998) or biofilm developing in waters with very low flow velocities tend to develop filamentous structures called “streamers” which undulate to increase turbulence and therby the transport rate of nutrients to the biofilm cells (Stewart, 2012; Gashti *et al.*, 2015). On the other hand, high levels of shear stress on the sediment and biofilm surface can constitute o stressor for biofilm formation. Not only does the shear force determine bacterial detachment (Fink *et al.*, 2015) and was shown to promote biofilm erosion/ sloughing (Moreira *et al.*, 2015), it also shapes the community structure of all kinds of other microbes such as algae and protists (Willkomm *et al.*, 2007; Pohlon, Marxsen & Kuesel, 2010). In addition, as shown in section 3.3.2, high levels of shear stress can strongly delay the formation of natural complex biofilms on fresh un-colonized sediment which was also reported by Coundoul et al. (2015). One possible explanation may be the complication of bacterial settlement and thus, the initial phase of biofilm formation (Lemos *et al.*, 2015). Although established bacterial biofilms may be able to react to present levels of shear stress and adapt their morphology (Peyton & Characklis, 1993; Choi & Morgenroth, 2003), if the initial bacterial settlement is impaired by very high shear forces near the substrate, the next succession steps in biofilm formation are impeded, too. A reason for this delay in biofilm formation may be found in a variety of complex interactions between bacteria and algae during the nascent stages of a biofilm. The initial

development of a bacterial biofilm as a “conditioning layer” on the sediment was described to be essential for a subsequent settlement of larger microbes such as diatoms (Blenkinsopp & Lock, 1994; Roeselers, Van Loosdrecht & Muyzer, 2007). Furthermore, impairment of the bacterial community can also strongly influence the community composition and morphology of the diatom community in a developed biofilm (D'Costa & Anil, 2014).

Considering these ambivalent impacts and as described in section 3.3.2, a medium level of flow velocity appears to constitute an optimal situation for biofilm growth (sufficient nutrient supply and tolerable shear forces). Interestingly, bacteria seem to be less susceptible to shear stress than diatoms (decrease in mean chlorophyll *a* contents with increasing flow velocity ca. 50% compared to approximately stable BCC under the highest BSS). A reason for this may be found in the fact that the amount of contact events of cells and substrate is increased with growing turbulence and flow velocity. Especially the first contact and the number of contact events of cells and the substrate are of great importance as the microbial cell itself first reacts as an inert particle (Hermansson, 1999) while near surface forces such as Brownian motion can augment surface drag to the substrate (Li, Tam & Tang, 2008). However, within seconds after contact to the substrate, bacterial cells can initiate multiple very fast and short reversible bindings which apparently trigger irreversible binding to the surface (Hoffman *et al.*, 2015). In this transition process of reversible to irreversible binding, bacterial flagella play an essential role as an instrument for the bacteria to actively control the attachment through rapid changes in genetic and metabolic pathways (Deweger *et al.*, 1987; Bruzaud *et al.*, 2015; Yoshihara *et al.*, 2015). Furthermore, the influence of flagella as important components for active attachment was extended by Gu et al. (2016) as it could be demonstrated that flagella response on microscale topography of the substrate and are a driving factor for the orientation of the attached cell. When single cells are irreversible attached to the surface and start to proliferate, their produced EPS augment the development cell-cell adhesiveness that can be even higher than the adhesion forces between cells and the substrate (Fang, Chan & Xu, 2000). This could explain, the erosion of greater biofilm sheets rather than small biofilm flocks during determination of erosion resistance via SETEG measuremts. These findings suggest that in comparison to other bigger microbes (such as diatoms), bacteria are adapted to faster colonization and attachment and a shorter residence time in the biofilm.

Furthermore, the hydraulic regime may have had an additional influence upon the biofilm development which can become clear when differentiating between large scaled and micro

scaled flow patterns. While the overall flow velocity and bed shear stress affects the general settlement and detachment of biofilm compounds as described above, micro scaled characteristics of the flow (e.g. micro stalls and eddies) and biofilm structures influence each other in a strong reciprocal way (Karimi *et al.*, 2015). As a consequence, the hydrodynamic regime should be seen as a driving factor for small scale heterogeneity of the biofilm: high shear stress may impair and delay biofilm development in a general way and prevent the biofilm from growing large structures into the running water due to mechanical forces ripping off too exposed components. However, the most important micro scale flow regime near the bed can be impacted by very small biofilm structures which in turn influences the boundary conditions (flow velocity, nutrient advection) for near spots on the sediment surface. These small changes can be very crucial as they may constitute the small difference determining if cells can stay in touch with the surface long enough to permanently adhere in that spot. Thus, biofilm patchiness is increased with flow velocity as a result of a higher impact of small scale topographical differences upon near bed current.

Illumination intensity and nutrients

In contrast to the effects of shear stress, the influence of light intensity and nutrient availability upon the development of the biofilm is rather convoluted because these two parameters cannot be regarded strictly separated as light is the primary energy source for autotrophic organisms and their photosynthesis is directly linked to the growth in biomass and the uptake of nutrients (Geider, MacIntyre & Kana, 1998; Li *et al.*, 2010; Prieto *et al.*, 2016). Other studies (Lange *et al.*, 2011; Bowes *et al.*, 2012) even emphasized that illumination intensity is the most important driving factor for the development of the algal community structure – even more important than nutrient (phosphorus) limitation or the effect of grazing. Although this was supported by Zippel and Neu (2005) who expanding these observations by the effects of illumination upon the overall biofilm structure, microalgae are not the only functional key players. The complex interactions between autotrophic and heterotrophic microbes have to be taken into account as they shape the overall biofilm system. As described above, pioneer bacteria are crucial for diatom settlement. However, this is just one small facet as a complex competition for nitrogen and phosphorus between diatoms and bacteria shapes the entire biofilm food web (Havskum *et al.*, 2003) and is apparently strongly affected by the amount of available organic carbon. Moreover, recent studies gathered insights in very specialized bacterial EPS utilization

(Sack, van der Wielen & van der Kooij, 2014) and a strong regulatory role of these EPS produced by diatoms upon bacterial development and activity (Agogue *et al.*, 2014).

The importance of nutrient limitation for river bed communities could recently be demonstrated via a large comprehensive set of field experiments (Reisinger, Tank & Dee, 2016) which could link the type of land use and resulting nutrients runoff into limnic systems to biofilm productivity. However, it is still hardly addressed in which way nutrient competition between different species and taxa impacts the community composition of biofilms. Jackson et al. (2001) suggested that resource competition may not be the primary driving force for the development of the bacterial community structure. In fact, in the mentioned study, bacteria were shown to react more sensitively to abiotic niches such as temperature gradients. In contrast to this, nutrient and light competition was described as essential for algal communities (Hillebrand & Sommer, 1997). In this context, the attachment form of the algal cells was demonstrated to be of major importance. Biofilm morphology and diffusion limited nutrient uptake and nutrient cycling often influence each other in a complex reciprocal feedback relationship (Mulholland *et al.*, 1994). As a consequence, erected species can access nutrients from the water column overlying the nutrient- depleted periphyton, while microalgae which live adnate to the surface (such as diatoms) might be excluded from the water column as nutrient source (Riber & Wetzel, 1987; Paul & Duthie, 1989; Burkholder, Wetzel & Klomparens, 1990; Mulholland *et al.*, 1994). Some diatom species adapted to this disadvantage by faster reproduction and the ability to colonize new habitats very quickly, before they are suppressed by later successional stages which are more competitive (Peterson, 1996). Other diatom species are able to avoid suppression via the ability to relatively fast active movement (Hay, Maitland & Paterson, 1993). These different life styles and attachment forms may have an essential impact upon the overall biofilm stability as flat attaching microalgae species were shown to exhibit significantly greater resistance to mechanical forces than erected specimen (Poff & Ward, 1995) and the movement of a great fraction of biofilm diatoms trough the matrix might have a destabilizing effect (see chapter 3.2.3). Taking into account these findings and the high complexity of the constantly changing and adapting biofilm community, the great importance of the temporal scale becomes plain as different organisms may be dominant at different points in time. This is the reason for the high temporal resolution during sampling which allowed a detailed insight into the microbial community.

Regarding the cultivation conditions in the flumes, as nutrient levels in the water taken from the River Enz were constantly low to moderate during the course of the year, erected species might have had an advantage after long biofilm development. However, during spring where biostabilization displayed a maximum, pioneer diatoms were dominant in the gathered river water which is a result of the natural seasonal succession in the river (Battarbee, 1986). In addition, these early successional stages such as pioneering small diatom species, such as the permanently attaching *Achnanthidium minutissimum*, had a competitive advantage on the fresh, un-colonized sediment surfaces due to their faster reproduction and in higher flow regimes due to their small profile and strong attachment. Thus, especially during spring and in the observed period of time during the experiments, early successional stages had all prerequisites to dominate the diatom community before later succession stages could establish dominance (which was indicated during autumn which a dominance of *Nitzschia* specimen in matured biofilms) The fact that only relatively few diatom species were dominant throughout the experiments might be due to experimental setup as a closed system without the addition of new fresh river water during running experiments. This closed system in combination with nutrient limitation may have led to the same development described by Law et al. (2014) who reported only two diatom species dominant in very oligotrophic conditions even if a large colonization pool could be found. This nutrient limitation was stated to be more important than flow velocity or grazing as it constitutes a selective factor favoring the most effective nutrient assimilation capacity. Furthermore, this might be one reason for the dominance of the bacterial community by *Leptolyngbya* whose ability to fixate great amounts of atmospheric nitrogen (Havens *et al.*, 1996) could have resulted in a decisive competitive advantage against other autotrophic microorganisms.

4.3.2 Potential indicators for biofilm stability

Considering bacterial species, as mentioned before, many examples were found independent of boundary conditions and in biofilms of varying biostabilization capacity which suggests that these microbes may have no direct influence upon biofilm stability. However, a comprehensive investigation of the microbial community composition across the different seasons and boundary condition revealed clearly distinct dominance patterns. In biofilms with very high stability, a variable combination of 6 different bacteria dominated the community. Among these 6 organisms were two cyanobacteria - *Leptolyngbya sp.* and *Pseudoanabaena biceps,* two alpha proteobacteria - *Paracoccus aminophilus* and *Rhodobacter capsulatus* – and two beta proteobacteria – *Rhodoferax saidenbachensis* and

Rubrivivax gelatinosus (see ***Table 16*** in the appendix). Interestingly, these species were not dominant or even absent in biofilms which exhibited significantly lower stability. Instead, three bacterial species clearly dominated biofilms with comparable low biostabilization capacity: *Pseudomonas fluorescens*, *Pseudomonas taeanensis* and *Caulobacter vibrioides*.

4.3.3 The role of microorganisms for biofilm stability

All bacterial species which were dominant in very stable biofilms had at least one of two common features: they were either phototrophic and/or had very versatile metabolisms. Besides the two cyanobacteria of the genus Leptolyngbya and Pseudoananaena, *Rhodobacter capsulatus* was shown to be capable of anoxic photosynthesis, N-fixation and various other metabolic pathways including different types of respiration (Tichi & Tabita, 2001). In addition, *Rubrivivax gelatinosus* was described as a very fast growing facultative photoheterotrophic microorganism (Wawrousek *et al.*, 2014). *Paracoccus aminophilus* and *Rhodoferax saidenbachensis* can be considered as very adaptable microorganisms with a broad range of different respiration pathways (Urakami *et al.*, 1990) and the ability to utilize various algal secondary metabolites such as sugar alcohols (Kaden *et al.*, 2014) which can be considered as constantly present in the EPS matrix due to secretion of algal cells or as a result of cell death and lysis. This metabolic versatility might be an essential prerequisite for these microbes to utilize different niches, e.g. gradient zones, in the biofilm system which became increasingly complex due to diatom development. In this context, it is interesting to note that *Rubrivivax gelatinosus* apparently dominated early and maturing biofilms developing under high illumination intensity up to a point where diatom proliferated in the system. In matured biofilms *Rubrivivax gelatinosus* apparently was outcompeted which was clearly visible on DGGE fingerprints. This replacement may be a result of the increasing competition for light as primary energy source due to the multiplying diatoms and other phototrophic bacteria like the detected cyanobacteria. However, besides other bacterial species *Rubrivivax gelatinosus* might play an essential role in conditioning the sediment surface facilitating a later diatom settlement and development as described for other heterotrophic bacteria (Roeselers, Van Loosdrecht & Muyzer, 2007).

Moreover, the fast growth and the potential for high EPS production of *Rhodobacter capsulatus* and *Paracoccus aminophilus* (Onder *et al.*, 2010; Brimacombe *et al.*, 2013; Dziewit *et al.*, 2014) may lead to a stable biofilm matrix tightly attached to the sediment grains – especially in crucial early development stages. This could be of major structural

importance for the overall biofilm system and lead to higher biofilm stability. Considering these structural aspects, the two detected dominant cyanobacteria might also be of high relevance as they form long filaments which are up to 5 mm long (Kanellopoulos *et al.*, 2015). On the on hand, these extended chains of single cells constitute an option to increase micro-turbelation and availability of nutrients. On the other hand, these fibers can act as anchor points for settling cells and can be linked and tangled up which can increase the stability of the overall biofilm network.

In contrast to biofilms displaying very high stability, biofilms with low biostabilization capacity were dominated by bacteria of the genus *Caulobacter* or *Pseudomonas*. A very important characteristic which differentiates members of these genus from the earlier mentioned species proliferation in very stable biofilms is a very high motility. This feature is essential for fast colonization of favorable substrates and new nutrient resources (de Weert *et al.*, 2002). However, the lifestyle of the detected dominant *Caulobacter vibrioides* might decrease overall biofilm stability as the anisomorph reproduction cycle with staked and swarmer cells (Henrici & Johnson, 1935) can be assumed to lead to a steadily shifting, comparable unstable cover with bacterial cells in opposite to a colonization by immotile bacteria such as *Paracoccus aminophilus* which forms stable clusters of cells (Urakami *et al.*, 1990). The swimming behavior of the two dominant monotrichous *Pseudomonas fluorescens* and *Pseudomonas taeanensis* was described as very similar to *Caulobacter* specimen (Ping, Birkenbeil & Monajembashi, 2013). However, in contrast to *Caulobacter*, for *Pseudomonas* the flagellum is of major importance for surface adhesion as the initial step of biofilm formation (Mastropaolo *et al.*, 2012). Moreover, Decoin et al. (2015) could demonstrate the expressional link between flagellum compounds such as flagellin and the production of antimicrobial agents and suggested that parallel motility and antibiotics production enhances competitiveness. The well described antibiotics production by different members of the genus *Pseudomonas* (Raaijmakers, Weller & Thomashow, 1997) may effectively suppress bacterial competitors and might be another explanation for the decreased stability of the biofilm matrix. Especially, the fact reported by Duffy and Defago (1999) that algal secondary metabolites such as sugar alcohols can be used by *Pseudomonas* to increase the antibiotic production is very interesting. Although the abiotic factors should not be ruled out as influencing factors, it might be possible that in a complex multispecies biofilm, the *Pseudomonas* specimen might start their chemical warfare against competing bacteria and thereby delay the maturation of the biofilm. First, the settlement of algae could be delayed in early biofilms due to a lack of sufficient bacteria creating favorable

surface features. Subsequently, when the biofilm system gradually changes by the development of algae, *Pseudomonas* might be able to increase their antibiotic production due to algal exudates. This may increasingly impact symbiotic bacteria of the algae (Amin, Parker & Armbrust, 2012) or the species found in dominant abundances in very stable biofilms. Structural consequences with implications for the stability of the overall biofilm system might be possible. An alternative might be a frequent migration to better suited sediment patches which could disrupt the biofilm matrix when a great fraction of bacteria changes into motile state and leaves the sediment surface.

4.3.4 The impact of the cultivation conditions in the used mesocosm setup

It can be expected that beside the strictly controlled and controllable boundary conditions during the experiments, a number of simplifications in the used setup and limitations in the approach additionally influenced the gathered results. These impact factors can be divided in abiotic and biotic boundary conditions during the experiments. The simplifications in abiotic conditions concerned the constant levels of temperature, illumination and flow velocity throughout the duration of the experiments which were essential for reproducibility of results. Generally, a highly dynamic system promotes a higher diversity due to the establishment of various spatial and temporal ecological niches. This may be a reason why a higher bacterial diversity could be detected in rivers than in lakes (Pascault *et al.*, 2014). Furthermore, niches and temperature gradients can be very important impact factors for the bacterial community (Lear *et al.*, 2014), whereas a lack of dynamics in terms of temperature and illumination may have a stabilizing effect on the community of diatoms (Carrick & Steinman, 2001). These findings suggest that the lack of dynamics promotes a clearer development of dominant species in a more homogenous environment. As these boundary conditions were kept constant, the main driving factor for small scaled heterogeneity was the hydrodynamic regime whose micro flow patterns created a highly diverse environment. This is supported by the fact that increasing flow velocity resulted in higher dynamics in the bacterial community (see chapter 3.3.2).

Another simplification was the utilization of one type of inert sediment (glass beads of a diameter of 100-200 µm) as substrate for biofilm cultivation. While the inert character of the glass beads was essential to exclude potential influences of organic matters present in the sediment upon the biostabilization capability of the biofilm, the grain size of the chosen substrate is an unneglectable parameter. Although studies are rare that precisely

investigated substrate preference of diatoms - which constitute the functional key players in matured biofilms - there have been indications that mobile species are more abundant in soft substrate while sessile species dominate hard substrates (Potapova & Charles, 2005). In addition to this preference for muddy sediments, diatom species with a small profile may develop clear dominance in disturbance-rich environment (Passy, 2007). Besides the fact that biostabilization is of no relevance on gravels, rocks or wood, which was the reason for choosing these glass beads as substrate, the high dynamics in fine sediments (comparably low resistance towards erosion) might give mobile diatom species an important survival and completion advantage e.g. as they are able to resurface after being buried during sediment erosion. However, this possible disadvantage for sessile diatom species could not prevent the dominance of pioneer species such as *Achnanthidium minutissimum* during the spring experiments nor the dominance of different species of non-motile *Fragilaria* during the summer experiments. Thus, the type and grain size of the utilized sediment apparently had a minor influence upon the development of the microbial community.

Concerning the biotic conditions during the experiments, the most apparent simplification during the mesocosm experiments was the lack of macrophytes in the flumes. As a consequence, microbial algae developing in the water column and on the sediment surface had no competition for light which can have a significant reducing effect upon biofilm development (Gette-Bouvarot *et al.*, 2015). This lack of an important inhibitory effect might have enhanced the development of phototroph microbes. As all phototroph microorganisms are affected in the same way, the general succession in the biofilm can be assumed to have been accelerated: first the pioneer species profit from the increased illumination intensity and their faster reproduction. However, pioneer species can be outcompeted fast by more competitive later succession stages. Thus, the microbial community composition should not have been influenced in a significant way.

Another very apparent simplification was the exclusion of macrozoobenthos during biofilm cultivation via filtration of the river water through gauze. Generally, different species of grazers have a significant reducing effect on the biomass of algae in biofilms (Sturt, Jansen & Harrison, 2011; Schneck, Schwarzbold & Melo, 2013; Martina *et al.*, 2014; Schossow, Arndt & Becker, 2016). In this context, the abundance of grazers is apparently of greater importance than grazer taxon (Lamberti *et al.*, 1995). Furthermore, as grazers were shown to prefer specific growth forms or physiological stages of microalgae (Steinman, Mulholland & Hill, 1992; De Troch *et al.*, 2012), the community structure of algae in biofilms can be

significantly influenced and shaped. As a result, algal communities are driven to early successional stages under grazing pressure (Poff & Ward, 1995) as species with rapid reproduction can regenerate faster after being grazed. Moreover, the migration patterns of the grazers may significantly increase the spatial heterogeneity of biomass distribution in biofilms (Hillebrand, 2008). These findings demonstrate the important impact of grazers upon the algal community of biofilms. However, in the absence of grazing macrozoobenthos, a clear dominance of diatoms was recorded in benthic biofilms – especially under high illumination intensities (Steinman, 1992). This observation, besides the annual succession within the community of microalgae in the river, may explain the development of the community structure of the biofilm algal communities during the flume experiments of this study - especially during spring. Thus, the exclusion of grazers in the performed flume experiments might have resulted in a competition advantage for slower reproducing microalgae. This may have promoted the growth of big diatom species such as the members of the family *Nitzschia* or the even bigger *Fragilaria*. As diatom migration may have had an adverse effect on biofilm stability (see section 3.2.3), biofilm under moderate grazing pressure might even establish higher stabilization potential, when the diatom community is driven to early successional stages consisting of sessile species such as *Achnanthidium minutissimum*.

4.3.4 Conclusions

This section demonstrated a significant impact of the investigated abiotic boundary conditions (light intensity and bed shear stress) on biofilm development. Besides having a clear effect on different biofilm parameters such as microbial biomass, produced EPS, and the microbial community composition, the environmental conditions significantly influenced biostabilization. In addition, the different levels of LI and BSS effected the biofilms in different ways. On the one hand, minimal LI resulted in a significant reduction of biofilm development as especially the growth of microalgae was impaired. This observation emphasizes the important role of primary production of autotrophic microbes in the biofilm matrix. On the other hand, the highest level of BSS caused a general delay in biofilm formation and biostabilization as the initial microbial settlement, especially of microalgae, was apparently limited while bacteria could adapt faster to the high dynamics in the habitat with very high flow velocity.

Moreover, it was possible for the first time to shed light on the role of different microbial key players and their respective mode of life during the process of biostabilization. In biofilms with very high stability, the dominant species among bacteria and diatoms were always sessile and/ or had the capacity to produce high amounts of EPS adhesives which is linked to fast reproduction and colonization of freshly exposed sediment surfaces. Thus, their lifestyle may have directly enhanced the stability of the biofilm system and the underlying fine sediment. In contrast to that, less stable biofilms were constantly dominated by opportunistic, very flexible microbes often associated with later successional stages. These microorganisms displayed a high degree of mobility and the potential for elaborated forms of chemical warfare. As a result, these specific species may have profited from these opportunistic strategies and had a selective advantage but their dominance apparently had adverse effects on other microbes with a higher stabilizing effect, and the overall integrity of the biofilm matrix. This observation may reflect the unstable, ever changing nature of a matured biofilm system which is characterized by a constantly high degree of attachment and detachment as well as biological, chemical and structural reorganization.

5. General Conclusion

In this experimental setup, no statistically significant differences could be detected in any biofilm parameters during inter- or intra- flume comparisons. Furthermore, biofilm formation as well as biostabilization were very similar under the same boundary conditions. It has to be emphasized how crucial this result is for all further considerations as this constituted the scientific proof for the reliability of this mesocosm: The fact that identical abiotic boundary conditions resulted in very similar biofilms was an essential prerequisite for the investigation of different influencing factors – as seen in the sections 3.2 and 3.3 – and allowed a first elucidation of some fundamental principles of riverine microbial biostabilization.

1. Microbial biostabilization is not only of essential relevance in marine and brackish habitats but also in lotic systems. Compared with biofilms from intertidal mudflats, biofilms in the presented flume mesocosmos displayed lower biomasses. Reasons for this may be higher nutrient levels and longer maturation of the biofilms on the researched brackish or marine sediments. However, throughout the experiments in the flume mesocosmos, a high degree of biostabilization could be detected in developing biofilms– even under boundary conditions of minimal nutrient supply - as inert glass beads were used as substrate and the river water had a minimal nutrient load. The tested sets of environmental conditions allowed only a first

but very important insight in the huge variety of different possible combinations of boundary conditions in the environment as these results demonstrated the significance of microbial stabilization of fine sediments in rivers.

2. The extracellular polymeric substances (EPS) play an essential role during the process of biostabilization. Presently, it is generally assumed that EPS are the main responsible factor for the gluing and stabilizing effect of biofilms on fine sediments. As carbohydrates generally contribute the majority to the EPS, the main focus of many studies laid on further investigations of these components e.g. by analysing sugar monomers or functional groups. However, in the experiments of the present work, correlations between EPS protein content and biofilm adhesiveness were clearly higher than between EPS carbohydrate content and biofilm stability. This indicated the importance of extracellular proteins e.g. adhesive proteins produced by sessile diatoms despite their lower quantitative proportion in the EPS matrix compared to extracellular carbohydrates. In addition, this observation may highlight the different roles of carbohydrates and proteins in the extracellular matrix where small amounts of secreted proteins e.g. extracellular enzymes produced by bacteria can play an important structural and functional role.

3. High microbial biomass and EPS production enhances biostabilization as especially microalgae produce high amounts of EPS with stabilizing effect. High correlations between biofilm adhesiveness and algal biomass as well as bacterial cell counts could be detected which is supported by various studies describing the link between microbial biomass, the produced EPS, and resulting biostabilization. However, as the dominant species of algae and bacteria could clearly be correlated to different levels of biofilm stability, the composition of the microbial community appears to be of greater significance for biofilm stability than mere biomass. Especially a dominance of sessile bacterial and diatom species might be beneficial for the stability of the biofilm matrix while motile specimen can have a disrupting effect on the biofilm system. Furthermore, the high correlation between functional organization of the bacterial community and adhesiveness in matured biofilms emphasize the role of the symbiotic interactions of bacteria and diatoms, and the resulting shaping influence upon the bacterial community composition.

4. The seasonal changing microbial community has a significant impact upon biofilm development and corresponding biostabilization. The seasonal changing microbial biocenosis in the water column of rivers in moderate climate, e.g. the succession of diatoms,

green algae and cyanobacteria during the course of the year, is well known. The investigations described in section 3.2 could demonstrate how this seasonality significantly influenced especially the algal community of the developing as well as matured biofilms. In this context, it was very remarkable that a change of pioneer species to later successional stages in the diatom community was already sufficient to induce a significant decrease in biofilm adhesiveness. Reasons for this development might be found in the different lifestyles of early and late successional stages and different requirements of adaptation during the course of the year e.g. the need and possibility for fast reproduction in a situation without macrophyte competition during spring versus high competition and predation risk during summer and autumn. Thus, these results should be taken into account during further experiments aiming towards a deeper investigation of impact factors on biostabilization in rivers.

5. Abiotic boundary conditions – especially light intensity- have a significant impact upon the microbial development, production and sediment stabilization as photosynthetic microbes dominate the metabolic pathways in the biofilm system. The two investigated abiotic boundary conditions – light intensity (LI) and bed shear stress (BSS) – clearly differed in their influence on biofilm development. On the one hand, algal biomass and biofilm adhesiveness displayed clear rising values with increasing levels of LI. Especially the very low biofilm stability detected under minimal LI growth conditions demonstrated the essential role of algal EPS production as a nutrient source for heterotrophic microbes during the process of biostabilization in this flume system where no additional nutrient sources existed. On the other hand, increasing levels of BSS delayed biofilm development and biostabilization. The microbial community might be able to adapt to a rather unstable environment under high levels of BSS to a certain degree, as indicated by the constantly high dynamics in the bacterial community and high quantitative fraction of mobile diatom species that were detected in the biofilm matrix. However, as high biostabilization seems linked to earlier successional stages it remains questionable if a considerably longer experimental duration can result in comparable stabilizing effect as observed under the lowest level of BSS.

6. Outlook

Hydropower plays an essential role in the context of the turnaround in energy politics as well as a central renewable energy source for the future on a global level (Bartle, 2002) as its output is much more stable and controllable compared to other renewable energy sources such as solar or wind energy. Nevertheless, there are increasing signs that reservoirs may be significantly involved in the emission of great amounts of greenhouse gases such as methane e.g. due to frequently fluctuating water levels (Deemer *et al.*, 2016). A possible counter strategy for this issue may either be the avoidance of ebullition of greenhouse gasses from the consolidated fine sediments or the prevention of anaerobic conditions in reservoir fine sediments which is a prerequisite for fermentative processes such as methanogenesis. The simplest way to implement this approach may be less frequent flushing of the reservoir and the installation of deep water aeration systems which may be used to enhance the water quality of bottom near water layer, and the oxygen saturation of the bed. However, less flushing would facilitate the buildup of high fine sediment deposits and the establishment of matured biofilms with significant stabilizing effect upon fine sediments – as demonstrated in this work. Admittedly, this approach would clearly collide with the aim of power plant effectiveness. This emphasizes clearly the requirement of a comprehensive and optimized sediment management strategy which takes all different stakeholders into account.

Besides producing renewable energy, reservoirs play a central role in the flood protection of downstream settlements. They can buffer seasonal changing discharges in alpine rivers due to snow melting in spring and possible heavy rain events during late summer/ autumn. However, when the capacity of a reservoir is reached water has to be discharged to downstream river section which can have dimensions of a flushing event if weather conditions result in very high discharges. The demonstrated clear seasonality of the stabilizing effect of lotic biofilms with a significant maximum in spring and decreasing biostabilization through the course of the year strongly suggests that reservoir flushing should be performed during autumn or winter when biofilm and fine sediment stability in the reservoir is minimal. However, as the spawning time of salmonids, such as black or brook trout, is from November until March flushing events during this time of the year could have devastating effects on the survival of affected fish populations. Flushing in autumn may

colmate the gravel spawning grounds and render them unappropriated so that the oviposition of the fish is affected but flushing in late winter can cause colmation of the gravel pores and reduce the oxygen content of the habitat of the new born fish larvae (Carling, 1984). The protection of river and floodplain ecology is statutory regulation enacted by the European Water Framework Directive which demands “the good ecological state” of rivers. In this context, not only an appropriate chemical quality of surface water in rivers is targeted but also a state of the river ecosystem that is as close as possible to a natural condition. In a situation where most European rivers are fragmented by numerous dams and weirs, different compensatory measures to improve the passage of fish and other aquatic organisms have been taken and are being implemented. Though, these procedures are often very expensive or not always fully satisfactory (Tummers *et al.*, 2016). In many cases an undisturbed migration is still not possible and different organizations try to compensate this issue via stocking measures.

The already discussed colmation of river sediments may not only impact the reproduction of gravel spawning fish, it could also greatly influence the community composition and the metabolism of sediment microbes parallel to the reported effects on affected benthic invertebrates (Descloux, Datry & Usseglio-Polatera, 2014). Thus, possible fermentative products as well as opportunistic pathogens might reach the groundwater via the hyporheic interstitial and may affect the quality of drinking water (Abia *et al.*, 2016). Moreover, high discharges and flow velocities - especially in straightened rivers sections- can cause massive sediment transport and deepening of the river bed which may result in a lowering of groundwater levels which can have effects on the vegetation of the catchment area and nearby agricultural areas. Thus, reservoir management may have great implications for human health as well as agriculture and has to account for the long-term assurance of ground water and drinking water quality. One possible alternative to avoid an overall flushing campaign would be the installation of underwater structures, e.g. propellers, to increase near bed turbulence and shear stress to avoid low flow velocity and to delay biostabilization. Thus, a series of smaller flushing events might be able to transport the deposited fine sediment load without major casualties in macrozoobenthos and fish or impacting the quantity and quality of ground water and drinking water.

However, the effectiveness and economic profitableness, the impact on afore mentioned greenhouse gas emissions, as well as the effects on the downstream river reaches of such a strategy should be thoroughly investigated beforehand. This dilemma reflects a small part

of a current and ongoing discussion about the hierarchy of different objects of protection and overall goals in environmental and energy politics where it can be very difficult to find a satisfying compromise for the interests of various different stakeholders. A sustainable solution should incorporate strategies to increase the passability of both, river sediments as well as migrating aquatic organisms, while guaranteeing the economical effectiveness of water power plants.

New impulses to optimize sediment management strategies might be created especially due to insights gathered during the seasonality experiments and during the investigations of the impacts of abiotic boundary conditions. This concerns all three essential steps in the process of developing a successful sediment management concept: measuring, monitoring and modelling. First, the effects of seasonality as well as different boundary conditions on the development of biofilms and their stabilization capacity were actually measurable. Not only was this crucial for any further, deeper analytic steps. This fact suggests that biofilm formation and stabilization of riverine fine sediments can be controlled by setting the boundary conditions. For further endpoint-orientated investigations this knowledge may already suffice e.g. due to time restrictions, the assessment of the impact of different nutrient levels could only be performed in smaller subprojects without a reliable statistical data base and therefore should be repeated. In addition, experiments should be performed with natural sediments and water from the corresponding river/ reservoir to account for the specific situation e.g. of the actual nutrient supply and the microbial community in this habitat.

A cost-effective, fast analysis in monitoring approaches may be facilitated by the finding that the stabilizing effect of the biofilms displayed strong correlations to other biofilm parameters. The straightforward determination of microbial biomass and EPS contents which displayed high correlations to biofilm adhesiveness (see ***Table 8*** and ***Table 15***) as well as the measurements of biofilm adhesiveness which in turn was highly correlated with the erosion resistance of the colonized substrate (Thom *et al.*, 2015a) may provide additional, more sensitive information than common techniques used in monitoring. Moreover, these investigations may even constitute appropriate surrogates for difficult and very work and time consuming standard measurements of sediment stability e.g. utilizing erosion flumes such as SETEG in areas which are hardly accessible or in cases where fragile fine sediment cores would have to be transported over long distances.

The final step in a sustainable sediment management strategy would be the establishment of a suitable model to simulate biofilm formation and corresponding biostabilization. This modelling approach would require a broad data base and a comprehensive as well as very detailed knowledge about all relevant processes and their role for the microbial stabilization of fine sediments. The presented work could provide a first starting point for this comprehensive understanding as a central research focus was the investigation of the microbial ecology of the cultivated biofilm. The fact that this work is the first to correlate the microbial ecology with the overall ecosystem function of the biofilm system demonstrates the requirement of further interdisciplinary research to unravel all essential processes. One of the most important findings of the microbiologic and genetic investigations might be the clear indication of specific dominance patterns among bacteria as well as diatoms which had a significant correlation with biofilm stability. This strongly suggests the essential role of microbial key players whose productivity and lifestyle displayed a significant effect upon the biofilm matrix during the process of biostabilization. Furthermore, this work gave first insight in the various influencing factors such as boundary conditions or successional stage which exhibited a major impact on the abundance of these microbial key organisms. However, in order to incorporate this knowledge of the importance of certain microbial key players into possible future modelling approaches, more research is still required: While diatom determination is well established and standardized, identification of bacterial key players could be optimized e.g. by meta genomic and third generation sequencing approaches which avoid possible polymerase biases.

In addition, the exact role of key players among bacteria and microalgae in the biofilm matrix should be further investigated. As an example, the determination of the biofilm adhesiveness could be coupled with an assessment of metabolic productivity e.g. via determination of the activity of intra and extracellular enzymes in different biofilm layers. Furthermore, the produced EPS matrix could be characterized in more detail e.g. proteins via SDS-PAGE and mass spectrometry of prominent bands. These and other investigations could constitute the foundation of future short- and long-term modelling approaches with the overall aim to predict the development of the biofilm microbial community under certain circumstances e.g. initial light intensity, initial bed shear stress, initial nutrient supply, initial biofilm pioneers (heterotrophic bacteria) and floating microbes in the water column.

To summarize, the insights into the fundamental principles of the process of biostabilization gathered during this work constitute important results for further fundamental research. The

first essential steps have been taken: the construction of a reliable unique setup, the evaluation of reproducible biofilm cultivation in this setup as well as first investigations of different driving factors during the process of biostabilization. These investigations paved the way for further studies e.g. to analyze currently hardly assessed boundary conditions such as different nutrient levels. Moreover, first links for possible applications of the gathered knowledge could be derived. In addition, specialized analytic approaches e.g. metagnomic and metabolomic techniques may be applied in order to set the foundation for future modelling approaches.

7. Appendix

Table 9: Detected diatom species with minimal abundance of 1% of total counted frustules.

Taxon	spring		summer		autumn	
	day 11	day 28	day 11	day 28	day 11	day 28
Achnanthidium minutissimum var. minutissimum	+	+	+	+	+	+
Amphora pediculus	+	+	+	+	+	
Cocconeis placentula					+	
Cyclotella menighiana			+	+	+	+
Cyclotella sp.			+	+	+	+
Diatoma moniliformis var. moniliformis	+					
Diatoma vulgaris	+					
Encyonema silesiacum	+	+			+	
Eolimna minima	+	+	+	+	+	+
Eolimna subminuscula	+		+	+		+
Fragilaria construens f. venter		+			+	
Fragilaria pararumpens		+			+	
Fragilaria pinnata					+	
Gomphonema olivaceum					+	
Mayamaea atomus var. permitis	+	+	+	+	+	+
Navicula gregaria	+	+	+		+	+
Navicula lanceolata	+				+	
Navicula reichardtiana					+	+
Navicula veneta					+	
Nitzschia abbreviata			+	+	+	+
Nitzschia acicularis	+		+	+	+	
Nitzschia amphibia						+
Nitzschia capitellata	+		+	+		
Nitzschia constricta			+		+	
Nitzschia dissipata var. dissipata	+	+	+	+	+	+
Nitzschia fonticola	+	+	+	+	+	+
Nitzschia inconspicua					+	
Nitzschia linearis	+			+		+
Nitzschia palea var. debilis	+		+	+	+	+
Nitzschia palea var. palea			+	+	+	
Nitzschia paleacea	+		+			+
Nitzschia sp.						+
Nitzschia supralitorea	+		+		+	+
Pennate unident.	+				+	
Planothidium frequentissimum	+		+	+	+	
Sellaphora seminulum	+	+	+	+	+	+
Surirella brebissonii var. brebissonii						+
Surirella brebissonii var. kuetzingii	+	+	+			+
Surirella minuta			+		+	+

Table 16: Bacterial community of differently stable biofilms.

Biofilm adhesiveness	Bacterial strain
Max	**Leptolyngbya sp. AM_398976.1**
Max	**Paracoccus aminophilus JCM 7686 plasmid pAMI1**
Max	**Pseudanabaena biceps PCC 7429 scaffold_00102**
Max	**Rhodobacter capsulatus SB 1003**
Max	**Rhodoferax saidenbachensis ED16 contig64**
Max	**Rubrivivax gelatinosus IL144 DNA,**
Max	Methylibium petroleiphilum PM1
Max	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C
Max	Rhizobium mesoamericanum STM3625 genomic scaffold, ANZ_1636
Max	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1
Max	Gemmatimonas phototrophica strain AP64 Contig_6
Max	Brevundimonas subvibrioides ATCC 15264
Max	Hydrogenophaga sp. Root209 contig_13
Max	Sphingopyxis sp. MC1 contigEBMC1_3
Max	Gemmatimonas aurantiaca T-27 DANN
Max	Hydrogenophaga sp. Root209 contig_13
Max	Gemmatimonas phototrophica strain AP64 Contig_6
Max	Beta proteobacterium AAP65 AAP65_Contigs_64
Max	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92
Max	Beta proteobacterium AAP65 AAP65_Contigs_64
Max	Aquabacterium sp. NJ1 contig01
Max	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92
Max	Methyloversatilis universalis Fam500 Metunv3DRAFT_Contig245.1_C
Max	Alpha proteobacterium AAP81b AAP81b_Contigs_75
Min	**Pseudomonas fluorescens SBW25**
Min	**Pseudomonas taeanensis MS-3 contig_13**
Min	**Caulobacter vibrioides strain T5M6 contig_129**
Min	Aquabacterium sp. NJ1 contig01
Min	Fluoribacter dumoffii NY 23 chromosome
Min	Microbacterium sp. UNCCL10 BR85DRAFT_scaffold00001.1_C
Min	Gemmatimonas aurantiaca T-27 DANN
Min	Hyphomicrobium zavarzinii ATCC 27496 strain ZV-622
Min	Reyranella massiliensis 521 genomic scaffold, Scaffold01
Min	Hyphomicrobium zavarzinii ATCC 27496 strain ZV-622
Min	Gemmatimonas phototrophica strain AP64 Contig_6
Min	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1

Identified bacterial strains: Max: the highest biofilm adhesiveness; Min: the lowest biofilm adhesiveness. Bold written bacterial strain were found to be dominant in corresponding biofilms.

Table 17: Bacterial strains of analysed prominent DGGE bands.

Sample	Organism	Max Score	Total Score	Query Cover	E value	Ident	Accession
May13_Qme_end1 Seq 1	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	894	894	100%	0.0	98%	NC_009379.1
May13_Qme_end1 Seq 2	Candidatus Odyssella thessalonicensis L13 HMO_scaffold00002	619	619	100%	2,00E-174	94%	NZ_AEWF01000006.1
May13_Qme_end1 Seq 3	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	900	900	100%	0.0	98%	NC_009379.1
May13_Qme_end2 Seq 1	Gemmatimonas aurantiaca T-27 DANN	689	689	100%	0.0	91%	NC_012489.1
May13_Qme_end2 Seq 2	Gemmatimonas phototrophica strain AP64 Contig_6	917	917	100%	0.0	99%	NZ_AUXF01000006.1
May13_Qme_end2 Seq 3	Gemmatimonas phototrophica strain AP64 Contig_6	872	872	100%	0.0	98%	NZ_AUXF01000006.1
May13_Qme Seq 1	Gemmatimonas aurantiaca T-27	689	689	100%	0.0	91%	NC_012489.1
May13_Qme Seq 2	Brevundimonas subvibrioides ATCC 15264	737	1475	100%	0.0	95%	NC_014375.1
May13_Qme Seq 3	Ideonella sp. B508-1 DNA, contig: IB508.C5	795	795	100%	0.0	94%	NZ_BADL01000005.1
May13_Qme_end3 Seq 1	Synechococcus sp. PCC 6312	542	542	99%	5,00E-151	87%	NC_019680.1
May13_Qme_end3 Seq 2	Synechococcus sp. PCC 6312	558	558	99%	5,00E-156	88%	NC_019680.1
May13_Qme_end3 Seq 3	Synechococcus sp. PCC 6312	569	569	99%	3,00E-159	88%	NC_019680.1
May13_Qme_mid Seq 1	Anabaena sp. 90 chromosome chANA01	536	2666	100%	3,00E-149	87%	NC_019427.1
May13_Qme_mid Seq 2	Anabaena sp. 90 chromosome chANA01	531	2638	100%	1,00E-147	87%	NC_019427.1
May13_Qme_mid Seq 3	Anabaena sp. 90 chromosome chANA01	525	2611	100%	6,00E-146	87%	NC_019427.1
May13_Qlo_Qme_end Seq 1	Hydrogenophaga sp. Root209 contig_13	944	944	99%	0.0	99%	NZ_LMIE01000005.1
May13_Qlo_Qme_end Seq 2	Hydrogenophaga sp. Root209 contig_13	939	939	99%	0.0	99%	NZ_LMIE01000005.1
May13_Qlo_Qme_end Seq 3	Hydrogenophaga sp. Root209 contig_13	926	926	99%	0.0	99%	NZ_LMIE01000005.1
May13_Qme_Qhi Seq 1	Anaeromyxobacter sp. Fw109-5	320	641	100%	3,00E-84	78%	NC_009675.1
May13_ub1 Seq 1	Cyanothece sp. CCY0110 1101676644568	573	573	100%	2,00E-160	88%	NZ_AAXW01000006.1
May13_ub1 Seq 2	Sphingomonas sp. Root241 contig_2	737	737	100%	0.0	95%	NZ_LMIV01000002.1
May13_ub1 Seq 3	Reyranella massiliensis 521 genomic scaffold	854	854	99%	0.0	99%	NZ_HE997181.1
May13_ub2 Seq 1	Pirellula staleyi DSM 6068, complete genome	536	536	100%	3,00E-149	86%	NC_013720.1
May13_ub2 Seq 2	Ideonella sp. 201-F6 DNA, contig: ISCTG090	878	878	100%	0.0	97%	NZ_BBYR01000090.1
May13_ub3 Seq 1	Nostoc azollae' 0708	580	2323	100%	1,00E-162	89%	NC_014248.1
May13_ub3 Seq 2	Rhizobacter sp. Root1221 contig_4	689	689	99%	0.0	91%	NZ_LMDI01000034.1
May13_Qhi_mid1 Seq 1	Pseudomonas taeanensis MS-3 contig_13	915	915	100%	0.0	98%	NZ_AWSQ01000013.1
May13_Qhi_mid1 Seq 2	Cyanothece sp. CCY0110 1101676644568	573	573	100%	2,00E-160	88%	NZ_AAXW01000006.1
May13_Qhi_mid2 Seq 1	Aquabacterium sp. NJ1 contig01	920	1841	100%	0.0	98%	NZ_JRKM01000001.1
May13_Qhi_mid2 Seq 2	Aquabacterium sp. NJ1 contig01	937	1874	100%	0.0	99%	NZ_JRKM01000001.1
May13_Qhi_mid2 Seq 3	Aquabacterium sp. NJ1 contig01	942	1885	100%	0.0	99%	NZ_JRKM01000001.1
May13_Qlo Seq 1	Sphingopyxis sp. MC1 contigEBMC1_3	760	760	100%	0.0	96%	NZ_AOUN01000003.1
May13_Qlo Seq 2	Brevundimonas subvibrioides ATCC 15264	821	1642	100%	0.0	98%	NC_014375.1
May13_Qlo Seq 3	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	704	704	100%	0.0	91%	NZ_JQKD01000107.1
May13_Qlo_end1 Seq 1	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	704	704	100%	0.0	91%	NZ_JQKD01000107.1
May13_Qlo_end1 Seq 2	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	704	704	100%	0.0	91%	NZ_JQKD01000107.1
May13_Qlo_end1 Seq 3	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	704	704	100%	0.1	91%	NZ_JQKD01000107.1
May13_Qlo_end2 Seq 1	Anabaena sp. 90 chromosome chANA01	501	2502	100%	1,00E-138	86%	NC_019427.1
May13_Qlo_end2 Seq 2	Anabaena sp. 90 chromosome chANA01	501	2502	100%	1E-138	0,86	NC_019427.1
May13_Qlo_end2 Seq 3	Anabaena sp. 90 chromosome chANA01	501	2502	100%	1E-138	0,86	NC_019427.1
Jul13_Lno_Lme1 Seq 1	Gemmobacter sp. LW-1 contig330	584	584	100%	7,00E-164	93%	NZ_LJSC01000026.1
Jul13_Lno_Lme1 Seq 2	Rheinheimera sp. KL1 NODE_33	750	750	100%	0.0	93%	NZ_LAMX01000036.1
Jul13_Lno Seq 1	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	839	839	100%	0.0	96%	NC_009379.1

Jul13_Lno Seq 2	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	845	845	100%	0.0	96%	NC_009379.1
Jul13_Lno Seq 3	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	850	850	100%	0.0	96%	NC_009379.1
Jul13_ub1 Seq 1	Devosia geojensis strain BD-c194 NODE_87	654	654	100%	0.0	92%	NZ_JZEX01000084.1
Jul13_ub1 Seq 2	Devosia geojensis strain BD-c194 NODE_87	649	649	100%	0.0	91%	NZ_JZEX01000084.1
Jul13_ub1 Seq 3	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	861	861	100%	0.0	96%	NC_009379.1
Jul13_Lno_Lme2 Seq 1	Fluoribacter dumoffii NY 23 chromosome	848	2546	100%	0.0	96%	NZ_CM001373.1
Jul13_Lno_Lme2 Seq 2	Flavobacterium tegetincola DSM 22377 H602DRAFT_scaffold00029.29_C	438	438	100%	8,00E-120	82%	NZ_AUDN01000041.1
Jul13_Lno_Lme2 Seq 3	Sorangium cellulosum 'So ce 56'	732	2929	100%	0.0	91%	NC_010162.1
Jul13_Lno_Lme3 Seq 1	Caulobacter vibrioides strain T5M6 contig_129	702	702	100%	0.0	93%	NZ_LNIY01000034.1
Jul13_Lno_Lme3 Seq 2	Microbacterium sp. UNCCL10 BR85DRAFT_scaffold00001.1_C	904	904	100%	0.0	99%	NZ_JQKT01000007.1
Jul13_Lno_Lme3 Seq 3	Gemmatimonas aurantiaca T-27 DANN	795	795	100%	0.0	95%	NC_012489.1
Jul13_Lno_mid_end Seq 1	Hyphomicrobium zavarzinii ATCC 27496 strain ZV-622	726	726	100%	0.0	94%	NZ_KB911255.1
Jul13_Lno_mid_end Seq 2	Reyranella massiliensis 521 genomic scaffold, Scaffold01	865	865	99%	0.0	99%	NZ_HE997181.1
Jul13_Lno_mid_end Seq 3	Hyphomicrobium zavarzinii ATCC 27496 strain ZV-622	721	721	100%	0.0	94%	NZ_KB911255.1
Jul13_Lme_mid Seq 1	Cyanothece sp. CCY0110 1101676644568	579	579	100%	4,00E-162	89%	NZ_AAXW01000006.1
Jul13_Lme_mid Seq 2	Cyanothece sp. CCY0110 1101676644568	579	579	100%	4,00E-162	89%	NZ_AAXW01000006.1
Jul13_Lme_mid Seq 3	Cyanothece sp. CCY0110 1101676644568	579	579	100%	4E-162	89%	NZ_AAXW01000006.1
Jul13_Lhi_end1 Seq 1	Synechococcus sp. PCC 6312	564	564	99%	1,00E-157	88%	NC_019680.1
Jul13_Lhi_end1 Seq 2	Synechococcus sp. PCC 6312, complete genome	569	569	99%	3,00E-159	88%	NC_019680.1
Jul13_Lhi_end1 Seq 3	Synechococcus sp. PCC 6312, complete genome	569	569	99%	3,00E-159	88%	NC_019680.1
Jul13_ub2 Seq 1	Afipia felis genospecies A WGS project CCAZ000000000 data, strain 76713	671	671	100%	0.0	92%	NZ_CCAZ020000001.1
Jul13_ub2 Seq 2	Cyanothece sp. CCY0110 1101676644568	571	571	100%	7,00E-160	88%	NZ_AAXW01000006.1
Jul13_ub2 Seq 3	Cyanothece sp. CCY0110 1101676644568	556	556	100%	2,00E-155	88%	NZ_AAXW01000006.1
Jul13_Lme_Lhi_mid Seq 1	Anabaena sp. 90 chromosome chANA01	553	2722	100%	3,00E-154	88%	NC_019427.1
Jul13_Lme_Lhi_mid Seq 2	Cyanothece sp. CCY0110 1101676644568	553	553	100%	3,00E-154	88%	NZ_AAXW01000006.1
Jul13_Lme_Lhi_mid Seq 3	Cyanothece sp. CCY0110 1101676644568	579	579	100%	4,00E-162	89%	NZ_AAXW01000006.1
Jul13_ub3 Seq 1	Flavobacterium succinicans LMG 10402 contig_01	833	833	100%	0.0	96%	NZ_JATV01000001.1
Jul13_ub3 Seq 2	Pseudomonas fuscovaginae UPB0736	808	808	100%	0.0	95%	NZ_JH605139.1
Jul13_ub3 Seq 3	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	850	850	100%	0.0	96%	NC_009379.1
Jul13_Lme_Lhi_end Seq 1	'Nostoc azollae' 0708, complete genome	536	2146	100%	3,00E-149	87%	NC_014248.1
Jul13_Lme_Lhi_end Seq 2	'Nostoc azollae' 0708, complete genome	542	2168	100%	5,00E-151	87%	NC_014248.1
Jul13_Lme_Lhi_end Seq 3	'Nostoc azollae' 0708, complete genome	525	2102	100%	6,00E-146	87%	NC_014248.1
Jul13_Lhi_end2 Seq 1	Gemmatimonas aurantiaca T-27 DANN	839	839	100%	0.0	97%	NC_012489.1
Jul13_Lhi_end2 Seq 2	Synechococcus sp. PCC 6312	545	545	99%	4,00E-152	88%	NC_019680.1
Jul13_Lhi_end2 Seq 3	Anabaena sp. 90 chromosome chANA01	531	2638	100%	1,00E-147	87%	NC_019427.1
Jul13_Lhi_end3 Seq 1	Hydrogenophaga sp. Root209 contig_13	944	944	99%	0.0	99%	NZ_LMIE01000005.1
Jul13_Lhi_end3 Seq 2	Hydrogenophaga sp. Root209 contig_13	747	747	100%	0.0	93%	NZ_LMIE01000005.1
Jul13_Lhi_end3 Seq 3	Hydrogenophaga sp. Root209 contig_13	939	939	99%	0.0	99%	NZ_LMIE01000005.1
Aug13_Lhi Seq 1	Gemmatimonas phototrophica strain AP64 Contig_6	878	878	100%	0.0	98%	NZ_AUXF01000006.1
Aug13_Lhi Seq 2	Gemmatimonas phototrophica strain AP64 Contig_6	922	922	100%	0.0	99%	NZ_AUXF01000006.1
Aug13_Lhi Seq 3	Gemmatimonas phototrophica strain AP64 Contig_6	867	867	100%	0.0	98%	NZ_AUXF01000006.1
Aug13_Lhi Seq 4	Gemmatimonas phototrophica strain AP64 Contig_6	867	867	100%	0.0	98%	NZ_AUXF01000006.1
Aug13_Lno_Lme Seq 1	Gemmatimonas phototrophica strain AP64 Contig_6	867	867	100%	0.0	98%	NZ_AUXF01000006.1
Aug13_Lno_Lme Seq 2	Gemmatimonas phototrophica strain AP64 Contig_6	933	933	100%	0.0	100%	NZ_AUXF01000006.1
Aug13_Lno_Lme Seq 3	Brevundimonas subvibrioides ATCC 15264	809	1619	100%	0.0	98%	NC_014375.1
Aug13_Lno_Lme Seq 4	Brevundimonas subvibrioides ATCC 15264	582	1165	100%	3,00E-163	89%	NC_014375.1
Aug13_Lmed Seq 1	Geitlerinema sp. PCC 7407	623	1859	100%	2,00E-175	91%	NC_019703.1
Aug13_Lmed Seq 2	Brevundimonas subvibrioides ATCC 15264	582	1165	100%	3,00E-163	89%	NC_014375.1
Aug13_Lmed Seq 3	Crocosphaera watsonii WH 8501 ctg258	573	573	100%	2,00E-160	89%	NZ_AADV02000093.1

Aug13_Lmed Seq 4	Crocosphaera watsonii WH 8501 ctg258	579	579	100%	4,00E-162	89%	NZ_AADV02000093.1
Aug13_Lno_Lme Seq 1	Gemmatimonas phototrophica strain AP64 Contig_6	928	928	100%	0.0	99%	NZ_AUXF01000006.1
Aug13_Lno_Lme Seq 2	Gemmatimonas phototrophica strain AP64 Contig_6	861	861	100%	0.0	99%	NZ_AUXF01000006.1
Aug13_Lno_Lme Seq 3	Gemmatimonas phototrophica strain AP64 Contig_6	640	640	100%	2,00E-180	90%	NZ_AUXF01000006.1
Aug13_Lno_Lme Seq 4	Planctomyces limnophilus DSM 3776	483	966	100%	4,00E-133	84%	NC_014148.1
Aug13_ub1 Seq 1	Flectobacillus major DSM 103 genomic scaffold FlemaDRAFT_FME.6	588	1759	100%	7,00E-165	88%	NZ_KE386491.1
Aug13_ub1 Seq 2	Acidovorax sp. JHL-3 K226DRAFT_scf7180000000013_quiver.1_C	946	3786	100%	0.0	99%	NZ_JAFU01000001.1
Aug13_ub1 Seq 3	Aquabacterium sp. NJ1 contig01	885	1771	100%	0.0	97%	NZ_JRKM01000001.1
Aug13_ub2 Seq 1	Pelomonas sp. Root662 contig_2	863	863	100%	0.0	97%	NZ_LMHE01000011.1
Aug13_ub2 Seq 2	Sphingopyxis fribergensis strain Kp5.2	787	787	100%	0.0	97%	NZ_CP009122.1
Aug13_ub2 Seq 3	Acetobacteraceae bacterium AT-5844	665	665	100%	0.0	92%	NZ_JH599943.1
Aug13_Lme_end1 Seq 1	Cyanothece sp. CCY0110 1101676644568	556	556	100%	2,00E-155	88%	NZ_AAXW01000006.1
Aug13_Lme_end1 Seq 2	Cyanothece sp. CCY0110 1101676644568	582	582	100%	3,00E-163	89%	NZ_AAXW01000006.1
Aug13_Lme_end1 Seq 3	Cyanothece sp. CCY0110 1101676644568	571	571	100%	7,00E-160	88%	NZ_AAXW01000006.1
Aug13_Lme_end1 Seq 4	Cyanothece sp. CCY0110 1101676644568	582	582	100%	3,00E-163	89%	NZ_AAXW01000006.1
Aug13_ub3 Seq 1	Cellvibrio mixtus subsp. mixtus J3-8 Scaffold25_1	787	787	100%	0.0	94%	NZ_ALBT01000125.1
Aug13_ub3 Seq 2	Cellvibrio mixtus subsp. mixtus J3-8 Scaffold25_1	815	815	100%	0.0	95%	NZ_ALBT01000125.1
Aug13_ub3 Seq 3	Cellvibrio mixtus subsp. mixtus J3-8 Scaffold25_1	804	804	100%	0.0	95%	NZ_ALBT01000125.1
Aug13_ub3 Seq 4	Aquabacterium sp. NJ1 contig01	885	1771	100%	0.0	97%	NZ_JRKM01000001.1
Aug13_Lme_end2 Seq 1	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	710	710	100%	0.0	91%	NZ_JQKD01000107.1
Aug13_Lme_end2 Seq 2	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	710	710	100%	0.0	91%	NZ_JQKD01000107.1
Aug13_Lme_end2 Seq 3	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	710	710	100%	0.0	91%	NZ_JQKD01000107.1
Aug13_Lme_end2 Seq 4	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	710	710	100%	0.0	91%	NZ_JQKD01000107.1
Aug13_Lno Seq 1	Flavobacterium succinicans LMG 10402 contig_01	909	909	100%	0.0	98%	NZ_JATV01000001.1
Aug13_Lno Seq 2	Flavobacterium succinicans LMG 10402 contig_01	909	909	100%	0.0	98%	NZ_JATV01000001.1
Mar13_ub_sta1 Seq 1	Sphingobium sp. Leaf26 contig_19	848	848	100%	0.0	99%	NZ_LMKV01000011.1
Mar13_ub_sta1 Seq 2	Pseudorhodobacter psychrotolerans strain PAMC 27389 ctg7180000022378	815	815	100%	0.0	98%	NZ_LGIC01000003.1
Mar13_ub_sta1 Seq 3	[Polyangium] brachysporum strain DSM 7029	699	2097	100%	0.0	91%	NZ_CP011371.1
Mar13_ub_sta1 Seq 4	Brevundimonas nasdae strain TPW30 Contig_38	787	787	100%	0.0	97%	NZ_JWSY01000038.1
Mar13_ub_sta1 Seq 5	Xylophilus sp. Leaf220 contig_3	808	808	100%	0.0	95%	NZ_LMKO01000022.1
Mar13_ub_sta2 Seq 1	Nostoc azollae' 0708	569	2279	100%	3,00E-159	88%	NC_014248.1
Mar13_ub_sta2 Seq 2	Anabaena sp. 90 chromosome chANA01	564	2805	100%	1,00E-157	88%	NC_019427.1
Mar13_ub_sta2 Seq 3	Cyanothece sp. CCY0110 1101676644568	573	573	100%	2,00E-160	88%	NZ_AAXW01000006.1
Mar13_ub_sta2 Seq 4	Synechococcus sp. PCC 6312	536	536	99%	3,00E-149	87%	NC_019680.1
Mar13_ub_sta2 Seq 5	Anabaena sp. 90 chromosome chANA01	534	2629	100%	9,00E-149	87%	NC_019427.1
Mar13_Qlo_Qme_end Seq 2	Bacteria/Cyanobacteria/Cyanobacteria/SubsectionIII_FamilyI/Leptolyngbya_2	945	944	99%	0.0	99%	AM_398976.1
Mar13_Qlo_Qme_end Seq 3	Bacteria/Cyanobacteria/Cyanobacteria/SubsectionIII_FamilyI/Leptolyngbya_2	927	927	99%	0.0	99%	AM_398976.1
Mar13_ub_sta4 Seq 3	Bacteria/Cyanobacteria/Cyanobacteria/SubsectionIII_FamilyI/Leptolyngbya_2	915	915	99%	0.0	99%	AM_398976.1
Mar13_ub_sta6 Seq 5	Bacteria/Cyanobacteria/Cyanobacteria/SubsectionIII_FamilyI/Leptolyngbya_2	934	934	99%	0.0	99%	AM_398976.1
Mar14_Lhi_end Seq 1	Bacteria/Cyanobacteria/Cyanobacteria/SubsectionIII_FamilyI/Leptolyngbya_2	933	933	99%	0.0	99%	AY_768526.1
Mar14_Lhi_end Seq 2	Bacteria/Cyanobacteria/Cyanobacteria/SubsectionIII_FamilyI/Leptolyngbya_2	924	924	99%	0.0	99%	AY_768526.1
Mar13_Qlo_mid Seq 1	Anabaena sp. 90 chromosome chANA01	538	2681	100%	7,00E-150	87%	NC_019427.1
Mar13_Qlo_mid Seq 2	Nostoc azollae' 0708	575	2301	100%	5,00E-161	89%	NC_014248.1
Mar13_Qlo_mid Seq 3	Cyanothece sp. CCY0110 1101676644568	579	579	100%	4,00E-162	89%	NZ_AAXW01000006.1
Mar13_Qlo_mid Seq 4	Synechococcus sp. PCC 6312	536	536	99%	3,00E-149	87%	NC_019680.1
Mar13_Qlo_mid Seq 5	Cyanothece sp. CCY0110 1101676644568	584	584	100%	9,00E-164	89%	NZ_AAXW01000006.1
Mar13_Qlo_end Seq 1	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	697	697	100%	0.0	91%	NZ_JQKD01000107.1
Mar13_Qlo_end Seq 2	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	704	704	100%	0.0	91%	NZ_JQKD01000107.1
Mar13_Qlo_end Seq 3	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	704	704	100%	0.0	91%	NZ_JQKD01000107.1

Mar13_Qlo_end Seq 4	Hydrogenophaga sp. Root209 contig_13	713	713	99%	0.0	92%	NZ_LMIE01000005.1
Mar13_Qlo_end Seq 5	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	699	699	100%	0.0	91%	NZ_JQKD01000107.1
Mar13_Qlo_Qme_end Seq 1	Beta proteobacterium AAP65 AAP65_Contigs_64	889	889	100%	0.0	98%	NZ_LJHW01000064.1
Mar13_Qlo_Qme_end Seq 2	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92	708	708	100%	0.0	94%	NZ_JJML01000087.1
Mar13_Qlo_Qme_end Seq 3	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92	725	725	100%	0.0	94%	NZ_JJML01000087.1
Mar13_Qlo_Qme_end Seq 4	Beta proteobacterium AAP65 AAP65_Contigs_64	933	933	100%	0.0	99%	NZ_LJHW01000064.1
Mar13_Qme_Qhi_mid Seq 1	Flavobacterium sp. 83 T410DRAFT_scf7180000000004_quiver_dupTrim_4441.1_C	876	5263	100%	0.0	97%	NZ_JQMS01000001.1
Mar13_Qme_Qhi_mid Seq 2	Flavobacterium sp. 83 T410DRAFT_scf7180000000004_quiver_dupTrim_4441.1_C	876	5263	100%	0.0	97%	NZ_JQMS01000001.1
Mar13_Qme_Qhi_mid Seq 3	Flavobacterium sp. 83 T410DRAFT_scf7180000000004_quiver_dupTrim_4441.1_C	887	5329	100%	0.0	98%	NZ_JQMS01000001.1
Mar13_Qme_Qhi_mid Seq 4	Flavobacterium sp. 83 T410DRAFT_scf7180000000004_quiver_dupTrim_4441.1_C	887	5329	100%	0.0	98%	NZ_JQMS01000001.1
Mar13_Qme_Qhi_mid Seq 5	Flavobacterium sp. 83 T410DRAFT_scf7180000000004_quiver_dupTrim_4441.1_C	887	5329	100%	0.0	98%	NZ_JQMS01000001.1
Mar13_ub_sta3 Seq 1	Pelomonas sp. Root662 contig_2	841	841	100%	0.0	96%	NZ_LMHE01000011.1
Mar13_ub_sta3 Seq 2	Pseudomonas fuscovaginae UPB0736	920	920	100%	0.0	98%	NZ_JH605139.1
Mar13_ub_sta3 Seq 3	Synechococcus sp. PCC 6312	531	531	99%	1,00E-147	87%	NC_019680.1
Mar13_ub_sta3 Seq 4	Methylotenera versatilis 79 MetveDRAFT_chromosome1.1_C	837	1422	100%	0.0	96%	NZ_ARVX01000001.1
Mar13_ub_sta3 Seq 5	Synechococcus sp. PCC 6312	556	556	99%	2,00E-155	88%	NC_019680.1
Mar13_ub_sta4 Seq 1	Flectobacillus major DSM 103	632	1892	100%	3,00E-178	89%	NZ_KE386491.1
Mar13_ub_sta4 Seq 2	Janthinobacterium lividum strain RIT308 contigs33	859	859	100%	0.0	96%	NZ_JFYR01000027.1
Mar13_ub_sta4 Seq 3	Flectobacillus major DSM 103	627	1875	100%	2,00E-176	89%	NZ_KE386491.1
Mar13_ub_sta4 Seq 4	Sphingopyxis macrogoltabida strain 203	832	832	100%	0.0	99%	NZ_CP009429.1
Mar13_ub_sta4 Seq 5	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92	708	708	100%	0.0	94%	NZ_JJML01000087.1
Mar13_ub_sta5 Seq 1	Flavobacterium succinicans LMG 10402 contig_01	865	865	100%	0.0	97%	NZ_JATV01000001.1
Mar13_ub_sta5 Seq 2	Methylotenera versatilis 79 MetveDRAFT_chromosome1.1_C	865	1477	100%	0.0	97%	NZ_ARVX01000001.1
Mar13_ub_sta5 Seq 3	Synechococcus sp. PCC 6312	575	575	99%	5,00E-161	89%	NC_019680.1
Mar13_ub_sta5 Seq 4	Methylotenera versatilis 79 MetveDRAFT_chromosome1.1_C	865	1477	100%	0.0	97%	NZ_ARVX01000001.1
Mar13_ub_sta5 Seq 5	Methylotenera versatilis 79 MetveDRAFT_chromosome1.1_C	859	1466	100%	0.0	96%	NZ_ARVX01000001.1
Mar13_ub_sta_mid1 Seq 1	Cyanothece sp. CCY0110 1101676644568	568	568	100%	9,00E-159	88%	NZ_AAXW01000006.1
Mar13_ub_sta_mid1 Seq 2	Cyanothece sp. CCY0110 1101676644568	568	568	100%	9,00E-159	88%	NZ_AAXW01000006.1
Mar13_ub_sta_mid1 Seq 3	Cyanothece sp. CCY0110 1101676644568	568	568	100%	9,00E-159	88%	NZ_AAXW01000006.1
Mar13_ub_sta_mid1 Seq 4	Cyanothece sp. CCY0110 1101676644568	573	573	100%	2,00E-160	88%	NZ_AAXW01000006.1
Mar13_Qme_mid Seq 1	Rhodoferax ferrireducens T118	819	1638	100%	0.0	95%	NC_007908.1
Mar13_Qme_mid Seq 2	Flectobacillus major DSM 103	627	1875	100%	2,00E-176	89%	NZ_KE386491.1
Mar13_Qme_mid Seq 3	Synechococcus sp. PCC 6312	547	547	99%	1,00E-152	88%	NC_019680.1
Mar13_Qme_mid Seq 4	Flectobacillus major DSM 103	627	1870	100%	2,00E-176	89%	NZ_KE386491.1
Mar13_Qme_mid Seq 5	Sphingomonas sanxanigenens NX02	754	2263	100%	0.0	96%	NZ_CP006644.1
Mar13_ub_sta_mid2 Seq 1	Sphingobium yanoikuyae ATCC 51230	832	3328	100%	0.0	99%	NZ_JH992904.1
Mar13_ub_sta_mid2 Seq 2	[Polyangium] brachysporum strain DSM 7029	822	2468	100%	0.0	95%	NZ_CP011371.1
Mar13_ub_sta_mid2 Seq 3	Sphingopyxis fribergensis strain Kp5.2	854	854	100%	0.0	99%	NZ_CP009122.1
Mar13_ub_sta_mid2 Seq 4	Croceicoccus naphthovorans strain PQ-2	760	760	100%	0.0	96%	NZ_CP011770.1
Mar13_ub_sta_mid2 Seq 5	Cyanothece sp. PCC 7822	425	1260	100%	6,00E-116	83%	NC_014501.1
Mar13_ub_mid_end Seq 1	Sphingopyxis sp. MC1 contigEBMC1_3	760	760	100%	0.0	96%	NZ_AOUN01000003.1
Mar13_ub_mid_end Seq 2	Krokinobacter sp. 4H-3-7-5	518	1534	100%	1,00E-143	85%	NC_015496.1
Mar13_ub_mid_end Seq 3	Synechococcus sp. PCC 6312	564	564	99%	1,00E-157	88%	NC_019680.1
Mar13_ub_mid_end Seq 4	Sediminibacterium salmoneum NBRC 103935	900	900	100%	0.0	98%	NZ_KI866530.1
Mar13_ub_mid_end Seq 5	Synechococcus sp. PCC 6312	564	564	99%	1,00E-157	88%	NC_019680.1
Mar13_ub1 Seq 1	Schlesneria paludicola DSM 18645 Scaffold9_1	556	556	100%	2,00E-155	86%	NZ_AHZR01000092.1
Mar13_ub1 Seq 2	Collimonas fungivorans Ter331	876	2629	100%	0.0	97%	NC_015856.1
Mar13_ub1 Seq 3	Flavobacterium psychrophilum JIP02/86	800	4793	100%	0.0	95%	NC_009613.3
Mar13_ub1 Seq 4	Flavobacterium sp. EM1308 contig00062	754	754	99%	0.0	93%	NZ_JNCP01000062.1

Mar13_ub1 Seq 5	Leadbetterella byssophila DSM 17132	628	1886	100%	4,00E-177	89%	NC_014655.1
Mar13_ub2 Seq 1	Reyranella massiliensis 521 genomic scaffold, Scaffold01	459	459	98%	5,00E-126	85%	NZ_HE997181.1
Mar13_ub2 Seq 2	Brevundimonas subvibrioides ATCC 15264	765	1531	100%	0.0	96%	NC_014375.1
Mar13_ub2 Seq 3	Pseudorhodobacter psychrotolerans strain PAMC 27389 ctg7180000022378	726	726	100%	0.0	94%	NZ_LGIC01000003.1
Mar13_ub2 Seq 4	Rhodobacter capsulatus SB 1003	752	3010	100%	0.0	96%	NC_014034.1
Mar13_ub2 Seq 5	Reyranella massiliensis 521 genomic scaffold, Scaffold01	824	824	99%	0.0	98%	NZ_HE997181.1
Mar13_Qme_mid Seq 1	Pseudanabaena biceps PCC 7429 scaffold_00102	809	809	99%	0.0	98%	NZ_ALWB01000102.1
Mar13_Qme_mid Seq 2	Cyanothece sp. CCY0110 1101676644568	579	579	100%	4,00E-162	89%	NZ_AAXW01000006.1
Mar13_Qme_mid Seq 3	Synechococcus sp. PCC 6312	569	569	99%	3,00E-159	88%	NC_019680.1
Mar13_Qme_mid Seq 4	Paracoccus aminophilus JCM 7686 plasmid pAMI1	708	708	100%	0.0	94%	NC_022042.1
Mar13_Qme_mid Seq 5	Alpha proteobacterium AAP81b AAP81b_Contigs_75	809	809	100%	0.0	98%	NZ_LJHX01000075.1
Mar13_ub_mid Seq 1	Variovorax paradoxus EPS	944	1889	100%	0.0	99%	NC_014931.1
Mar13_ub_mid Seq 2	Pseudomonas stutzeri A1501	843	3333	100%	0.0	96%	NC_009434.1
Mar13_ub3 Seq 1	Sphingopyxis sp. MC1 contigEBMC1_3	771	771	100%	0.0	96%	NZ_AOUN01000003.1
Mar13_ub3 Seq 2	Massilia sp. WF1 contig10	828	828	100%	0.0	95%	NZ_LELH02000010.1
Mar13_ub3 Seq 3	Pseudorhodobacter psychrotolerans strain PAMC 27389 ctg7180000022378	837	837	100%	0.0	99%	NZ_LGIC01000003.1
Mar13_ub3 Seq 4	Novosphingobium sp. Leaf2 contig_1	793	793	100%	0.0	97%	NZ_LMJY01000001.1
Mar13_ub3 Seq 5	Synechococcus sp. PCC 6312	558	558	99%	5,00E-156	88%	NC_019680.1
Mar13_ub_end1 Seq 1	Synechococcus sp. PCC 6312	575	575	99%	5,00E-161	89%	NC_019680.1
Mar13_ub_end1 Seq 2	Synechococcus sp. PCC 6312	575	575	99%	5,00E-161	89%	NC_019680.1
Mar13_ub_end1 Seq 3	Rhodoferax saidenbachensis ED16 contig64	472	472	100%	4,00E-130	96%	NZ_AWQR01000064.1
Mar13_ub_end1 Seq 4	Rhodoferax saidenbachensis ED16 contig64	472	472	100%	4,00E-130	96%	NZ_AWQR01000064.1
Mar13_ub_end1 Seq 5	Synechococcus sp. PCC 6312	575	575	99%	5,00E-161	89%	NC_019680.1
Mar13_ub_end2 Seq 1	Rhizobacter sp. Root29 contig_40	887	887	100%	0.0	97%	NZ_LMCN01000034.1
Mar13_ub_end2 Seq 2	Rhizobacter sp. Root29 contig_40	887	887	100%	0.0	97%	NZ_LMCN01000034.1
Mar13_ub_end2 Seq 3	Deinococcus soli' Cha et al. 2014 strain N5	713	2141	100%	0.0	93%	NZ_CP011389.1
Mar13_ub_end2 Seq 4	Geopsychrobacter electrodiphilus DSM 16401 D888DRAFT	379	379	100%	5,00E-102	81%	NZ_ARWE01000001.1
Mar13_ub_sta6 Seq 1	Acidovorax sp. JHL-3 K226DRAFT_scf7180000000013_quiver.1_C	946	3786	100%	0.0	99%	NZ_JAFU01000001.1
Mar13_ub_sta6 Seq 2	Pseudomonas stutzeri A1501	843	3333	100%	0.0	96%	NC_009434.1
Mar13_ub_sta6 Seq 3	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92	697	697	100%	0.0	93%	NZ_JJML01000087.1
Nov13_ub_mid_end Seq 1	Phenylobacterium sp. Root1290 contig_1	854	854	100%	0.0	99%	NZ_LMEE01000001.1
Nov13_ub_mid_end Seq 2	Brevundimonas diminuta ATCC 11568 genomic scaffold BDIM_scaffold00008	837	837	100%	0.0	99%	NZ_GL883089.1
Nov13_ub_mid_end Seq 3	Brevundimonas diminuta ATCC 11568 genomic scaffold BDIM_scaffold00008	815	815	100%	0.0	98%	NZ_GL883089.1
Nov13_ub_mid_end Seq 4	Brevundimonas diminuta ATCC 11568 genomic scaffold BDIM_scaffold00008	843	843	100%	0.0	99%	NZ_GL883089.1
Nov13_ub Seq 1	Cyanothece sp. CCY0110 1101676644568	573	573	100%	2,00E-160	88%	NZ_AAXW01000006.1
Nov13_ub Seq 2	Rhodobacteraceae bacterium PD-2 genomic scaffold scaffold25	845	845	100%	0.0	98%	NZ_KN714923.1
Nov13_ub Seq 3	Pseudomonas fluorescens SBW25	893	4465	100%	0.0	98%	NC_012660.1
Nov13_ub Seq 4	Pseudomonas fluorescens SBW25	887	4437	100%	0.0	97%	NC_012660.1
Nov13_Qhi Seq 1	Acinetobacter johnsonii CIP 64.6 genomic scaffold acLZl-supercont1.9	922	922	100%	0.0	98%	NZ_KB849238.1
Nov13_Qhi Seq 2	Acinetobacter johnsonii CIP 64.6 genomic scaffold acLZl-supercont1.9	754	754	100%	0.0	93%	NZ_KB849238.1
Nov13_Qhi Seq 3	Acinetobacter johnsonii CIP 64.6 genomic scaffold acLZl-supercont1.8	939	1872	100%	0.0	99%	NZ_KB849237.1
Nov13_ub_end Seq 1	Anabaena sp. 90 chromosome chANA01	558	2749	100%	5,00E-156	88%	NC_019427.1
Nov13_ub_end Seq 2	Anabaena sp. 90 chromosome chANA01	558	2749	100%	5,00E-156	88%	NC_019427.1
Nov13_ub_end Seq 3	Gemmata obscuriglobus UQM 2246 gcontig_1106221704925	508	508	98%	6,00E-141	86%	NZ_ABGO01000325.1
Nov13_Qmed Seq 1	Nostoc azollae' 0708	558	2235	100%	5,00E-156	88%	NC_014248.1
Nov13_Qmed Seq 2	Nostoc azollae' 0708	558	2235	100%	5,00E-156	88%	NC_014248.1
Nov13_Qmed Seq 3	Nostoc azollae' 0708	558	2235	100%	5,00E-156	88%	NC_014248.1
Mar14_ub1 Seq 1	Pseudomonas taeanensis MS-3 contig_13	915	915	100%	0.0	98%	NZ_AWSQ01000013.1
Mar14_ub1 Seq 2	Pseudomonas taeanensis MS-3 contig_13	898	898	100%	0.0	98%	NZ_AWSQ01000013.1

Mar14_ub2 Seq 1	Pelomonas sp. Root662 contig_2	835	835	100%	0.0	96%	NZ_LMHE01000011.1
Mar14_ub2 Seq 2	Thioalkalivibrio sulfidophilus HL-EbGr7	608	608	100%	6,00E-171	88%	NC_011901.1
Mar14_ub2 Seq 3	Dechloromonas aromatica RCB	793	3173	100%	0.0	94%	NC_007298.1
Mar14_ub_mid_end Seq 1	Phenylobacterium sp. Root1290 contig_1	865	865	100%	0.0	99%	NZ_LMEE01000001.1
Mar14_ub_mid_end Seq 2	Brevundimonas diminuta ATCC 11568 genomic scaffold BDIM_scaffold00008	832	832	100%	0.0	99%	NZ_GL883089.1
Mar14_ub_mid_end Seq 3	Phenylobacterium sp. Root1290 contig_1	865	865	100%	0.0	99%	NZ_LMEE01000001.1
Mar14_Lno_end Seq 1	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	850	850	100%	0.0	96%	NC_009379.1
Mar14_Lno_end Seq 2	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	856	856	100%	0.0	96%	NC_009379.1
Mar14_Lno_end Seq 3	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1	750	750	100%	0.0	93%	NC_009379.1
Mar14_ub3 Seq 1	Flavobacterium sp. 83 T410DRAFT_scf7180000000004_quiver_dupTrim_4441.1_C	843	5060	100%	0.0	96%	NZ_JQMS01000001.1
Mar14_ub3 Seq 2	Acidovorax sp. JHL-3 K226DRAFT_scf7180000000013_quiver.1_C	835	3343	100%	0.0	96%	NZ_JAFU01000001.1
Mar14_ub4 Seq 1	Pseudomonas taeanensis MS-3 contig_13	909	909	100%	0.0	98%	NZ_AWSQ01000013.1
Mar14_ub4 Seq 2	Pseudomonas taeanensis MS-3 contig_13	893	893	100%	0.0	98%	NZ_AWSQ01000013.1
Mar14_ub4 Seq 3	Pseudomonas taeanensis MS-3 contig_13	898	898	100%	0.0	98%	NZ_AWSQ01000013.1
Mar14_ub5 Seq 1	Synechococcus sp. NKBG15041c DNA, contig; Contig37	579	579	100%	4,00E-162	89%	NZ_BAUB01000037.1
Mar14_ub5 Seq 2	Anabaena sp. 90 chromosome chANA01	558	2749	100%	5,00E-156	88%	NC_019427.1
Mar14_Lme_Lhi_end Seq 1	Methylibium petroleiphilum PM1	876	876	100%	0.0	97%	NC_008825.1
Mar14_Lme_Lhi_end Seq 2	Xenophilus azovorans DSM 13620 Q392DRAFT_scaffold00102.102_C	782	782	100%	0.0	94%	NZ_JQKD01000107.1
Mar14_Lme_Lhi_end Seq 3	Rhizobium mesoamericanum STM3625 genomic scaffold, ANZ_1636	547	547	100%	1,00E-152	91%	NZ_HF536772.1
Mar14_Lme_end Seq 1	Synechococcus sp. PCC 6312	569	569	99%	3,00E-159	88%	NC_019680.1
Mar14_Lme_end Seq 2	Nostoc azollae' 0708	569	2279	100%	2,00E-159	89%	NC_014248.1
Mar14_Lme_end Seq 3	Nostoc azollae' 0708	575	2301	100%	5,00E-161	89%	NC_014248.1
Mar14_Lhi_sta_mid Seq 1	Rubrivivax gelatinosus IL144 DNA,	813	2435	100%	0.0	95%	NC_017075.1
Mar14_Lhi_sta_mid Seq 2	Rhodoferax saidenbachensis ED16 contig64	894	977	100%	0.0	98%	NZ_AWQR01000064.1
Mar14_Lhi_sta_mid Seq 3	Aquabacterium sp. NJ1 contig01	948	1896	100%	0.0	99%	NZ_JRKM01000001.1
Mar14_Lhi_end Seq 1	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92	713	713	100%	0.0	94%	NZ_JJML01000087.1
Mar14_Lhi_end Seq 2	Neosynechococcus sphagnicola sy1 strain CAUP A 1101 synech5_c92	713	713	100%	0.0	94%	NZ_JJML01000087.1
Mar14_Lhi_end Seq 3	Devosia limi DSM 17137 NODE_157	573	573	100%	2,00E-160	89%	NZ_LAJF01000157.1
Mar14_Lme_Lhi_end Seq 1	Methyloversatilis universalis Fam500 Metunv3DRAFT_Contig245.1_C	857	2573	100%	0.0	96%	NZ_ARWH01000001.1
Mar14_Lme_Lhi_end Seq 2	Methyloversatilis universalis Fam500 Metunv3DRAFT_Contig245.1_C	857	2573	100%	0.0	96%	NZ_ARWH01000001.1
Mar14_Lme_Lhi_end Seq 3	Methyloversatilis universalis Fam500 Metunv3DRAFT_Contig245.1_C	846	2540	100%	0.0	96%	NZ_ARWH01000001.1

Tables

Table 1: Overview over the single experiments.

Table 2: Abiotic boundary conditions set in the experiments.

Table 3**:** Intra flume comparison: mean values of EPS (carbohydrates and protein) and chlorophyll *a* contents (n=144), bacterial cell counts (n=24) and surface adhesiveness (n=162) during the experiment and 6 flumes (with STDev).

Table 4**:** Inter flume comparison: mean values of EPS (carbohydrates and protein) and chlorophyll *a* contents (n=120), bacterial cell counts (n=40) and surface adhesiveness (n=135) during the experiment and 5 flumes (with STDev).

Table 5**:** Results of the Kruskall Wallis tests: intra-flume and inter-flume comparisons of the measured data.

Table 6**:** Algae species of the mature biofilms (22nd day): displayed as proportional abundances; species with a relative abundance of less than 2.0% were summarised as “others”.

Table 7: Seasonal comparison of biofilm productivity, microbial community and functionality (mean values and corresponding StDEV).

Table 8**:** Correlations of biofilm parameters during spring.

Table 9: Detected diatom species with minimal abundance of 1% of total counted frustules.

Table 10: Summarized comparison of the different boundary conditions.

Table 11**:** Temporal biofilm development under different levels of bed shear stress.

Table 12**:** Temporal biofilm development under different levels of light intensity.

Table 13: Comparison of biofilm grown under different levels of bed shear stress.

Table 14: Comparison of biofilm cultivated under different levels of illumination intensity.

Table 15: Comprehensive overview over correlations of biofilm parameters.

Table 16: Bacterial community of differently stable biofilms.

Table 17: Bacterial strains of analysed prominent DGGE bands.

Figures

Figure 1: Stages of biofilm development modified after Monroe (2007): 1. Initial attachment; 2. Irreversible attachment; 3. Early maturation; 4. Late maturation; 5. Dispersal.

Figure 2: ESEM image of a natural biofilm in an intertidal mudflat habitat (de Winder et al., 1999): the thick layer of EPS produced by the biofilm consisting of different diatoms has glued the sediment grains together so that no individual grains remain visible; the scale bar: 250 µm.

Figure 3: Images of two exemplary, very distinct morphologies and physical properties of natural biofilms cultivated during the presented thesis: left: very flexible, elastic biofilm/sediment mat; right: robust, compacted biofilm/ sediment sheet; scale bars: 1 cm.

Figure 4: AFM images of produced EPS network of the diatom Cylindrotheca closterium (Pletikapic et al., 2011); left: freshly produced polymers attached to the apex of the cell rostrum; right: self-aggregated EPS network around one cell of Cylindrotheca closterium.

Figure 5: Figure 5: ESEM image of the freshwater diatom Achnanthidium minutissimum and the secreted EPS capsule (Leinweber & Kroth, 2015); left: the EPS capsule is required for bacterial attachment and adhesion to the substrate (arrows indicate EPS attachment stalks); scale bar: 2 µm; right: detail image of produced EPS stalks required for attachment to the substratum; theses stalks exhibit high mechanical stability and longitudinal strength due to their composition of single parallel EPS fibrils (arrows); scale bar: 1 µm.

Figure 6: Experimental setup. top: image of three equivalent straight flumes installed in one containers; bottom: schematic image of one straight flume (a) outflow tank, (b) pump, (c) inlet flow section with baffles, (d) biofilm cultivation section, (e) outlet flow section, (f) weir, (g) fluorescent tubes, (h) sediment cartridges, (i) bypass, (j) current abatement, (k) fine tuning valve (after Thom et al., 2012b).

***Figure 7*:** Development of the colloidal EPS of the biofilms. Left: mean carbohydrate contents; right: mean protein contents (for both n=15; with corresponding STDev).

***Figure 8*:** Development of the microbial biomass in the biofilms. Left: mean chlorophyll a contents (n = 15; with STDev); right: mean bacterial cell counts (n = 5; with STDev).

Figure 9: Dynamics within the bacterial community. Left: DGGE band patterns of 16S rDNA gene diversity of biofilms exemplified shown for flume 1 (stained with ethidium bromide; inverted picture; numbers represent days of growth); right: moving window analysis of the bacterial community's dynamics in the same flume (crosses) based on densitometry similarity matrices. Additionally shown are the maximal (round dots) and minimal (triangular) change rates over the residual flumes.

***Figure 10*:** Functional organization of bacterial community expressed as Parento-Lorenz curves obtained via densitometry and normalization of DGGE peak patterns.

***Figure 11*:** Development of the adhesiveness of the biofilms displayed as mean determined current [mA] (n = 15; with STDev).

Figure 12: Temporal development of selected biofilm features (mean values with corresponding STDev): spring (▲) as well as summer (●) n=4; autumn (■) n=2. Upper left: EPS carbohydrate contents - per g dry weight of the sediment (DW); upper right: EPS protein contents; lower left: chlorophyll a contents; lower right: biofilm adhesiveness (T3) – with logarithmic ordinate.

Figure 13: Temporal development of the bacterial community. Inverted image of DGGE band patterns taken during spring from the experiment “May”. The indicated numbers represent days of growth. The developing specialization of the bacterial community and dominance of single bands became very plain.

***Figure 14*:** DCA of the diatom communities. Triangles: spring; circles: summer; squares: autumn. Numbers represent sampling days. A: “August”, J: “July”, M: “May”, m: “March”, N: “November”. The additional “a” and “b” represent the two different flumes or replicates run during one single sediment.

***Figure 15*:** Relative abundances of diatom genera [%] of early and late successional biofilm stages during all seasons. Genera with a relative abundance of less than 3.0 % were added up and summarized as 'others'.

Figure 16: Adhesiveness of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.

***Figure 17*:** Content of EPS components of early and late biofilm stages under different levels of LI; left: EPS carbohydrates; right: EPS proteins.

***Figure 18*:** Microbial biomass of early and late biofilm stages. Upper left: Algal biomass under different levels of bed shear stress; upper right: Bacterial biomass under different levels of bed shear stress; lower left: algal biomass under different levels of light intensity; lower right: Bacterial biomass under different levels of light intensity.

Figure 19: Range weighted richness (Rr) of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.

Figure 20: Functional organization (Fo) of the bacterial community of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.

Figure 21: Dynamics within the bacterial community of early and late biofilm stages. Left: under different levels of BSS; right: under different levels of LI.

Figure 22: Composition of early and late diatom communities under different levels of bed shear stress. Genera with a relative abundance less than 3% were summarized as “others”.

Figure 23**:** Temporal development of the diatom community under different light intensities: no algal growth was detectable under minimal illumination. Genera with a relative abundance less than 3% were summarized as “others”.

References

Abdullahi A.S., Underwood G.J.C. & Gretz M.R. (2006) Extracellular matrix assembly in diatoms (bacillariophyceae). V. Environmental effects on polysaccharide synthesis in the model diatom, Phaeodactylum tricornutum. *Journal of Phycology,* **42,** 363-378.

Aberle J., Dittrich A., Fischer-Antze T., Gölz E., Koll K., Schindler M., Stephan U., Wirtz C., Zanke U. & Zarn B. (2013) Merkblatt DWA-M 526 Grundlagen morphodynamischer Phänomene in Fließgewässern. Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e.V., Hennef.

Abia A.L.K., Ubomba-Jaswa E., Genthe B. & Momba M.N.B. (2016) Quantitative microbial risk assessment (QMRA) shows increased public health risk associated with exposure to river water under conditions of riverbed sediment resuspension. *Science of the Total Environment,* **566,** 1143-1151.

Agogue H., Mallet C., Orvain F., De Crignis M., Mornet F. & Dupuy C. (2014) Bacterial dynamics in a microphytobenthic biofilm: A tidal mesocosm approach. *Journal of Sea Research,* **92,** 36-45.

Amin S.A., Parker M.S. & Armbrust E.V. (2012) Interactions between Diatoms and Bacteria. *Microbiology and Molecular Biology Reviews,* **76,** 667-+.

Amos C.L., Bergamasco A., Umgiesser G., Cappucci S., Cloutier D., Denat L., Flindt M., Bonardi M. & Cristante S. (2004) The stability of tidal flats in Venice Lagoon - The results of in-situ measurements using two benthic, annular flumes. *Journal of Marine Systems,* **51,** 211-241.

Anderson A., Spears B., Lubarsky H., Davidson I., Gerbersdorf S. & Paterson D. (2011) Magnetic particle induction and its importance in biofilm research. In: *Biomedical Engineering - Frontiers and Challenges.* (Ed R. Fazel-Rezai).

Apoya-Horton M.D., Yin L., Underwood G.J.C. & Gretz M.R. (2006) Movement modalities and responses to environmental changes of the mudflat diatom Cylindrotheca closterium (Bacillariophyceae). *Journal of Phycology,* **42,** 379-390.

Araya R., Yamaguchi N., Tani K. & Nasu M. (2003) Change in the bacterial community of natural river biofilm during biodegradation of aniline-derived compounds determined by denaturing gradient gel electrophoresis. *Journal of Health Science,* **49,** 379-385.

Arenas J. & Tommassen J. (2017) Meningococcal Biofilm Formation: Let's Stick Together. *Trends in Microbiology,* **25,** 113-124.

Artigas J., Fund K., Kirchen S., Morin S., Obst U., Romani A.M., Sabater S. & Schwartz T. (2012) Patterns of biofilm formation in two streams from different bioclimatic regions: analysis of microbial community structure and metabolism. *Hydrobiologia,* **695,** 83-96.

Audry S., Schafer J., Blanc G. & Jouanneau J.M. (2004) Fifty-year sedimentary record of heavy metal pollution (Cd, Zn, Cu, Pb) in the Lot River reservoirs (France). *Environmental Pollution,* **132,** 413-426.

Austen I., Andersen T.J. & Edelvang K. (1999) The influence of benthic diatoms and invertebrates on the erodibility of an intertidal a mudflat, the Danish Wadden Sea. *Estuarine Coastal and Shelf Science,* **49,** 99-111.

Barmuta L.A. & Lake P.S. (1982) On the value of the river continuum concept. *New Zealand Journal of Marine and Freshwater Research,* **16,** 227-229.

Bartle A. (2002) Hydropower potential and development activities. *Energy Policy,* **30,** 1231-1239.

Batalla R.J. & Vericat D. (2009) HYDROLOGICAL AND SEDIMENT TRANSPORT DYNAMICS OF FLUSHING FLOWS: IMPLICATIONS FOR MANAGEMENT IN LARGE MEDITERRANEAN RIVERS. *River Research and Applications,* **25,** 297-314.

Battarbee R. (1986) Diatom analysis. Handbook of Holocene Palaeoecology and Palaeohydology. *John Wiley & Sons Ltd*.

Battin T.J., Kaplan L.A., Newbold J.D. & Hansen C.M.E. (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature,* **426,** 439-442.

Battin T.J., Sloan W.T., Kjelleberg S., Daims H., Head I.M., Curtis T.P. & Eberl L. (2007) Microbial landscapes: new paths to biofilm research. *Nature Reviews Microbiology,* **5,** 76-81.

Becker K. (1996) Exopolysaccharide production and attachment strength of bacteria and diatoms on substrates with different surface tensions. *Microbial Ecology,* **32,** 23-33.

Beier S., Witzel K.-P. & Marxsen J. (2008) Bacterial community composition in central European running waters examined by temperature gradient gel electrophoresis and sequence analysis of 16S rRNA genes. *Applied and Environmental Microbiology,* **74,** 188-199.

Bernard C.S., Giraud C., Spagnolo J. & De Bentzmann S. (2012) Biofilms: The Secret Story of Microbial Communities. In: *Bacterial Pathogenesis: Molecular and Cellular Mechanisms.* (Eds C. Locht & M. Simonet), pp. 129-168. Caister Academic Press, 32 Hewitts Lane, Wymondham Nr 18 0ja, Uk.

Besemer K., Hoedl I., Singer G. & Battin T.J. (2009) Architectural differentiation reflects bacterial community structure in stream biofilms. *Isme Journal,* **3,** 1318-1324.

Besemer K., Singer G., Limberger R., Chlup A.-K., Hochedlinger G., Hoedl I., Baranyi C. & Battin T.J. (2007) Biophysical controls on community succession in stream biofilms. *Applied and Environmental Microbiology,* **73,** 4966-4974.

Black K.S., Tolhurst T.J., Paterson D.M. & Hagerthey S.E. (2002) Working with natural cohesive sediments. *Journal of Hydraulic Engineering-Asce,* **128,** 2-8.

Blenkinsopp S.A. & Lock M.A. (1994) The impact of storm-flow on river biofilm architecture. *Journal of Phycology,* **30,** 807-818.

Böckelmann U., Manz W., Neu T.R. & Szewzyk U. (2000) Characterization of the microbial community of lotic organic aggregates ('river snow') in the Elbe River of Germany by cultivation and molecular methods. *Fems Microbiology Ecology,* **33,** 157-170.

Bohorquez J., Mcgenity T.J., Papaspyrou S., Garcia-Robledo E., Corzo A. & Underwood G.J.C. (2017) Different Types of Diatom-Derived Extracellular Polymeric Substances Drive Changes in Heterotrophic Bacterial Communities from Intertidal Sediments. *Frontiers in Microbiology,* **8,** 16.

Bowes M.J., Ings N.L., Mccall S.J., Warwick A., Barrett C., Wickham H.D., Harman S.A., Armstrong L.K., Scarlett P.M., Roberts C., Lehmann K. & Singer A.C. (2012) Nutrient and light limitation of periphyton in the River Thames: Implications for catchment management. *Science of the Total Environment,* **434,** 201-212.

Brimacombe C.A., Stevens A., Jun D., Mercer R., Lang A.S. & Beatty J.T. (2013) Quorum-sensing regulation of a capsular polysaccharide receptor for the Rhodobacter capsulatus gene transfer agent (RcGTA). *Molecular Microbiology,* **87,** 802-817.

Bruckner C.G., Rehm C., Grossart H.-P. & Kroth P.G. (2011) Growth and release of extracellular organic compounds by benthic diatoms depend on interactions with bacteria. *Environmental Microbiology,* **13,** 1052-1063.

Brümmer I.H.M., Fehr W. & Wagner-Döbler I. (2000) Biofilm community structure in polluted rivers: Abundance of dominant phylogenetic groups over a complete annual cycle. *Applied and Environmental Microbiology,* **66,** 3078-3082.

Brümmer I.H.M., Felske A. & Wagner-Döbler I. (2003) Diversity and seasonal variability of beta-proteobacteria in biofilms of polluted rivers: Analysis by temperature gradient gel electrophoresis and cloning. *Applied and Environmental Microbiology,* **69,** 4463-4473.

Brunke M. & Gonser T. (1997) The ecological significance of exchange processes between rivers and groundwater. *Freshwater Biology,* **37,** 1-33.

Bruzaud J., Tarrade J., Coudreuse A., Canette A., Herry J.M., De Givenchy E.T., Darmanin T., Guittard F., Guilbaud M. & Bellon-Fontaine M.N. (2015) Flagella but not type IV pili are involved in the initial adhesion of Pseudomonas aeruginosa PAO1 to hydrophobic or superhydrophobic surfaces. *Colloids and Surfaces B-Biointerfaces,* **131,** 59-66.

Buhmann M.T., Schulze B., Forderer A., Schleheck D. & Kroth P.G. (2016) BACTERIA MAY INDUCE THE SECRETION OF MUCIN-LIKE PROTEINS BY THE DIATOM PHAEODACTYLUM TRICORNUTUM. *Journal of Phycology,* **52,** 463-474.

Burke M., Jorde K. & Buffington J.M. (2009) Application of a hierarchical framework for assessing environmental impacts of dam operation: Changes in streamflow, bed mobility and recruitment of riparian trees in a western North American river. *Journal of Environmental Management,* **90,** S224-S236.

Burkholder J.M., Wetzel R.G. & Klomparens K.L. (1990) Direct comparison of phosphate-uptake by adnate and loosely attached microalgae within an intact biofilm matrix. *Applied and Environmental Microbiology,* **56,** 2882-2890.

Carling P.A. (1984) DEPOSITION OF FINE AND COARSE SAND IN AN OPEN-WORK GRAVEL BED. *Canadian Journal of Fisheries and Aquatic Sciences,* **41,** 263-280.

Carrick H.J. & Steinman A.D. (2001) Variation in periphyton biomass and species composition in Lake Okeechobee, Florida (USA): Distribution of algal guilds along environmental gradients. *Archiv Fur Hydrobiologie,* **152,** 411-438.

Chavant P., Martinie B., Meylheuc T., Bellon-Fontaine M.N. & Hebraud M. (2002) Listeria monocytogenes LO28: Surface physicochemical properties and ability to form biofilms at different temperatures and growth phases. *Applied and Environmental Microbiology,* **68,** 728-737.

Chiovitti A., Bacic A., Burke J. & Wetherbee R. (2003) Heterogeneous xylose-rich glycans are associated with extracellular glycoproteins from the biofouling diatom Craspedostauros australis (Bacillariophyceae). *European Journal of Phycology,* **38,** 351-360.

Chiovitti A., Dugdale T.M. & Wetherbee R. (2006) Diatom adhesives: Molecular and mechanical properties. In: *Biological Adhesives.* (Eds Smith Am & Callow Ja), pp. 79-103. Springer, Heidelberg.

Choi Y.C. & Morgenroth E. (2003) Monitoring biofilm detachment under dynamic changes in shear stress using laser-based particle size analysis and mass fractionation. *Water Science and Technology,* **47,** 69-76.

Chrismas N.a.M., Barker G., Anesio A.M. & Sanchez-Baracaldo P. (2016) Genomic mechanisms for cold tolerance and production of exopolysaccharides in the Arctic cyanobacterium Phormidesmis priestleyi BC1401. *Bmc Genomics,* **17,** 14.

Coma R., Ribes M., Gili J.M. & Zabala M. (2000) Seasonality in coastal benthic ecosystems. *Trends in Ecology & Evolution,* **15,** 448-453.

Coundoul F., Bonometti T., Graba M., Sauvage S., Perez J.M.S. & Moulin F.Y. (2015) Role of local flow conditions in river biofilm colonization and early growth. *River Research and Applications,* **31,** 350-367.

Crump B.C. & Hobbie J.E. (2005) Synchrony and seasonality in bacterioplankton communities of two temperate rivers. *Limnology and Oceanography,* **50,** 1718-1729.

Cuadrado D.G., Carmona N.B. & Bournod C. (2011) Biostabilization of sediments by microbial mats in a temperate siliciclastic tidal flat, Bahia Blanca estuary (Argentina). *Sedimentary Geology,* **237,** 95-101.

D'costa P.M. & Anil A.C. (2014) Penicillin-mediated changes in viable benthic diatom assemblages - insights about the relevance of bacteria across spatial and seasonal scales. *Marine and Freshwater Research,* **65,** 437-452.

De Angelis M., Siragusa S., Campanella D., Di Cagno R. & Gobbetti M. (2015) Comparative proteomic analysis of biofilm and planktonic cells of Lactobacillus plantarum DB200. *Proteomics,* **15,** 2244-2257.

De Brouwer J.F.C. & Stal L.J. (2002) Daily fluctuations of exopolymers in cultures of the benthic diatoms Cylindrotheca closterium and Nitzschia sp (Bacillariophyceae). *Journal of Phycology,* **38,** 464-472.

De Brouwer J.F.C., Wolfstein K., Ruddy G.K., Jones T.E.R. & Stal L.J. (2005) Biogenic stabilization of intertidal sediments: The importance of extracellular polymeric substances produced by benthic diatoms. *Microbial Ecology,* **49,** 501-512.

De Deckere E., Tolhurst T.J. & De Brouwer J.F.C. (2001) Destabilization of cohesive intertidal sediments by infauna. *Estuarine Coastal and Shelf Science,* **53,** 665-669.

De Troch M., Vergaerde I., Cnudde C., Vanormelingen P., Vyverman W. & Vincx M. (2012) The taste of diatoms: the role of diatom growth phase characteristics and associated bacteria for benthic copepod grazing. *Aquatic Microbial Ecology,* **67,** 47-58.

De Weert S., Vermeiren H., Mulders I.H.M., Kuiper I., Hendrickx N., Bloemberg G.V., Vanderleyden J., De Mot R. & Lugtenberg B.J.J. (2002) Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by Pseudomonas fluorescens. *Molecular Plant-Microbe Interactions,* **15,** 1173-1180.

De Winder B., Staats N., Stal L.J. & Paterson D.M. (1999) Carbohydrate secretion by phototrophic communities in tidal sediments. *Journal of Sea Research,* **42,** 131-146.

Decoin V., Gallique M., Barbey C., Le Mauff F., Poc C.D., Feuilloley M.G.J., Orange N. & Merieau A. (2015) A Pseudomonas fluorescens type 6 secretion system is related to mucoidy, motility and bacterial competition. *Bmc Microbiology,* **15**.

Deemer B.R., Harrison J.A., Li S.Y., Beaulieu J.J., Delsontro T., Barros N., Bezerra-Neto J.F., Powers S.M., Dos Santos M.A. & Vonk J.A. (2016) Greenhouse Gas Emissions from Reservoir Water Surfaces: A New Global Synthesis. *Bioscience,* **66,** 949-964.

Denicola D.M. & Mcintire C.D. (1990) Effects of substrate relief on the distribution of periphyton in laboratory streams. 1. Hydrology. *Journal of Phycology,* **26,** 624-633.

Descloux S., Datry T., Philippe M. & Marmonier P. (2010) Comparison of Different Techniques to Assess Surface and Subsurface Streambed Colmation with Fine Sediments. *International Review of Hydrobiology,* **95,** 520-540.

Descloux S., Datry T. & Usseglio-Polatera P. (2014) Trait-based structure of invertebrates along a gradient of sediment colmation: Benthos versus hyporheos responses. *Science of the Total Environment,* **466,** 265-276.

Deweger L.A., Vandervlugt C.I.M., Wijfjes A.H.M., Bakker P., Schippers B. & Lugtenberg B. (1987) Flagella of a plant-growth-simulating pseudomonas- fluorescens strains are required for colonization of potato roots. *Journal of Bacteriology,* **169,** 2769-2773.

Di Meglio L., Busalmen J.P., Pastore J.I., Ballarin V.L. & Nercessian D. (2014) Hyperhalophilic archaeal biofilms: growth kinetics, structure, and antagonistic interaction in continuous culture. *Biofouling,* **30,** 237-245.

Donlan R.M. (2002) Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases,* **8,** 881-890.

Droppo I.G. (2001) Rethinking what constitutes suspended sediment. *Hydrological Processes,* **15,** 1551-1564.

Droppo I.G. (2004) Structural controls on floc strength and transport. *Canadian Journal of Civil Engineering,* **31,** 569-578.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. & Smith F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry,* **28,** 350-356.

Duffy B.K. & Defago G. (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by Pseudomonas fluorescens biocontrol strains. *Applied and Environmental Microbiology,* **65,** 2429-2438.

Dugdale T.M., Dagastine R., Chiovitti A., Mulvaney P. & Wetherbee R. (2005) Single adhesive nanofibers from a live diatom have the signature fingerprint of modular proteins. *Biophysical Journal,* **89,** 4252-4260.

Dugdale T.M., Dagastine R., Chiovitti A. & Wetherbee R. (2006) Diatom adhesive mucilage contains distinct supramolecular assemblies of a single modular protein. *Biophysical Journal,* **90,** 2987-2993.

Dugdale T.M., Willis A. & Wetherbee R. (2006) Adhesive modular proteins occur in the extracellular mucilage of the motile, pennate diatom Phaeodactylum tricornutum. *Biophysical Journal,* **90,** L58-L60.

Dvorak P., Hindak F., Hasler P., Hindakova A. & Poulickova A. (2014) Morphological and molecular studies of Neosynechococcus sphagnicola, gen. et sp nov (Cyanobacteria, Synechococcales). *Phytotaxa,* **170,** 24-34.

Dyhrman S.T., Jenkins B.D., Rynearson T.A., Saito M.A., Mercier M.L., Alexander H., Whitney L.P., Drzewianowski A., Bulygin V.V., Bertrand E.M., Wu Z., Benitez-Nelson C. & Heithoff A. (2012) The Transcriptome and Proteome of the Diatom Thalassiosira pseudonana Reveal a Diverse Phosphorus Stress Response. *Plos One,* **7**.

Dziewit L., Czarnecki J., Wibberg D., Radlinska M., Mrozek P., Szymczak M., Schlueter A., Puehler A. & Bartosik D. (2014) Architecture and functions of a multipartite genome of the methylotrophic bacterium Paracoccus aminophilus JCM 7686, containing primary and secondary chromids. *Bmc Genomics,* **15**.

Elhariry H., Gherbawy Y., El-Deeb B. & Altalhi A. (2012) Molecular Identification and Biofilm-Forming Ability of Culturable Aquatic Bacteria In Microbial Biofilms Formed in Drinking Water Distribution Networks. *Geomicrobiology Journal,* **29,** 561-569.

Emge P., Moeller J., Jang H., Rusconi R., Yawata Y., Stocker R. & Vogel V. (2016) Resilience of bacterial quorum sensing against fluid flow. *Scientific Reports,* **6,** 10.

Emtiazi F., Schwartz T., Marten S.M., Krolla-Sidenstein P. & Obst U. (2004) Investigation of natural biofilms formed during the production of drinking water from surface water embankment filtration. *Water Research,* **38,** 1197-1206.

Espeland E.M., Francoeur S.N. & Wetzel R.G. (2001) Influence of algal photosynthesis on biofilm bacterial production and associated glucosidase and xylosidase activities. *Microbial Ecology,* **42,** 524-530.

Fang H.H.P., Chan K.Y. & Xu L.C. (2000) Quantification of bacterial adhesion forces using atomic force microscopy (AFM). *Journal of Microbiological Methods,* **40,** 89-97.

Fenchel T. & Glud R.N. (2000) Benthic primary production and O-2-CO2 dynamics in a shallow-water sediment: Spatial and temporal heterogeneity. *Ophelia,* **53,** 159-171.

Fink R., Oder M., Rangus D., Raspor P. & Bohinc K. (2015) Microbial adhesion capacity. Influence of shear and temperature stress. *International Journal of Environmental Health Research,* **25,** 656-669.

Flemming H.-C. & Wingender J. (2010) The biofilm matrix. *Nature Reviews Microbiology,* **8,** 623-633.

Flemming H., Wingender J., Mayer C.K., V. & Borchard W. (2000a) Cohesiveness in biofilm matrix polymers. In: *SGM Symposium*

Flemming H.C. & Wingender J. (2001) Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects. *Water Science and Technology,* **43,** 1-8.

Flemming H.C., Wingender J., Griebe T. & Mayer C. (2000b) Physio-Chemcal Properties of Biofilms. In: *Biofilms: Recent Advances in their Study and Control.* (Ed L.V. Evans), p. 20. CRC Press.

Flemming H.C., Wingender J., Szewzyk U., Steinberg P., Rice S.A. & Kjelleberg S. (2016) Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology,* **14,** 563-575.

Fogg G.E. (1983) The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Marina,* **26,** 3-14.

Förstner U. (2004) Sediment dynamics and pollutant mobility in rivers: An interdisciplinary approach. *Lakes & Reservoirs Research and Management,* **9,** 25-40.

Frolund B., Palmgren R., Keiding K. & Nielsen P.H. (1996) Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research,* **30,** 1749-1758.

Gaerdes A., Iversen M.H., Grossart H.-P., Passow U. & Ullrich M.S. (2011) Diatom-associated bacteria are required for aggregation of Thalassiosira weissflogii. *Isme Journal,* **5,** 436-445.

Gao Y., Jiang X.Q., Lin D.J., Chen Y.H. & Tong Z.C. (2016) The Starvation Resistance and Biofilm Formation of Enterococcus faecalis in Coexistence with Candida albicans, Streptococcus gordonii, Actinomyces viscosus, or Lactobacillus acidophilus. *Journal of Endodontics,* **42,** 1233-1238.

Garnier J., Billen G. & Coste M. (1995) Seasonal succession of diatoms and chlorophyceae in the drainage network of the Seine river - observations and modelling. *Limnology and Oceanography,* **40,** 750-765.

Gashti M.P., Bellavance J., Kroukamp O., Wolfaardt G., Taghavi S.M. & Greener J. (2015) Live-streaming: Time-lapse video evidence of novel streamer formation mechanism and varying viscosity. *Biomicrofluidics,* **9,** 4.

Gawne B., Wang Y., Hoagland K.D. & Gretz M.R. (1998) Role of bacteria and bacterial exopolymer in the attachment of Achnanthes longipes (Bacillariophyceae). *Biofouling,* **13,** 137-156.

Geider R.J., Macintyre H.L. & Kana T.M. (1998) A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. *Limnology and Oceanography,* **43,** 679-694.

Gerbersdorf S.U., Bittner R., Lubarsky H., Manz W. & Paterson D.M. (2009) Microbial assemblages as ecosystem engineers of sediment stability. *Journal of Soils and Sediments,* **9,** 640-652.

Gerbersdorf S.U., Jancke T., Westrich B. & Paterson D.M. (2008) Microbial stabilization of riverine sediments by extracellular polymeric substances. *Geobiology,* **6,** 57-69.

Gerbersdorf S.U., Manz W. & Paterson D.M. (2008) The engineering potential of natural benthic bacterial assemblages in terms of the erosion resistance of sediments. *Fems Microbiology Ecology,* **66,** 282-294.

Gerbersdorf S.U. & Schubert H. (2011) Vertical migration of phytoplankton in coastal waters with different UVR transparency. *Environmental Sciences Europe,* **23,** 36.

Gerbersdorf S.U. & Wieprecht S. (2015) Biostabilization of cohesive sediments: revisiting the role of abiotic conditions, physiology and diversity of microbes, polymeric secretion, and biofilm architecture. *Geobiology,* **13,** 68-97.

Gette-Bouvarot M., Mermillod-Blondin F., Lemoine D., Delolme C., Danjean M., Etienne L. & Volatier L. (2015) The potential control of benthic biofilm growth by macrophytes-A mesocosm approach. *Ecological Engineering,* **75,** 178-186.

Gieg L.M., Fowler S.J. & Berdugo-Clavijo C. (2014) Syntrophic biodegradation of hydrocarbon contaminants. *Current Opinion in Biotechnology,* **27,** 21-29.

Glud R.N., Kuhl M., Kohls O. & Ramsing N.B. (1999) Heterogeneity of oxygen production and consumption in a photosynthetic microbial mat as studied by planar optodes. *Journal of Phycology,* **35,** 270-279.

Gölz E., Grebmayer T., Hartmann S., Knoblauch H., Koll K., Schaipp B., Schmidt A., Vollmer S., Wieprecht S., Faulhaber P., Habersack H., Harreiter H., Keller M., Liedermann M., Schimpf H., Sieben A. & Spreafico M. (2010) Merkblatt DWA-M 525 Sedimentmanagement in Fließgewässern - Grundlagen, Methoden, Fallbeispiele. Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e.V.

Grabowski R.C., Droppo I.G. & Wharton G. (2011) Erodibility of cohesive sediment: The importance of sediment properties. *Earth-Science Reviews,* **105,** 101-120.

Gu H., Chen A., Song X.R., Brasch M.E., Henderson J.H. & Ren D.C. (2016) How Escherichia coli lands and forms cell clusters on a surface: a new role of surface topography. *Scientific Reports,* **6,** 14.

Haddad A., Jensen V., Becker T. & Haussler S. (2009) The Pho regulon influences biofilm formation and type three secretion in Pseudomonas aeruginosa. *Environmental Microbiology Reports,* **1,** 488-494.

Hanlon A.R.M., Bellinger B., Haynes K., Xiao G., Hofmann T.A., Gretz M.R., Ball A.S., Osborn A.M. & Underwood G.J.C. (2006) Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom-dominated, microalgal biofilm over a tidal emersion-immersion period. *Limnology and Oceanography,* **51,** 79-93.

Harimawan A., Rajasekar A. & Ting Y.P. (2011) Bacteria attachment to surfaces - AFM force spectroscopy and physicochemical analyses. *Journal of Colloid and Interface Science,* **364,** 213-218.

Harimawan A. & Ting Y.P. (2016) Investigation of extracellular polymeric substances (EPS) properties of P. aeruginosa and B. subtillis and their role in bacterial adhesion. *Colloids and Surfaces B-Biointerfaces,* **146,** 459-467.

Havens K.E., East T.L., Meeker R.H., Davis W.P. & Steinman A.D. (1996) Phytoplankton and periphyton responses to in situ experimental nutrient enrichment in a shallow subtropical lake. *Journal of Plankton Research,* **18,** 551-566.

Havskum H., Thingstad T.F., Scharek R., Peters F., Berdalet E., Sala M.M., Alcaraz M., Bangsholt J.C., Zweifel U.L., Hagstrom A., Perez M. & Dolan J.R. (2003) Silicate and labile DOC interfere in structuring the microbial food web via algal-bacterial competition for mineral nutrients: Results of a mesocosm experiment. *Limnology and Oceanography,* **48,** 129-140.

Hay S.I., Maitland T.C. & Paterson D.M. (1993) The speed of diatom migration through natural and artificial substrata. *Diatom Research,* **8,** 371-384.

He X.Y., Wang J.P., Abdoli L. & Li H. (2016) Mg2+/Ca2+ promotes the adhesion of marine bacteria and algae and enhances following biofilm formation in artificial seawater. *Colloids and Surfaces B-Biointerfaces,* **146,** 289-295.

Henrici A.T. & Johnson D.E. (1935) Studies of Freshwater Bacteria: II. Stalked Bacteria, a New Order of Schizomycetes. *Journal of Bacteriology,* **30,** 61-93.

Hermansson M. (1999) The DLVO theory in microbial adhesion. *Colloids and Surfaces B-Biointerfaces,* **14,** 105-119.

Heywood M.J.T. & Walling D.E. (2007) The sedimentation of salmonid spawning gravels in the Hampshire Avon catchment, UK: implications for the dissolved oxygen content of intragravel water and embryo survival. *Hydrological Processes,* **21,** 770-788.

Higgins M.J., Crawford S.A., Mulvaney P. & Wetherbee R. (2002) Characterization of the adhesive mucilages secreted by live diatom cells using atomic force microscopy. *Protist,* **153,** 25-38.

Higgins M.J., Sader J.E., Mulvaney P. & Wetherbee R. (2003) Probing the surface of living diatoms with atomic force microscopy: The nanostructure and nanomechanical properties of the mucilage layer. *Journal of Phycology,* **39,** 722-734.

Hillebrand H. (2008) Grazing regulates the spatial variability of periphyton biomass. *Ecology,* **89,** 165-173.

Hillebrand H., Durselen C.D., Kirschtel D., Pollingher U. & Zohary T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology,* **35,** 403-424.

Hillebrand H. & Sommer U. (1997) Response of epilithic microphytobenthos of the Western Baltic Sea to in situ experiments with nutrient enrichment. *Marine Ecology Progress Series,* **160,** 35-46.

Hoffman M.D., Zucker L.I., Brown P.J.B., Kysela D.T., Brun Y.V. & Jacobson S.C. (2015) Timescales and Frequencies of Reversible and Irreversible Adhesion Events of Single Bacterial Cells. *Analytical Chemistry,* **87,** 12032-12039.

Hoffmann B., Volkmer E., Kokott A., Augat P., Ohnmacht M., Sedlmayr N., Schieker M., Claes L., Mutschler W. & Ziegler G. (2009) Characterisation of a new bioadhesive system based on polysaccharides with the potential to be used as bone glue. *Journal of Materials Science-Materials in Medicine,* **20,** 2001-2009.

Hofmann G., Werum M. & Lange-Bertalot H. (2011) Diatomeen im Süßwasser-Benthos von Mitteleuropa. *A.R.G. Gantner Verlag K.G.*: Ruggell.

Holtappels M. & Lorke A. (2011) Estimating turbulent diffusion in a benthic boundary layer. *Limnology and Oceanography-Methods,* **9,** 29-41.

Horn H. & Hempel D.C. (1998) Modeling mass transfer and substrate utilization in the boundary layer of biofilm systems. *Water Science and Technology,* **37,** 139-147.

Hudek L., Premachandra D., Webster W.a.J. & Brau L. (2016) Role of Phosphate Transport System Component PstB1 in Phosphate Internalization by Nostoc punctiforme. *Applied and Environmental Microbiology,* **82,** 6344-6356.

Jackson C.R., Churchill P.F. & Roden E.E. (2001) Successional changes in bacterial assemblage structure during epilithic biofilm development. *Ecology,* **82,** 555-566.

Jermy A. (2010) BIOFILMS eDNA limits biofilm attachment. *Nature Reviews Microbiology,* **8,** 612-612.

Jones R.T., Sanchez-Contreras M., Vlisidou I., Amos M.R., Yang G.W., Munoz-Berbel X., Upadhyay A., Potter U.J., Joyce S.A., Ciche T.A., Jenkins A.T.A., Bagby S., Ffrench-Constant R.H. & Waterfield N.R. (2010) Photorhabdus adhesion modification protein (Pam) binds extracellular polysaccharide and alters bacterial attachment. *Bmc Microbiology,* **10,** 13.

Kaden R., Sproeer C., Beyer D. & Krolla-Sidenstein P. (2014) Rhodoferax saidenbachensis sp nov., a psychrotolerant, very slowly growing bacterium within the family Comamonadaceae, proposal of appropriate taxonomic position of Albidiferax ferrireducens strain T118(T) in the genus Rhodoferax and emended description of the genus Rhodoferax. *International Journal of Systematic and Evolutionary Microbiology,* **64,** 1186-1193.

Kalmbach S., Manz W., Wecke J. & Szewzyk U. (1999) Aquabacterium gen. nov., with description of Aquabacterium citratiphilum sp. nov., Aquabacterium parvum sp. nov. and Aquabacterium commune sp. nov., three in situ dominant bacterial species from the Berlin drinking water system. *International Journal of Systematic Bacteriology,* **49,** 769-777.

Kanellopoulos C., Lamprinou V., Mitropoulos P. & Voudouris P. (2015) Thermogenic travertine deposits in Thermopylae hot springs (Greece) in association with cyanobacterial microflora. *Carbonates Evaporites*.

Karickhoff S.W., Brown D.S. & Scott T.A. (1979) Sorption of hydrophobic pollutants on natural sediments. *Water Research,* **13,** 241-248.

Karimi A., Karig D., Kumar A. & Ardekani A.M. (2015) Interplay of physical mechanisms and biofilm processes: review of microfluidic methods. *Lab on a Chip,* **15,** 23-42.

Karrasch B., Mehrens M., Rosenlöcher Y. & Peters K. (2001) The dynamics of phytoplankton, bacteria and heterotrophic flagellates at two banks near Magdeburg in the River Elbe (Germany). *Limnologica,* **31,** 93-107.

Kendrick M.R. & Huryn A.D. (2015) Discharge, legacy effects and nutrient availability as determinants of temporal patterns in biofilm metabolism and accrual in an arctic river. *Freshwater Biology,* **60,** 2323-2336.

Khandeparker R.D.S. & Bhosle N.B. (2001) Extracellular polymeric substances of the marine fouling diatom Amphora rostrata Wm.Sm. *Biofouling,* **17,** 117-127.

Kopmann R., Mewis P., Nujic M., Rüther N. & Schmautz M. (2014) Merkblatt DWA-M 540 Feststofftransportmodelle. Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e.V., Hennef.

Krammer K. & Lange-Bertalot H. (1986-1991) Bacillariophyceae. In: *Süßwasserflora von Mitteleuropa. Band 1-4.* (Eds H. Ettl & J. Gerloff & H. Heyning & D. Mollenhauer). Gustav Fischer Verlag, Jena.

Kudo I., Miyamoto M., Noiri Y. & Maita Y. (2000) Combined effects of temperature and iron on the growth and physiology of the marine diatom, Phaeodactylum tricornutum (Bacillariophyceae). *Journal of Phycology,* **36,** 1096-1102.

Kwon K., Shim H., Bae W., Oh J. & Bae J. (2016) Simultaneous biodegradation of carbon tetrachloride and trichloroethylene in a coupled anaerobic/aerobic biobarrier. *Journal of Hazardous Materials,* **313,** 60-67.

Lai C.Y., Zhong L., Zhang Y., Chen J.X., Wen L.L., Shi L.D., Sun Y.P., Ma F., Rittmann B.E., Zhou C., Tang Y.N., Zheng P. & Zhao H.P. (2016) Bioreduction of Chromate in a Methane-Based Membrane Biofilm Reactor. *Environmental Science & Technology,* **50,** 5832-5839.

Lamberti G.A., Gregory S.V., Ashkenas L.R., Li J.L., Steinman A.D. & Mcintire C.D. (1995) Influence of grazer type and abundance on plant- herbivore interactions in streams. *Hydrobiologia,* **306,** 179-188.

Länderarbeitsgemeinschaft Wasser. (1998) *Beurteilung der Wasserbeschaffenheit von Fließgewässern in der Bundesrepublik Deutschland – Chemische Gewässergüteklassifikation,* LAWA, Berlin.

Lane D. (1990) 16S/23S rRNA sequencing. In: *Nucleic Acid Sequencing Techniques in Bacterial Systematics.* (Eds E. Stackebrandt & M. Goodfellow), pp. 115–148. Wiley, New York, USA.

Lange K., Liess A., Piggott J.J., Townsend C.R. & Matthaei C.D. (2011) Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology,* **56,** 264-278.

Larson F., Lubarsky H., Gerbersdorf S.U. & Paterson D.M. (2009) Surface adhesion measurements in aquatic biofilms using magnetic particle induction: MagPI. *Limnology and Oceanography-Methods,* **7,** 490-497.

Law R.J., Elliott J.A., Jones I.D. & Page T. (2014) The influence of different environmental conditions upon the initial development and ecological dynamics of phytobenthic communities. *Fundamental and Applied Limnology,* **185,** 139-153.

Lawrence J.R., Chenier M.R., Roy R., Beaumier D., Fortin N., Swerhone G.D.W., Neu T.R. & Greer C.W. (2004) Microscale and molecular assessment of impacts of nickel, nutrients, and oxygen level on structure and function of river biofilm communities. *Applied and Environmental Microbiology,* **70,** 4326-4339.

Lear G., Anderson M.J., Smith J.P., Boxen K. & Lewis G.D. (2008) Spatial and temporal heterogeneity of the bacterial communities in stream epilithic biofilms. *Fems Microbiology Ecology,* **65,** 463-473.

Lear G., Bellamy J., Case B.S., Lee J.E. & Buckley H.L. (2014) Fine-scale spatial patterns in bacterial community composition and function within freshwater ponds. *Isme Journal,* **8,** 1715-1726.

Leinweber K. & Kroth P.G. (2015) Capsules of the diatom Achnanthidium minutissimum arise from fibrillar precursors and foster attachment of bacteria. *Peerj,* **3,** 17.

Lemon K.P., Higgins D.E. & Kolter R. (2007) Flagellar motility is critical for Listeria monocytogenes biofilm formation. *Journal of Bacteriology,* **189,** 4418-4424.

Lemos M., Mergulhao F., Melo L. & Simoes M. (2015) The effect of shear stress on the formation and removal of Bacillus cereus biofilms. *Food and Bioproducts Processing,* **93,** 242-248.

Li G.L., Smith C.S., Brun Y.V. & Tang J.X. (2005) The elastic properties of the Caulobacter crescentus adhesive holdfast are dependent on oligomers of N-acetylglucosamine. *Journal of Bacteriology,* **187,** 257-265.

Li G.L., Tam L.K. & Tang J.X. (2008) Amplified effect of Brownian motion in bacterial near-surface swimming. *Proceedings of the National Academy of Sciences of the United States of America,* **105,** 18355-18359.

Li X., Hu H.Y., Gan K. & Sun Y.X. (2010) Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. *Bioresource Technology,* **101,** 5494-5500.

Lind J.L., Heimann K., Miller E.A., Vanvliet C., Hoogenraad N.J. & Wetherbee R. (1997) Substratum adhesion and gliding in a diatom are mediated by extracellular proteoglycans. *Planta,* **203,** 213-221.

Lorenz M.O. (1905) Methods of measuring the concentration of wealth. *Journal of the American Statistical Association,* **9,** 209-219.

Lubarsky H. (2011) The impact of microbial extracellular polymeric substances on sediment stability. *doctoral dissertation, University of St Andrews*.

Lubarsky H.V., Gerbersdorf S.U., Hubas C., Behrens S., Ricciardi F. & Paterson D.M. (2012) Impairment of the Bacterial Biofilm Stability by Triclosan. *Plos One,* **7**.

Lubarsky H.V., Hubas C., Chocholek M., Larson F., Manz W., Paterson D.M. & Gerbersdorf S.U. (2010) The Stabilisation Potential of Individual and Mixed Assemblages of Natural Bacteria and Microalgae. *Plos One,* **5**.

Lundkvist M., Gangelhof U., Lunding J. & Flindt M.R. (2007) Production and fate of extracellular polymeric substances produced by benthic diatoms and bacteria: A laboratory study. *Estuarine Coastal and Shelf Science,* **75,** 337-346.

Lyautey E., Jackson C.R., Cayrou J., Rols J.L. & Garabetian F. (2005) Bacterial community succession in natural river biofilm assemblages. *Microbial Ecology,* **50,** 589-601.

Magaletti E., Urbani R., Sist P., Ferrari C.R. & Cicero A.M. (2004) Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom Cylindrotheca fusiformis under N- and P-limitation. *European Journal of Phycology,* **39,** 133-142.

Malarkey J., Baas J.H., Hope J.A., Aspden R.J., Parsons D.R., Peakall J., Paterson D.M., Schindler R.J., Ye L.P., Lichtman I.D., Bass S.J., Davies A.G., Manning A.J. & Thorne P.D. (2015) The pervasive role of biological cohesion in bedform development. *Nature Communications,* **6,** 6.

Mann E.E., Rice K.C., Boles B.R., Endres J.L., Ranjit D., Chandramohan L., Tsang L.H., Smeltzer M.S., Horswill A.R. & Bayles K.W. (2009) Modulation of eDNA Release and Degradation Affects Staphylococcus aureus Biofilm Maturation. *Plos One,* **4,** 12.

Manz W., Wendt-Potthoff K., Neu T.R., Szewzyk U. & Lawrence J.R. (1999) Phylogenetic composition, spatial structure, and dynamics of lotic bacterial biofilms investigated by fluorescent in situ hybridization and confocal laser scanning microscopy. *Microbial Ecology,* **37,** 225-237.

Marcarelli A.M., Bechtold H.A., Rugenski A.T. & Inouye R.S. (2009) Nutrient limitation of biofilm biomass and metabolism in the Upper Snake River basin, southeast Idaho, USA. *Hydrobiologia,* **620,** 63-76.

Martina L.C., Marquez J., Principe R., Gari N. & Albarino R. (2014) Does grazing change algal communities from grassland and pine afforested streams?: A laboratory approach. *Limnologica,* **49,** 26-32.

Marzorati M., Wittebolle L., Boon N., Daffonchio D. & Verstraete W. (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. *Environmental Microbiology,* **10,** 1571-1581.

Mason C.F., Underwood G.J.C., Baker N.R., Davey P.A., Davidson I., Hanlon G., Long S.P., Oxborough K., Paterson D.M. & Watson A. (2003) The role of herbicides in the erosion of salt marshes in eastern England. *Environmental Pollution,* **122,** 41-49.

Mastropaolo M.D., Silby M.W., Nicoll J.S. & Levy S.B. (2012) Novel Genes Involved in Pseudomonas fluorescens Pf0-1 Motility and Biofilm Formation. *Applied and Environmental Microbiology,* **78,** 4318-4329.

Mcgenity T.J. (2014) Hydrocarbon biodegradation in intertidal wetland sediments. *Current Opinion in Biotechnology,* **27,** 46-54.

Mckay R.M.L., Geider R.J. & Laroche J. (1997) Physiological and biochemical response of the photosynthetic apparatus of two marine diatoms to Fe stress. *Plant Physiology,* **114,** 615-622.

Mckew B.A., Dumbrell A.J., Taylor J.D., Mcgenity T.J. & Underwood G.J.C. (2013) Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments. *Fems Microbiology Ecology,* **84,** 495-509.

Mckew B.A., Taylor J.D., Mcgenity T.J. & Underwood G.J.C. (2011) Resistance and resilience of benthic biofilm communities from a temperate saltmarsh to desiccation and rewetting. *Isme Journal,* **5,** 30-41.

Mills J.V., Antler G. & Turchyn A.V. (2016) Geochemical evidence for cryptic sulfur cycling in salt marsh sediments. *Earth and Planetary Science Letters,* **453,** 23-32.

Molino P.J., Hodson O.M., Quinn J.F. & Wetherbee R. (2006) Utilizing QCM-D to characterize the adhesive mucilage secreted by two marine diatom species in-situ and in real-time. *Biomacromolecules,* **7,** 3276-3282.

Monroe D. (2007) Looking for chinks in the armor of bacterial biofilms. *Plos Biology,* **5,** 2458-2461.

Moreira J.M.R., Fulgencio R., Oliveira F., Machado I., Bialuch I., Melo L.F., Simoes M. & Mergulhao F.J. (2016) Evaluation of SICON (R) surfaces for biofouling mitigation in critical process areas. *Food and Bioproducts Processing,* **98,** 173-180.

Moreira J.M.R., Simoes M., Melo L.F. & Mergulhao F.J. (2015) The combined effects of shear stress and mass transfer on the balance between biofilm and suspended cell dynamics. *Desalination and Water Treatment,* **53,** 3348-3354.

Mortimer C.H. (1941) The exchange of dissolved substances between mud and water in lakes. *Journal of Ecology,* **29,** 280-329.

Moss J.A., Nocker A., Lepo J.E. & Snyder R.A. (2006) Stability and change in estuarine biofilm bacterial community diversity. *Applied and Environmental Microbiology,* **72,** 5679-5688.

Mueller-Steinhagen H., Malayeri M.R. & Watkinson A.P. (2011) Heat Exchanger Fouling: Mitigation and Cleaning Strategies. *Heat Transfer Engineering,* **32,** 189-196.

Mueller M., Pander J., Wild R., Lueders T. & Geist J. (2013) The effects of stream substratum texture on interstitial conditions and bacterial biofilms: Methodological strategies. *Limnologica,* **43,** 106-113.

Mulholland P.J., Steinman A.D., Marzolf E.R., Hart D.R. & Deangelis D.L. (1994) Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. *Oecologia,* **98,** 40-47.

Muyzer G., Dewaal E.C. & Uitterlinden A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology,* **59,** 695-700.

Mykytczuk N.C.S., Lawrence J.R., Omelon C.R., Southam G. & Whyte L.G. (2016) Microscopic characterization of the bacterial cell envelope of Planococcus halocryophilus Or1 during subzero growth at-15 degrees C. *Polar Biology,* **39,** 701-712.

Nejidat A., Saadi I. & Ronen Z. (2008) Effect of flagella expression on adhesion of Achromobacter piechaudii to chalk surfaces. *Journal of Applied Microbiology,* **105,** 2009-2014.

Nichols C.a.M., Nairn K.M., Glattauer V., Blackburn S.I., Ramshaw J.a.M. & Graham L.D. (2009) Screening Microalgal Cultures in Search of Microbial Exopolysaccharides with Potential as Adhesives. *Journal of Adhesion,* **85,** 97-125.

Noack M., Gerbersdorf S.U., Hillebrand G. & Wieprecht S. (2015) Combining Field and Laboratory Measurements to Determine the Erosion Risk of Cohesive Sediments Best. *Water,* **7,** 5061-5077.

Olapade O.A. & Leff L.G. (2004) Seasonal dynamics of bacterial assemblages in epilithic biofilms in a northeastern Ohio stream. *Journal of the North American Benthological Society,* **23,** 686-700.

Onder O., Aygun-Sunar S., Selamoglu N. & Daldal F. (2010) A Glimpse into the Proteome of Phototrophic Bacterium Rhodobacter capsulatus. *Recent Advances in Phototrophic Prokaryotes,* **675,** 179-209.

Otto K. & Silhavy T.J. (2002) Surface sensing and adhesion of Escherichia coli controlled by the Cpx-signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 2287-2292.

Pascault N., Roux S., Artigas J., Pesce S., Leloup J., Tadonleke R.D., Debroas D., Bouchez A. & Humbert J.F. (2014) A high-throughput sequencing ecotoxicology study of freshwater bacterial communities and their responses to tebuconazole. *Fems Microbiology Ecology,* **90,** 563-574.

Passarelli C., Meziane T., Thiney N., Boeuf D., Jesus B., Ruivo M., Jeanthon C. & Hubas C. (2015) Seasonal variations of the composition of microbial biofilms in sandy tidal flats: Focus of fatty acids, pigments and exopolymers. *Estuarine Coastal and Shelf Science,* **153,** 29-37.

Passarelli C., Olivier F., Paterson D.M. & Hubas C. (2012) Impacts of biogenic structures on benthic assemblages: microbes, meiofauna, macrofauna and related ecosystem functions. *Marine Ecology Progress Series,* **465,** 85-97.

Passy S.I. (2007) Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany,* **86,** 171-178.

Paterson D.M. (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. *Limnology and Oceanography,* **34,** 223-234.

Paterson D.M. & Black K.S. (2000) Siliciclastic intertidal microbial sediments. In: *Microbial Sediments.* (Eds R.E. Riding & S.M. Awramik), pp. 217-225. Springer, Berlin.

Paul B.J. & Duthie H. (1989) Nutrient cycling in the epilithon of running waters. *Canadian Journal of Botany,* **67,** 2302-2309.

Pennisi E. (2002) Materials science - Biology reveals new ways to hold on tight. *Science,* **296,** 250-251.

Perkins R.G., Underwood G.J.C., Brotas V., Snow G.C., Jesus B. & Ribeiro L. (2001) Responses of microphytobenthos to light: primary production and carbohydrate allocation over an emersion period. *Marine Ecology Progress Series,* **223,** 101-112.

Peterson C.G. (1996) Response of benthic algal communities to natural physical disturbance. In: *Aquatic Ecology Series; Algal ecology: Freshwater benthic ecosystems.* (Eds R.J. Stevenson & M.L. Bothwell & R.L. Lowe), pp. 375-402. Academic Press, Inc., San Diego.

Peyton B.M. & Characklis W.G. (1993) A statistical-analysis of the effect of substrate utilization and shear-stress on the kinetics of biofilm detachment. *Biotechnology and Bioengineering,* **41,** 728-735.

Pierre G., Zhao J.-M., Orvain F., Dupuy C., Klein G.L., Graber M. & Maugard T. (2014) Seasonal dynamics of extracellular polymeric substances (EPS) in surface sediments of a diatom-dominated intertidal mudflat (Marennes-Oleron, France). *Journal of Sea Research,* **92,** 26-35.

Ping L.Y., Birkenbeil J. & Monajembashi S. (2013) Swimming behavior of the monotrichous bacterium Pseudomonas fluorescens SBW25. *Fems Microbiology Ecology,* **86,** 36-44.

Pletikapic G., Radic T.M., Zimmermann A.H., Svetlicic V., Pfannkuchen M., Maric D., Godrijan J. & Zutic V. (2011) AFM imaging of extracellular polymer release by marine diatom Cylindrotheca closterium (Ehrenberg) Reiman & J.C. Lewin. *Journal of Molecular Recognition,* **24,** 436-445.

Poff N.L. & Ward J.V. (1995) Herbivory under different flow regimes - a field experiment and test of a model with a benthic stream insect. *Oikos,* **72,** 179-188.

Pohlon E., Marxsen J. & Kuesel K. (2010) Pioneering bacterial and algal communities and potential extracellular enzyme activities of stream biofilms. *Fems Microbiology Ecology,* **71,** 364-373.

Potapova M. & Charles D.F. (2005) Choice of substrate in algae-based water-quality assessment. *Journal of the North American Benthological Society,* **24,** 415-427.

Potapova M. & Hamilton P.B. (2007) Morphological and ecological variation within the Achnanthidium minutissimum (Bacillariophyceae) species complex. *Journal of Phycology,* **43,** 561-575.

Power M.E., Parker M.S. & Dietrich W.E. (2008) Seasonal reassembly of a river food web: Floods, droughts, and impacts of fish. *Ecological Monographs,* **78,** 263-282.

Prieto D.M., Devesa-Rey R., Rubinos D.A., Diaz-Fierros F. & Barral M.T. (2016) Biofilm Formation on River Sediments Under Different Light Intensities and Nutrient Inputs: A Flume Mesocosm Study. *Environmental Engineering Science,* **33,** 250-260.

Proctor C.R. & Hammes F. (2015) Drinking water microbiology - from measurement to management. *Current Opinion in Biotechnology,* **33,** 87-94.

Pruesse E., Quast C., Knittel K., Fuchs B.M., Ludwig W.G., Peplies J. & Glockner F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research,* **35,** 7188-7196.

Raaijmakers J.M., Weller D.M. & Thomashow L.S. (1997) Frequency of antibiotic-producing Pseudomonas spp. in natural environments. *Applied and Environmental Microbiology,* **63,** 881-887.

Rasmussen K. & Lewandowski Z. (1998) Microelectrode measurements of local mass transport rates in heterogeneous biofilms. *Biotechnology and Bioengineering,* **59,** 302-309.

Raunkjaer K., Hvitvedjacobsen T. & Nielsen P.H. (1994) Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. *Water Research,* **28,** 251-262.

Reisinger A.J., Tank J.L. & Dee M.M. (2016) Regional and seasonal variation in nutrient limitation of river biofilms. *Freshwater Science,* **35,** 474-489.

Rendueles O. & Ghigo J.M. (2012) Multi-species biofilms: how to avoid unfriendly neighbors. *Fems Microbiology Reviews,* **36,** 972-989.

Riber H.H. & Wetzel R.G. (1987) Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton'. *Limnology and Oceanography,* **32,** 1181-1194.

Righetti M. & Lucarelli C. (2007) May the Shields theory be extended to cohesive and adhesive benthic sediments? *Journal of Geophysical Research,* **112**.

Rochex A. & Lebeault J.M. (2007) Effects of nutrients on biofilm formation and detachment of a Pseudomonas putida strain isolated from a paper machine. *Water Research,* **41,** 2885-2892.

Roeselers G., Van Loosdrecht M.C.M. & Muyzer G. (2007) Heterotrophic pioneers facilitate phototrophic biofilm development. *Microbial Ecology,* **54,** 578-585.

Rogers J., Dowsett A.B., Dennis P.J., Lee J.V. & Keevil C.W. (1994) Influence of temperature and plumbing material selection on biofilm formation and growth of Legionella pneumophila in a model potable water system containing complex microbial flora. *Applied and Environmental Microbiology,* **60,** 1585-1592.

Romani A.M., Fund K., Artigas J., Schwartz T., Sabater S. & Obst U. (2008) Relevance of polymeric matrix enzymes during biofilm formation. *Microbial Ecology,* **56,** 427-436.

Romani A.M., Giorgi A., Acuna V. & Sabater S. (2004) The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. *Limnology and Oceanography,* **49,** 1713-1721.

Roy J.J., Sun L.H. & Ji L.H. (2014) Microalgal proteins: a new source of raw material for production of plywood adhesive. *Journal of Applied Phycology,* **26,** 1415-1422.

Sack E.L.W., Van Der Wielen P. & Van Der Kooij D. (2014) Polysaccharides and Proteins Added to Flowing Drinking Water at Microgram-per-Liter Levels Promote the Formation of Biofilms Predominated by Bacteroidetes and Proteobacteria. *Applied and Environmental Microbiology,* **80,** 2360-2371.

Sahu P.K., Iyer P.S., Oak A.M., Pardesi K.R. & Chopade B.A. (2012) Characterization of eDNA from the Clinical Strain Acinetobacter baumannii AIIMS 7 and Its Role in Biofilm Formation. *Scientific World Journal***,** 1-10.

Schlesner H. & Stackebrandt E. (1986) Assignment of the genera Planctomyces and Pirella to a new family Planctomycetaceae fam-nov and description of the order Planctomycetales ord-nov. *Systematic and Applied Microbiology,* **8,** 174-176.

Schmidt H., Thom M., King L., Wieprecht S. & Gerbersdorf S.U. (2016) The effect of seasonality upon the development of lotic biofilms and microbial biostabilisation. *Freshwater Biology*.

Schmidt H., Thom M., Matthies K., Behrens S., Obst U., Wieprecht S. & Gerbersdorf S.U. (2015) A multi-disciplinarily designed mesocosm to address the complex flow-sediment-ecology tripartite relationship on the microscale. *Environmental Science Europe,* **27**.

Schneck F., Schwarzbold A. & Melo A.S. (2013) Substrate roughness, fish grazers, and mesohabitat type interact to determine algal biomass and sediment accrual in a high-altitude subtropical stream. *Hydrobiologia,* **711,** 165-173.

Schossow M., Arndt H. & Becker G. (2016) Response of gastropod grazers to food conditions, current velocity, and substratum roughness. *Limnologica,* **58,** 49-58.

Schreiber R.A. & Pennock J.R. (1995) The relative contribution of benthic microalgae to total microalgal production in a shallow subtidal estuarine environment. *Ophelia,* **42,** 335-352.

Sear D., Frostick L., Rollinson G. & Lisle T. (2008) The significance and mechanics of fine–sediment infiltration and accumulation in gravel spawning beds. In: *Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation.* (Eds D. Sear & P. Devries), pp. 149–174. American Fisheries Society Symposium 65, Bethesda, MD.

Sekar R., Venugopalan V.P., Nandakumar K., Nair K.V.K. & Rao V.N.R. (2004) Early stages of biofilm succession in a lentic freshwater environment. *Hydrobiologia,* **512,** 97-108.

Seminara A., Angelini T.E., Wilking J.N., Vlamakis H., Ebrahim S., Kolter R., Weitz D.A. & Brenner M.P. (2012) Osmotic spreading of Bacillus subtilis biofilms driven by an extracellular matrix. *Proceedings of the National Academy of Sciences of the United States of America,* **109,** 1116-1121.

Sfriso A., Pavoni B. & Marcomini A. (1995) Nutrient distribution in the surface sediment of the central lagoon of Venice. *Science of the Total Environment,* **172,** 21-35.

Shannon C.E. & Weaver W. (1963) The mathematical theory of communication. *The mathematical theory of communication***,** 117p.-117p.

Shields A. (1936) Application of similarity principles and turbulence research to bed-load movement. In: *Mitt Preuss Verschsanst, Berlin. Wasserbau Schiffbau.* (Eds W. Ott & J. Uchelen), pp. 43-58. California Institute of Technology, Pasadena.

Shrout J.D., Chopp D.L., Just C.L., Hentzer M., Givskov M. & Parsek M.R. (2006) The impact of quorum sensing and swarming motility on Pseudomonas aeruginosa biofilm formation is nutritionally conditional. *Molecular Microbiology,* **62,** 1264-1277.

Singer G., Besemer K., Hoedl I., Chlup A., Hochedlinger G., Stadler P. & Battin T.J. (2006) Microcosm design and evaluation to study stream microbial biofilms. *Limnology and Oceanography-Methods,* **4,** 436-447.

Singer G., Besemer K., Schmitt-Kopplin P., Hoedl I. & Battin T.J. (2010) Physical Heterogeneity Increases Biofilm Resource Use and Its Molecular Diversity in Stream Mesocosms. *Plos One,* **5**.

Sinsabaugh R.L., Repert D., Weiland T., Golladay S.W. & Linkins A.E. (1991) Exoenzyme accumulation in epilithic biofilms. *Hydrobiologia,* **222,** 29-37.

Smith D.J. & Underwood G.J.C. (1998) Exopolymer production by intertidal epipelic diatoms. *Limnology and Oceanography,* **43,** 1578-1591.

Smith D.J. & Underwood G.J.C. (2000) The production of extracellular carbohydrates by estuarine benthic diatoms: The effects of growth phase and light and dark treatment. *Journal of Phycology,* **36,** 321-333.

Somerfield P.J. (2008) Identification of the Bray-Curtis similarity index: Comment on Yoshioka (2008). *Marine Ecology Progress Series,* **372,** 303-306.

Spears B.M., Saunders J.E., Davidson I. & Paterson D.M. (2008) Microalgal sediment biostabilisation along a salinity gradient in the Eden Estuary, Scotland: unravelling a paradox. *Marine and Freshwater Research,* **59,** 313-321.

Staats N., De Winder B., Stal L.J. & Mur L.R. (1999) Isolation and characterization of extracellular polysaccharides from the epipelic diatoms Cylindrotheca closterium and Navicula salinarum. *European Journal of Phycology,* **34,** 161-169.

Stal L.J. (2003) Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiology Journal,* **20,** 463-478.

Stal L.J. (2010) Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecological Engineering,* **36,** 236-245.

Steen I.H., Dahle H., Stokke R., Roalkvam I., Daae F.L., Rapp H.T., Pedersen R.B. & Thorseth I.H. (2016) Novel Barite Chimneys at the Loki's Castle Vent Field Shed Light on Key Factors Shaping Microbial Communities and Functions in Hydrothermal Systems. *Frontiers in Microbiology,* **6,** 13.

Steinman A.D. (1992) Does an increase in irradiance influence periphyton in a heavily-grazed woodland stream? *Oecologia,* **91,** 163-170.

Steinman A.D., Mulholland P.J. & Hill W.R. (1992) Functional-responses associated with growth form in stream algae. *Journal of the North American Benthological Society,* **11,** 229-243.

Stewart P.S. (2012) Mini-review: Convection around biofilms. *Biofouling,* **28,** 187-198.

Stewart P.S. & Franklin M.J. (2008) Physiological heterogeneity in biofilms. *Nature Reviews Microbiology,* **6,** 199-210.

Stoodley P., Dodds I., De Beer D., Scott H.L. & Boyle J.D. (2005) Flowing biofilms as a transport mechanism for biomass through porous media under laminar and turbulent conditions in a laboratory reactor system. *Biofouling,* **21,** 161-168.

Sturt M.M., Jansen M.a.K. & Harrison S.S.C. (2011) Invertebrate grazing and riparian shade as controllers of nuisance algae in a eutrophic river. *Freshwater Biology,* **56,** 2580-2593.

Sun J. & Liu D.Y. (2003) Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research,* **25,** 1331-1346.

Sutherland I.W. (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology-Uk,* **147,** 3-9.

Sutherland T.F., Grant J. & Amos C.L. (1998) The effect of carbohydrate production by the diatom Nitzschia curvilineata on the erodibility of sediment. *Limnology and Oceanography,* **43,** 65-72.

Sveden J.B., Walve J., Larsson U. & Elmgren R. (2016) The bloom of nitrogen-fixing cyanobacteria in the northern Baltic Proper stimulates summer production. *Journal of Marine Systems,* **163,** 102-112.

Svetlicic V., Zutic V., Pletikapic G. & Radic T.M. (2013) Marine Polysaccharide Networks and Diatoms at the Nanometric Scale. *International Journal of Molecular Sciences,* **14,** 20064-20078.

Taylor I.S., Paterson D.M. & Mehlert A. (1999) The quantitative variability and monosaccharide composition of sediment carbohydrates associated with intertidal diatom assemblages. *Biogeochemistry,* **45,** 303-327.

Taylor J.D., Mckew B.A., Kuhl A., Mcgenity T.J. & Underwood G.J.C. (2013) Microphytobenthic extracellular polymeric substances (EPS) in intertidal sediments fuel both generalist and specialist EPS-degrading bacteria. *Limnology and Oceanography,* **58,** 1463-1480.

Tena A., Vericat D. & Batalla R.J. (2014) Suspended sediment dynamics during flushing flows in a large impounded river (the lower River Ebro). *Journal of Soils and Sediments,* **14,** 2057-2069.

Ter Braak C.J.F. (1987-1992) CANOCO - a FORTRAN program for Canocial Community Ordination. Microcomputer Power: Ithanca, New York.

Ter Braak C.J.F. & Smilauer P. (1998) CANOCO Reference manual and users guide to CANOCO for windows: Software for community ordination (vers. 4.0). Microcomputer Power: Ithanca, New York.

Thom M., Schmidt H., Gerbersdorf S.U. & Wieprecht S. (2015a) Seasonal biostabilization and erosion behavior of fluvial biofilms under different hydrodynamic and light conditions. *International Journal of Sediment Research,* **30,** 273-284.

Thom M., Schmidt H., Wieprecht S. & Gerbersdorf S. (2012a) Dimensionierung von Fließrinnen zum Aufwuchs von Biofilm unter definierten hydraulischen Randbedingungen. DGL-Jahrestagung (24. - 28. September 2012, Koblenz).

Thom M., Schmidt H., Wieprecht S. & Gerbersdorf S. (2012b) Physikalische Modellversuche zur Untersuchung des Einflusses von Biofilm auf die Sohlenstabilität. *Wasserwirtschaft,* **6,** 32-36.

Thom M., Schmidt H., Wieprecht S. & Gerbersdorf S. (2015b) Biostabilization of fluvial sediments: An improved device to address an old problem. In: *IAHR*, Den Haag, Netherlands.

Tichi M.A. & Tabita F.R. (2001) Interactive control of Rhodobacter capsulatus redox-balancing systems during phototrophic metabolism. *Journal of Bacteriology,* **183,** 6344-6354.

Tolhurst J., Gust G. & Paterson D. (2002) The influence of an extracellular polymeric substance (EPS) on cohesive sediment stability. **5,** 409-425.

Tolhurst T.J., Consalvey M. & Paterson D.M. (2008) Changes in cohesive sediment properties associated with the growth of a diatom biofilm. *Hydrobiologia,* **596,** 225-239.

Tolhurst T.J., Defew E.C., De Brouwer J.F.C., Wolfstein K., Stal L.J. & Paterson D.M. (2006) Small-scale temporal and spatial variability in the erosion threshold and properties of cohesive intertidal sediments. *Continental Shelf Research,* **26,** 351-362.

Trevors J.T. (2010) One gram of soil: a microbial biochemical gene library. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* **97,** 99-106.

Trueba A., Garca S., Otero F.M., Vega L.M. & Madariaga E. (2015) Influence of flow velocity on biofilm growth in a tubular heat exchanger-condenser cooled by seawater. *Biofouling,* **31,** 527-534.

Tseng B.S., Majerczyk C.D., Da Silva D.P., Chandler J.R., Greenberg E.P. & Parsek M.R. (2016) Quorum Sensing Influences Burkholderia thailandensis Biofilm Development and Matrix Production. *Journal of Bacteriology,* **198,** 2643-2650.

Tummers J.S., Winter E., Silva S., O'brien P., Jang M.H. & Lucas M.C. (2016) Evaluating the effectiveness of a Larinier super active baffle fish pass for European river lamprey Lampetra fluviatilis before and after modification with wall-mounted studded tiles. *Ecological Engineering,* **91,** 183-194.

Underwood G.J.C. (2002) Adaptations of tropical marine microphytobenthic assemblages along a gradient of light and nutrient availability in Suva Lagoon, Fiji. *European Journal of Phycology,* **37,** 449-462.

Underwood G.J.C., Boulcott M., Raines C.A. & Waldron K. (2004) Environmental effects on exopolymer production by marine benthic diatoms: Dynamics, changes in composition, and pathways of production. *Journal of Phycology,* **40,** 293-304.

Underwood G.J.C. & Paterson D.M. (1993) Seasonal changes in diatom biomass, sediment stability and biogenic stabilization in the Severn Estuary. *Journal of the Marine Biological Association of the United Kingdom,* **73,** 871-887.

Underwood G.J.C. & Paterson D.M. (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. *Advances in Botanical Research, Vol 40,* **40,** 183-240.

Urakami T., Araki H., Oyanagi H., Suzuki K.I. & Komagata K. (1990) Paracoccus - aminophilus sp-nov and Paracoccus - aminovorans sp-nov, which utilize N,N-dimethylformamide. *International Journal of Systematic Bacteriology,* **40,** 287-291.

Vancanneyt M., Segers P., Abraham W. & De Vos P. (2009) Brevundimonas. In: *Bergey's manual of systematic bacteriology.* (Ed G. Garrity), pp. 308-316. Springer, New York.

Verneuil L., Silvestre J., Randrianjatovo I., Marcato-Romain C.E., Girbal-Neuhauser E., Mouchet F., Ce E.F., Gauthier L. & Pinelli E. (2015) Double walled carbon nanotubes promote the overproduction of extracellular protein-like polymers in Nitzschia palea: An adhesive response for an adaptive issue. *Carbon,* **88,** 113-125.

Volle C.B., Ferguson M.A., Aidala K.E., Spain E.M. & Nunez M.E. (2008) Spring constants and adhesive properties of native bacterial biofilm cells measured by atomic force microscopy. *Colloids and Surfaces B-Biointerfaces,* **67,** 32-40.

Wagner K., Besemer K., Burns N.R., Battin T.J. & Bengtsson M.M. (2015) Light availability affects stream biofilm bacterial community composition and function, but not diversity. *Environmental Microbiology,* **17,** 5036-5047.

Wang J., Cao S., Du C. & Chen D. (2013) Underwater locomotion strategy by a benthic pennate diatom Navicula sp. *Protoplasma,* **250,** 1203-1212.

Wang S.S.Y. & Wu W. River sedimentation and morphology modeling – the state of the art and future development In: *Proceedings of the Ninth International Symposium on River Sedimentation*2004.

Wang S.W., Liu X., Liu H.S., Zhang L., Guo Y., Yu S., Wozniak D.J. & Ma L.Y.Z. (2015) The exopolysaccharide Psl-eDNA interaction enables the formation of a biofilm skeleton in Pseudomonas aeruginosa. *Environmental Microbiology Reports,* **7,** 330-340.

Wang Y., Chen Y., Lavin C. & Gretz M.R. (2000) Extracellular matrix assembly in diatoms (Bacillariophyceae). IV. Ultrastructure of Achnanthes longipes and Cymbella cistula as revealed by high-pressure freezing/freeze substituton and cryo-field emission scanning electron microscopy. *Journal of Phycology,* **36,** 367-378.

Waring J., Baker N.R. & Underwood G.J.C. (2007) Responses of estuarine intertidal microphytobenthic algal assemblages to enhanced ultraviolet B radiation. *Global Change Biology,* **13,** 1398-1413.

Waters C.M. & Bassler B.L. (2005) Quorum sensing: Cell-to-cell communication in bacteria. *Annual Review of Cell and Developmental Biology,* **21,** 319-346.

Wawrousek K., Noble S., Korlach J., Chen J., Eckert C., Yu J.P. & Maness P.C. (2014) Genome Annotation Provides Insight into Carbon Monoxide and Hydrogen Metabolism in Rubrivivax gelatinosus. *Plos One,* **9**.

Wey J.K., Juergens K. & Weitere M. (2012) Seasonal and Successional Influences on Bacterial Community Composition Exceed That of Protozoan Grazing in River Biofilms. *Applied and Environmental Microbiology,* **78,** 2013-2024.

Widdows J., Brinsley M.D., Salkeld P.N. & Lucas C.H. (2000) Influence of biota on spatial and temporal variation in sediment erodability and material flux on a tidal flat (Westerschelde, The Netherlands). *Marine Ecology Progress Series,* **194,** 23-37.

Willis A., Chiovitti A., Dugdale T.M. & Wetherbee R. (2013) Characterization of the extracellular matrix of Phaeodactylum Tricornutum (Bacillariophyceae): structure, composition, and adhesive characteristics. *Journal of Phycology,* **49,** 937-949.

Willis A., Eason-Hubbard M., Hodson O., Maheswari U., Bowler C. & Wetherbee R. (2014) ADHESION MOLECULES FROM THE DIATOM PHAEODACTYLUM TRICORNUTUM (BACILLARIOPHYCEAE): GENOMIC IDENTIFICATION BY AMINO-ACID PROFILING AND IN VIVO ANALYSIS. *Journal of Phycology,* **50,** 837-849.

Willkomm M., Schlussell A., Reiz E. & Arndt H. (2007) Effects of microcurrents in the boundary layer on the attachment of benthic heterotrophic nanoflagellates. *Aquatic Microbial Ecology,* **48,** 169-174.

Wimpenny J., Manz W. & Szewzyk U. (2000) Heterogeneity in biofilms. *Fems Microbiology Reviews,* **24,** 661-671.

Windler M., Leinweber K., Bartulos C.R., Philipp B. & Kroth P.G. (2015) Biofilm and capsule formation of the diatom Achnanthidium minutissimum are affected by a bacterium *Journal of Phycology,* **51,** 343-355.

Witt O. & Westrich B. (2003) Quantification of erosion rates for undisturbed contaminated cohesive sediment cores by image analysis. *Hydrobiologia,* **494,** 271-276.

Woese C.R. (1987) Bacterial evolution. *Microbiological Reviews,* **51,** 221-271.

Wotton R.S. (2004) The ubiquity and many roles of exopolymers (EPS) in aquatic systems. *Scientia Marina,* **68,** 13-21.

Wu W.M., Wang S.S.Y. & Jia Y.F. (2000) Nonuniform sediment transport in alluvial rivers. *Journal of Hydraulic Research,* **38,** 427-434.

Wustman B.A., Gretz M.R. & Hoagland K.D. (1997) Extracellular matrix assembly in diatoms (Bacillariophyceae) .1. A model of adhesives based on chemical characterization and localization of polysaccharides from the marine diatom Achnanthes longipes and other diatoms. *Plant Physiology,* **113,** 1059-1069.

Wustman B.A., Lind J., Wetherbee R. & Gretz M.R. (1998) Extracellular matrix assembly in diatoms - (Bacillariophyceae) - III. Organization of fucoglucuronogalactans within the adhesive stalks of Achnanthes longipes. *Plant Physiology,* **116,** 1431-1441.

Xu Y., Jiang H., Chu F. & Liu C. (2014) Fractal model for surface erosion of cohesive sediments. *Fractals-Complex Geometry Patterns and Scaling in Nature and Society,* **22**.

Yallop M.L., Paterson D.M. & Wellsbury P. (2000) Interrelationships between rates of microbial production, exopolymer production, microbial biomass, and sediment stability in biofilms of intertidal sediments. *Microbial Ecology,* **39,** 116-127.

Yannarell A.C. & Triplett E.W. (2005) Geographic and environmental sources of variation in lake bacterial community composition. *Applied and Environmental Microbiology,* **71,** 227-239.

Yoshihara A., Nobuhira N., Narahara H., Toyoda S., Tokumoto H., Konishi Y. & Nomura T. (2015) Estimation of the adhesive force distribution for the flagellar adhesion of Escherichia coli on a glass surface. *Colloids and Surfaces B-Biointerfaces,* **131,** 67-72.

Yoshioka T., Wada E. & Hayashi H. (1994) A stable-isotope study on seasonal food-web dynamics in a eutrophic lake. *Ecology,* **75,** 835-846.

Zeng Y., Baumbach J., Vieira Barbosa E.G., Azevedo V., Zhang C. & Koblizek M. (2016) Metagenomic evidence for the presence of phototrophic Gemmatimonadetes bacteria in diverse environments. *Environmental Microbiology Reports,* **8,** 139-149.

Zeng Y., Selyanin V., Lukes M., Dean J., Kaftan D., Feng F. & Koblizek M. (2015) Characterization of the microaerophilic, bacteriochlorophyll a-containing bacterium Gemmatimonas phototrophica sp nov., and emended descriptions of the genus Gemmatimonas and Gemmatimonas aurantiaca. *International Journal of Systematic and Evolutionary Microbiology,* **65,** 2410-2419.

Zgair A.K. & Chhibber S. (2013) Stenotrophomonas maltophilia flagellin is involved in bacterial adhesion and biofilm formation. *Microbiology,* **82,** 647-651.

Zhang W.B., Seminara A., Suaris M., Brenner M.P., Weitz D.A. & Angelini T.E. (2014) Nutrient depletion in Bacillus subtilis biofilms triggers matrix production. *New Journal of Physics,* **16,** 13.

Zippel B. & Neu T.R. (2005) Growth and structure off phototrophic biofilms under controlled light conditions. *Water Science and Technology,* **52,** 203-209.

Institut für Wasser- und Umweltsystemmodellierung
Universität Stuttgart

Pfaffenwaldring 61
70569 Stuttgart (Vaihingen)
Telefon (0711) 685 - 64717/64749/64752/64679
Telefax (0711) 685 - 67020 o. 64746 o. 64681
E-Mail: iws@iws.uni-stuttgart.de
http://www.iws.uni-stuttgart.de

Verzeichnis der Mitteilungshefte

1 Röhnisch, Arthur: *Die Bemühungen um eine Wasserbauliche Versuchsanstalt an der Technischen Hochschule Stuttgart*, und
Fattah Abouleid, Abdel: *Beitrag zur Berechnung einer in lockeren Sand gerammten, zweifach verankerten Spundwand*, 1963

2 Marotz, Günter: *Beitrag zur Frage der Standfestigkeit von dichten Asphaltbelägen im Großwasserbau*, 1964

3 Gurr, Siegfried: *Beitrag zur Berechnung zusammengesetzter ebener Flächentrag-werke unter besonderer Berücksichtigung ebener Stauwände, mit Hilfe von Rand-wert- und Lastwertmatrizen*, 1965

4 Plica, Peter: Ein Beitrag zur Anwendung von Schalenkonstruktionen im Stahlwasserbau, und Petrikat, Kurt: Möglichkeiten und Grenzen des wasserbaulichen Versuchswesens, 19665

5 Plate, Erich: *Beitrag zur Bestimmung der Windgeschwindigkeitsverteilung in der durch eine Wand gestörten bodennahen Luftschicht*, und
Röhnisch, Arthur; Marotz, Günter: *Neue Baustoffe und Bauausführungen für den Schutz der Böschungen und der Sohle von Kanälen, Flüssen und Häfen; Gestehungskosten und jeweilige Vorteile*, sowie Unny, T.E.: *Schwingungsuntersuchungen am Kegelstrahlschieber*, 1967

6 Seiler, Erich: *Die Ermittlung des Anlagenwertes der bundeseigenen Binnenschiffahrts-straßen und Talsperren und des Anteils der Binnenschiffahrt an diesem Wert*, 1967

7 *Sonderheft anläßlich des 65. Geburtstages von Prof. Arthur Röhnisch mit Beiträgen von* Benk, Dieter; Breitling, J.; Gurr, Siegfried; Haberhauer, Robert; Honekamp, Hermann; Kuz, Klaus Dieter; Marotz, Günter; Mayer-Vorfelder, Hans-Jörg; Miller, Rudolf; Plate, Erich J.; Radomski, Helge; Schwarz, Helmut; Vollmer, Ernst; Wildenhahn, Eberhard; 1967

8 Jumikis, Alfred: *Beitrag zur experimentellen Untersuchung des Wassernachschubs in einem gefrierenden Boden und die Beurteilung der Ergebnisse*, 1968

9 Marotz, Günter: *Technische Grundlagen einer Wasserspeicherung im natürlichen Untergrund*, 1968

10 Radomski, Helge: *Untersuchungen über den Einfluß der Querschnittsform wellenförmiger Spundwände auf die statischen und rammtechnischen Eigenschaften*, 1968

11 Schwarz, Helmut: *Die Grenztragfähigkeit des Baugrundes bei Einwirkung vertikal gezogener Ankerplatten als zweidimensionales Bruchproblem*, 1969

12 Erbel, Klaus: *Ein Beitrag zur Untersuchung der Metamorphose von Mittelgebirgsschneedecken unter besonderer Berücksichtigung eines Verfahrens zur Bestimmung der thermischen Schneequalität*, 1969

13 Westhaus, Karl-Heinz: *Der Strukturwandel in der Binnenschiffahrt und sein Einfluß auf den Ausbau der Binnenschiffskanäle*, 1969

14 Mayer-Vorfelder, Hans-Jörg: *Ein Beitrag zur Berechnung des Erdwiderstandes unter Ansatz der logarithmischen Spirale als Gleitflächenfunktion*, 1970

15 Schulz, Manfred: *Berechnung des räumlichen Erddruckes auf die Wandung kreiszylindrischer Körper*, 1970

16 Mobasseri, Manoutschehr: *Die Rippenstützmauer. Konstruktion und Grenzen ihrer Standsicherheit*, 1970

17 Benk, Dieter: *Ein Beitrag zum Betrieb und zur Bemessung von Hochwasserrückhaltebecken*, 1970

18 Gàl, Attila: *Bestimmung der mitschwingenden Wassermasse bei überströmten Fischbauchklappen mit kreiszylindrischem Staublech*, 1971, *vergriffen*

19 Kuz, Klaus Dieter: *Ein Beitrag zur Frage des Einsetzens von Kavitationserscheinungen in einer Düsenströmung bei Berücksichtigung der im Wasser gelösten Gase*, 1971, *vergriffen*

20 Schaak, Hartmut: *Verteilleitungen von Wasserkraftanlagen*, 1971

21 *Sonderheft zur Eröffnung der neuen Versuchsanstalt des Instituts für Wasserbau der Universität Stuttgart mit Beiträgen von* Brombach, Hansjörg; Dirksen, Wolfram; Gàl, Attila; Gerlach, Reinhard; Giesecke, Jürgen; Holthoff, Franz-Josef; Kuz, Klaus Dieter; Marotz, Günter; Minor, Hans-Erwin; Petrikat, Kurt; Röhnisch, Arthur; Rueff, Helge; Schwarz, Helmut; Vollmer, Ernst; Wildenhahn, Eberhard; 1972

22 Wang, Chung-su: *Ein Beitrag zur Berechnung der Schwingungen an Kegelstrahlschiebern*, 1972

23 Mayer-Vorfelder, Hans-Jörg: *Erdwiderstandsbeiwerte nach dem Ohde-Variationsverfahren*, 1972

24 Minor, Hans-Erwin: *Beitrag zur Bestimmung der Schwingungsanfachungsfunktionen überströmter Stauklappen*, 1972, *vergriffen*

25 Brombach, Hansjörg: *Untersuchung strömungsmechanischer Elemente (Fluidik) und die Möglichkeit der Anwendung von Wirbelkammerelementen im Wasserbau*, 1972, *vergriffen*

26 Wildenhahn, Eberhard: *Beitrag zur Berechnung von Horizontalfilterbrunnen*, 1972

27 Steinlein, Helmut: *Die Eliminierung der Schwebstoffe aus Flußwasser zum Zweck der unterirdischen Wasserspeicherung, gezeigt am Beispiel der Iller*, 1972

28 Holthoff, Franz Josef: *Die Überwindung großer Hubhöhen in der Binnenschiffahrt durch Schwimmerhebewerke*, 1973

29 Röder, Karl: *Einwirkungen aus Baugrundbewegungen auf trog- und kastenförmige Konstruktionen des Wasser- und Tunnelbaues*, 1973

30 Kretschmer, Heinz: *Die Bemessung von Bogenstaumauern in Abhängigkeit von der Talform*, 1973

31 Honekamp, Hermann: *Beitrag zur Berechnung der Montage von Unterwasserpipelines*, 1973

32 Giesecke, Jürgen: *Die Wirbelkammertriode als neuartiges Steuerorgan im Wasserbau*, und Brombach, Hansjörg: *Entwicklung, Bauformen, Wirkungsweise und Steuereigenschaften von Wirbelkammerverstärkern*, 1974

33 Rueff, Helge: *Untersuchung der schwingungserregenden Kräfte an zwei hintereinander angeordneten Tiefschützen unter besonderer Berücksichtigung von Kavitation*, 1974

34 Röhnisch, Arthur: *Einpreßversuche mit Zementmörtel für Spannbeton - Vergleich der Ergebnisse von Modellversuchen mit Ausführungen in Hüllwellrohren*, 1975

35 *Sonderheft anläßlich des 65. Geburtstages von Prof. Dr.-Ing. Kurt Petrikat mit Beiträgen von*: Brombach, Hansjörg; Erbel, Klaus; Flinspach, Dieter; Fischer jr., Richard; Gàl, Attila; Gerlach, Reinhard; Giesecke, Jürgen; Haberhauer, Robert; Hafner Edzard; Hausenblas, Bernhard; Horlacher, Hans-Burkhard; Hutarew, Andreas; Knoll, Manfred; Krummet, Ralph; Marotz, Günter; Merkle, Theodor; Miller, Christoph; Minor, Hans-Erwin; Neumayer, Hans; Rao, Syamala; Rath, Paul; Rueff, Helge; Ruppert, Jürgen; Schwarz, Wolfgang; Topal-Gökceli, Mehmet; Vollmer, Ernst; Wang, Chung-su; Weber, Hans-Georg; 1975

36 Berger, Jochum: *Beitrag zur Berechnung des Spannungszustandes in rotationssymmetrisch belasteten Kugelschalen veränderlicher Wandstärke unter Gas- und Flüssigkeitsdruck durch Integration schwach singulärer Differentialgleichungen*, 1975

37 Dirksen, Wolfram: *Berechnung instationärer Abflußvorgänge in gestauten Gerinnen mittels Differenzenverfahren und die Anwendung auf Hochwasserrückhaltebecken*, 1976

38 Horlacher, Hans-Burkhard: *Berechnung instationärer Temperatur- und Wärmespannungsfelder in langen mehrschichtigen Hohlzylindern*, 1976

39 Hafner, Edzard: *Untersuchung der hydrodynamischen Kräfte auf Baukörper im Tiefwasserbereich des Meeres*, 1977, ISBN 3-921694-39-6

40 Ruppert, Jürgen: *Über den Axialwirbelkammerverstärker für den Einsatz im Wasserbau*, 1977, ISBN 3-921694-40-X

41 Hutarew, Andreas: *Beitrag zur Beeinflußbarkeit des Sauerstoffgehalts in Fließgewässern an Abstürzen und Wehren*, 1977, ISBN 3-921694-41-8, *vergriffen*

42 Miller, Christoph: *Ein Beitrag zur Bestimmung der schwingungserregenden Kräfte an unterströmten Wehren*, 1977, ISBN 3-921694-42-6

43 Schwarz, Wolfgang: *Druckstoßberechnung unter Berücksichtigung der Radial- und Längsverschiebungen der Rohrwandung*, 1978, ISBN 3-921694-43-4

44 Kinzelbach, Wolfgang: *Numerische Untersuchungen über den optimalen Einsatz variabler Kühlsysteme einer Kraftwerkskette am Beispiel Oberrhein*, 1978, ISBN 3-921694-44-2

45 Barczewski, Baldur: *Neue Meßmethoden für Wasser-Luftgemische und deren Anwendung auf zweiphasige Auftriebsstrahlen*, 1979, ISBN 3-921694-45-0

46 Neumayer, Hans: *Untersuchung der Strömungsvorgänge in radialen Wirbelkammerverstärkern*, 1979, ISBN 3-921694-46-9

47 Elalfy, Youssef-Elhassan: *Untersuchung der Strömungsvorgänge in Wirbelkammerdioden und -drosseln*, 1979, ISBN 3-921694-47-7

48 Brombach, Hansjörg: *Automatisierung der Bewirtschaftung von Wasserspeichern*, 1981, ISBN

3-921694-48-5

49 Geldner, Peter: *Deterministische und stochastische Methoden zur Bestimmung der Selbstdichtung von Gewässern*, 1981, ISBN 3-921694-49-3, *vergriffen*

50 Mehlhorn, Hans: *Temperaturveränderungen im Grundwasser durch Brauchwassereinleitungen*, 1982, ISBN 3-921694-50-7, *vergriffen*

51 Hafner, Edzard: *Rohrleitungen und Behälter im Meer*, 1983, ISBN 3-921694-51-5

52 Rinnert, Bernd: *Hydrodynamische Dispersion in porösen Medien: Einfluß von Dichteunterschieden auf die Vertikalvermischung in horizontaler Strömung*, 1983, ISBN 3-921694-52-3, *vergriffen*

53 Lindner, Wulf: *Steuerung von Grundwasserentnahmen unter Einhaltung ökologischer Kriterien*, 1983, ISBN 3-921694-53-1, *vergriffen*

54 Herr, Michael; Herzer, Jörg; Kinzelbach, Wolfgang; Kobus, Helmut; Rinnert, Bernd: *Methoden zur rechnerischen Erfassung und hydraulischen Sanierung von Grundwasserkontaminationen*, 1983, ISBN 3-921694-54-X

55 Schmitt, Paul: *Wege zur Automatisierung der Niederschlagsermittlung*, 1984, ISBN 3-921694-55-8, *vergriffen*

56 Müller, Peter: *Transport und selektive Sedimentation von Schwebstoffen bei gestautem Abfluß*, 1985, ISBN 3-921694-56-6

57 El-Qawasmeh, Fuad: *Möglichkeiten und Grenzen der Tropfbewässerung unter besonderer Berücksichtigung der Verstopfungsanfälligkeit der Tropfelemente*, 1985, ISBN 3-921694-57-4, *vergriffen*

58 Kirchenbaur, Klaus: *Mikroprozessorgesteuerte Erfassung instationärer Druckfelder am Beispiel seegangsbelasteter Baukörper*, 1985, ISBN 3-921694-58-2

59 Kobus, Helmut (Hrsg.): *Modellierung des großräumigen Wärme- und Schadstofftransports im Grundwasser*, Tätigkeitsbericht 1984/85 (DFG-Forschergruppe an den Universitäten Hohenheim, Karlsruhe und Stuttgart), 1985, ISBN 3-921694-59-0, *vergriffen*

60 Spitz, Karlheinz: *Dispersion in porösen Medien: Einfluß von Inhomogenitäten und Dichteunterschieden*, 1985, ISBN 3-921694-60-4, *vergriffen*

61 Kobus, Helmut: *An Introduction to Air-Water Flows in Hydraulics*, 1985, ISBN 3-921694-61-2

62 Kaleris, Vassilios: *Erfassung des Austausches von Oberflächen- und Grundwasser in horizontalebenen Grundwassermodellen*, 1986, ISBN 3-921694-62-0

63 Herr, Michael: *Grundlagen der hydraulischen Sanierung verunreinigter Porengrundwasserleiter*, 1987, ISBN 3-921694-63-9

64 Marx, Walter: *Berechnung von Temperatur und Spannung in Massenbeton infolge Hydratation*, 1987, ISBN 3-921694-64-7

65 Koschitzky, Hans-Peter: *Dimensionierungskonzept für Sohlbelüfter in Schußrinnen zur Vermeidung von Kavitationsschäden*, 1987, ISBN 3-921694-65-5

66 Kobus, Helmut (Hrsg.): *Modellierung des großräumigen Wärme- und Schadstofftransports im Grundwasser*, Tätigkeitsbericht 1986/87 (DFG-Forschergruppe an den Universitäten Hohenheim, Karlsruhe und Stuttgart) 1987, ISBN 3-921694-66-3

67 Söll, Thomas: *Berechnungsverfahren zur Abschätzung anthropogener Temperaturanomalien im Grundwasser*, 1988, ISBN 3-921694-67-1

68 Dittrich, Andreas; Westrich, Bernd: *Bodenseeufererosion, Bestandsaufnahme und Bewertung*, 1988, ISBN 3-921694-68-X, *vergriffen*

69 Huwe, Bernd; van der Ploeg, Rienk R.: *Modelle zur Simulation des Stickstoffhaushaltes von Standorten mit unterschiedlicher landwirtschaftlicher Nutzung*, 1988, ISBN 3-921694-69-8, *vergriffen*

70 Stephan, Karl: *Integration elliptischer Funktionen*, 1988, ISBN 3-921694-70-1

71 Kobus, Helmut; Zilliox, Lothaire (Hrsg.): *Nitratbelastung des Grundwassers, Auswirkungen der Landwirtschaft auf die Grundwasser- und Rohwasserbeschaffenheit und Maßnahmen zum*

Schutz des Grundwassers. Vorträge des deutsch-französischen Kolloquiums am 6. Oktober 1988, Universitäten Stuttgart und Louis Pasteur Strasbourg (Vorträge in deutsch oder französisch, Kurzfassungen zweisprachig), 1988, ISBN 3-921694-71-X

72 Soyeaux, Renald: *Unterströmung von Stauanlagen auf klüftigem Untergrund unter Berücksichtigung laminarer und turbulenter Fließzustände*,1991, ISBN 3-921694-72-8

73 Kohane, Roberto: *Berechnungsmethoden für Hochwasserabfluß in Fließgewässern mit überströmten Vorländern*, 1991, ISBN 3-921694-73-6

74 Hassinger, Reinhard: *Beitrag zur Hydraulik und Bemessung von Blocksteinrampen in flexibler Bauweise*, 1991, ISBN 3-921694-74-4, *vergriffen*

75 Schäfer, Gerhard: *Einfluß von Schichtenstrukturen und lokalen Einlagerungen auf die Längsdispersion in Porengrundwasserleitern*, 1991, ISBN 3-921694-75-2

76 Giesecke, Jürgen: *Vorträge, Wasserwirtschaft in stark besiedelten Regionen; Umweltforschung mit Schwerpunkt Wasserwirtschaft*, 1991, ISBN 3-921694-76-0

77 Huwe, Bernd: *Deterministische und stochastische Ansätze zur Modellierung des Stickstoffhaushalts landwirtschaftlich genutzter Flächen auf unterschiedlichem Skalenniveau*, 1992, ISBN 3-921694-77-9, *vergriffen*

78 Rommel, Michael: *Verwendung von Kluftdaten zur realitätsnahen Generierung von Kluftnetzen mit anschließender laminar-turbulenter Strömungsberechnung*, 1993, ISBN 3-92 1694-78-7

79 Marschall, Paul: *Die Ermittlung lokaler Stofffrachten im Grundwasser mit Hilfe von Einbohrloch-Meßverfahren*, 1993, ISBN 3-921694-79-5, *vergriffen*

80 Ptak, Thomas: *Stofftransport in heterogenen Porenaquiferen: Felduntersuchungen und stochastische Modellierung*, 1993, ISBN 3-921694-80-9, *vergriffen*

81 Haakh, Frieder: *Transientes Strömungsverhalten in Wirbelkammern,* 1993, ISBN 3-921694-81-7

82 Kobus, Helmut; Cirpka, Olaf; Barczewski, Baldur; Koschitzky, Hans-Peter: *Versuchseinrichtung zur Grundwasser und Altlastensanierung VEGAS, Konzeption und Programmrahmen*, 1993, ISBN 3-921694-82-5

83 Zang, Weidong: *Optimaler Echtzeit-Betrieb eines Speichers mit aktueller Abflußregenerierung*, 1994, ISBN 3-921694-83-3, *vergriffen*

84 Franke, Hans-Jörg: *Stochastische Modellierung eines flächenhaften Stoffeintrages und Transports in Grundwasser am Beispiel der Pflanzenschutzmittelproblematik*, 1995, ISBN 3-921694-84-1

85 Lang, Ulrich: *Simulation regionaler Strömungs- und Transportvorgänge in Karstaquiferen mit Hilfe des Doppelkontinuum-Ansatzes: Methodenentwicklung und Parameteridenti-fikation*, 1995, ISBN 3-921694-85-X, *vergriffen*

86 Helmig, Rainer: *Einführung in die Numerischen Methoden der Hydromechanik*, 1996, ISBN 3-921694-86-8, *vergriffen*

87 Cirpka, Olaf: *CONTRACT: A Numerical Tool for Contaminant Transport and Chemical Transformations - Theory and Program Documentation -*, 1996, ISBN 3-921694-87-6

88 Haberlandt, Uwe: *Stochastische Synthese und Regionalisierung des Niederschlages für Schmutzfrachtberechnungen*, 1996, ISBN 3-921694-88-4

89 Croisé, Jean: *Extraktion von flüchtigen Chemikalien aus natürlichen Lockergesteinen mittels erzwungener Luftströmung*, 1996, ISBN 3-921694-89-2, *vergriffen*

90 Jorde, Klaus: *Ökologisch begründete, dynamische Mindestwasserregelungen bei Ausleitungskraftwerken*, 1997, ISBN 3-921694-90-6, *vergriffen*

91 Helmig, Rainer: *Gekoppelte Strömungs- und Transportprozesse im Untergrund - Ein Beitrag zur Hydrosystemmodellierung-*, 1998, ISBN 3-921694-91-4, *vergriffen*

92 Emmert, Martin: *Numerische Modellierung nichtisothermer Gas-Wasser Systeme in porösen*

Medien, 1997, ISBN 3-921694-92-2

93 Kern, Ulrich: *Transport von Schweb- und Schadstoffen in staugeregelten Fließgewässern am Beispiel des Neckars*, 1997, ISBN 3-921694-93-0, *vergriffen*

94 Förster, Georg: *Druckstoßdämpfung durch große Luftblasen in Hochpunkten von Rohrleitungen 1997*, ISBN 3-921694-94-9

95 Cirpka, Olaf: *Numerische Methoden zur Simulation des reaktiven Mehrkomponenten-transports im Grundwasser*, 1997, ISBN 3-921694-95-7, *vergriffen*

96 Färber, Arne: *Wärmetransport in der ungesättigten Bodenzone: Entwicklung einer thermischen In-situ-Sanierungstechnologie*, 1997, ISBN 3-921694-96-5

97 Betz, Christoph: *Wasserdampfdestillation von Schadstoffen im porösen Medium: Entwicklung einer thermischen In-situ-Sanierungstechnologie*, 1998, SBN 3-921694-97-3

98 Xu, Yichun: *Numerical Modeling of Suspended Sediment Transport in Rivers*, 1998, ISBN 3-921694-98-1, *vergriffen*

99 Wüst, Wolfgang: *Geochemische Untersuchungen zur Sanierung CKW-kontaminierter Aquifere mit Fe(0)-Reaktionswänden,* 2000, ISBN 3-933761-02-2

100 Sheta, Hussam: *Simulation von Mehrphasenvorgängen in porösen Medien unter Einbeziehung von Hysterese-Effekten*, 2000, ISBN 3-933761-03-4

101 Ayros, Edwin: *Regionalisierung extremer Abflüsse auf der Grundlage statistischer Verfahren*, 2000, ISBN 3-933761-04-2, *vergriffen*

102 Huber, Ralf: *Compositional Multiphase Flow and Transport in Heterogeneous Porous Media*, 2000, ISBN 3-933761-05-0

103 Braun, Christopherus: *Ein Upscaling-Verfahren für Mehrphasenströmungen in porösen Medien*, 2000, ISBN 3-933761-06-9

104 Hofmann, Bernd: *Entwicklung eines rechnergestützten Managementsystems zur Beurteilung von Grundwasserschadensfällen*, 2000, ISBN 3-933761-07-7

105 Class, Holger: *Theorie und numerische Modellierung nichtisothermer Mehrphasenprozesse in NAPL-kontaminierten porösen Medien,* 2001, ISBN 3-933761-08-5

106 Schmidt, Reinhard: *Wasserdampf- und Heißluftinjektion zur thermischen Sanierung kontaminierter Standorte*, 2001, ISBN 3-933761-09-3

107 Josef, Reinhold: *Schadstoffextraktion mit hydraulischen Sanierungsverfahren unter Anwendung von grenzflächenaktiven Stoffen*, 2001, ISBN 3-933761-10-7

108 Schneider, Matthias: *Habitat- und Abflussmodellierung für Fließgewässer mit unscharfen Berechnungsansätzen*, 2001, ISBN 3-933761-11-5

109 Rathgeb, Andreas: *Hydrodynamische Bemessungsgrundlagen für Lockerdeckwerke an überströmbaren Erddämmen*, 2001, ISBN 3-933761-12-3

110 Lang, Stefan: *Parallele numerische Simulation instätionärer Probleme mit adaptiven Methoden auf unstrukturierten Gittern*, 2001, ISBN 3-933761-13-1

111 Appt, Jochen; Stumpp Simone: *Die Bodensee-Messkampagne 2001, IWS/CWR Lake Constance Measurement Program 2001*, 2002, ISBN 3-933761-14-X

112 Heimerl, Stephan: *Systematische Beurteilung von Wasserkraftprojekten,* 2002, ISBN 3-933761-15-8, *vergriffen*

113 Iqbal, Amin: *On the Management and Salinity Control of Drip Irrigation,* 2002, ISBN 3-933761-16-6

114 Silberhorn-Hemminger, Annette: *Modellierung von Kluftaquifersystemen: Geostatistische Analyse und deterministisch-stochastische Kluftgenerierung,* 2002, ISBN 3-933761-17-4

115 Winkler, Angela: *Prozesse des Wärme- und Stofftransports bei der In-situ-Sanierung mit festen Wärmequellen,* 2003, ISBN 3-933761-18-2

116 Marx, Walter: *Wasserkraft, Bewässerung, Umwelt - Planungs- und Bewertungsschwerpunkte der Wasserbewirtschaftung,* 2003, ISBN 3-933761-19-0

117 Hinkelmann, Reinhard: *Efficient Numerical Methods and Information-Processing Techniques in Environment Water,* 2003, ISBN 3-933761-20-4

118 Samaniego-Eguiguren, Luis Eduardo: *Hydrological Consequences of Land Use / Land Cover and Climatic Changes in Mesoscale Catchments*, 2003, ISBN 3-933761-21-2

119 Neunhäuserer, Lina: *Diskretisierungsansätze zur Modellierung von Strömungs- und Transportprozessen in geklüftet-porösen Medien*, 2003, ISBN 3-933761-22-0

120 Paul, Maren: *Simulation of Two-Phase Flow in Heterogeneous Poros Media with Adaptive Methods*, 2003, ISBN 3-933761-23-9

121 Ehret, Uwe: *Rainfall and Flood Nowcasting in Small Catchments using Weather Radar*, 2003, ISBN 3-933761-24-7

122 Haag, Ingo: *Der Sauerstoffhaushalt staugeregelter Flüsse am Beispiel des Neckars - Analysen, Experimente, Simulationen -,* 2003, ISBN 3-933761-25-5

123 Appt, Jochen: *Analysis of Basin-Scale Internal Waves in Upper Lake Constance,* 2003, ISBN 3-933761-26-3

124 Hrsg.: Schrenk, Volker; Batereau, Katrin; Barczewski, Baldur; Weber, Karolin und Koschitzky, Hans-Peter: *Symposium Ressource Fläche und VEGAS - Statuskolloquium 2003, 30. September und 1. Oktober 2003,* 2003, ISBN 3-933761-27-1

125 Omar Khalil Ouda: *Optimisation of Agricultural Water Use: A Decision Support System for the Gaza Strip,* 2003, ISBN 3-933761-28-0

126 Batereau, Katrin: *Sensorbasierte Bodenluftmessung zur Vor-Ort-Erkundung von Schadensherden im Untergrund*, 2004, ISBN 3-933761-29-8

127 Witt, Oliver: *Erosionsstabilität von Gewässersedimenten mit Auswirkung auf den Stofftransport bei Hochwasser am Beispiel ausgewählter Stauhaltungen des Oberrheins*, 2004, ISBN 3-933761-30-1

128 Jakobs, Hartmut: *Simulation nicht-isothermer Gas-Wasser-Prozesse in komplexen Kluft-Matrix-Systemen*, 2004, ISBN 3-933761-31-X

129 Li, Chen-Chien: *Deterministisch-stochastisches Berechnungskonzept zur Beurteilung der Auswirkungen erosiver Hochwasserereignisse in Flussstauhaltungen,* 2004, ISBN 3-933761-32-8

130 Reichenberger, Volker; Helmig, Rainer; Jakobs, Hartmut; Bastian, Peter; Niessner, Jennifer: *Complex Gas-Water Processes in Discrete Fracture-Matrix Systems: Up-scaling, Mass-Conservative Discretization and Efficient Multilevel Solution,* 2004, ISBN 3-933761-33-6

131 Hrsg.: Barczewski, Baldur; Koschitzky, Hans-Peter; Weber, Karolin; Wege, Ralf: *VEGAS - Statuskolloquium 2004,* Tagungsband zur Veranstaltung am 05. Oktober 2004 an der Universität Stuttgart, Campus Stuttgart-Vaihingen, 2004, ISBN 3-933761-34-4

132 Asie, Kemal Jabir: *Finite Volume Models for Multiphase Multicomponent Flow through Porous Media*. 2005, ISBN 3-933761-35-2

133 Jacoub, George: *Development of a 2-D Numerical Module for Particulate Contaminant Transport in Flood Retention Reservoirs and Impounded Rivers,* 2004, ISBN 3-933761-36-0

134 Nowak, Wolfgang: *Geostatistical Methods for the Identification of Flow and Transport Parameters in the Subsurface,* 2005, ISBN 3-933761-37-9

135 Süß, Mia: *Analysis of the influence of structures and boundaries on flow and transport processes in fractured porous media*, 2005, ISBN 3-933761-38-7

136 Jose, Surabhin Chackiath: *Experimental Investigations on Longitudinal Dispersive Mixing in Heterogeneous Aquifers,* 2005, ISBN: 3-933761-39-5

137 Filiz, Fulya: *Linking Large-Scale Meteorological Conditions to Floods in Mesoscale Catchments,* 2005, ISBN 3-933761-40-9

138 Qin, Minghao: *Wirklichkeitsnahe und recheneffiziente Ermittlung von Temperatur und Spannungen bei großen RCC-Staumauern*, 2005, ISBN 3-933761-41-7

139 Kobayashi, Kenichiro: *Optimization Methods for Multiphase Systems in the Subsurface -*

Application to Methane Migration in Coal Mining Areas, 2005, ISBN 3-933761-42-5

140 Rahman, Md. Arifur: *Experimental Investigations on Transverse Dispersive Mixing in Heterogeneous Porous Media,* 2005, ISBN 3-933761-43-3

141 Schrenk, Volker: *Ökobilanzen zur Bewertung von Altlastensanierungsmaßnahmen,* 2005, ISBN 3-933761-44-1

142 Hundecha, Hirpa Yeshewatesfa: *Regionalization of Parameters of a Conceptual Rainfall-Runoff Model*, 2005, ISBN: 3-933761-45-X

143 Wege, Ralf: *Untersuchungs- und Überwachungsmethoden für die Beurteilung natürlicher Selbstreinigungsprozesse im Grundwasser,* 2005, ISBN 3-933761-46-8

144 Breiting, Thomas: *Techniken und Methoden der Hydroinformatik - Modellierung von komplexen Hydrosystemen im Untergrund*, 2006, ISBN 3-933761-47-6

145 Hrsg.: Braun, Jürgen; Koschitzky, Hans-Peter; Müller, Martin: *Ressource Untergrund: 10 Jahre VEGAS: Forschung und Technologieentwicklung zum Schutz von Grundwasser und Boden,* Tagungsband zur Veranstaltung am 28. und 29. September 2005 an der Universität Stuttgart, Campus Stuttgart-Vaihingen, 2005, ISBN 3-933761-48-4

146 Rojanschi, Vlad: *Abflusskonzentration in mesoskaligen Einzugsgebieten unter Berücksichtigung des Sickerraumes,* 2006, ISBN 3-933761-49-2

147 Winkler, Nina Simone: *Optimierung der Steuerung von Hochwasserrückhaltebecken-systemen,* 2006, ISBN 3-933761-50-6

148 Wolf, Jens: *Räumlich differenzierte Modellierung der Grundwasserströmung alluvialer Aquifere für mesoskalige Einzugsgebiete*, 2006, ISBN: 3-933761-51-4

149 Kohler, Beate: *Externe Effekte der Laufwasserkraftnutzung*, 2006, ISBN 3-933761-52-2

150 Hrsg.: Braun, Jürgen; Koschitzky, Hans-Peter; Stuhrmann, Matthias: VEGAS-Statuskol-loquium 2006, Tagungsband zur Veranstaltung am 28. September 2006 an der Universität Stuttgart, Cam¬pus Stuttgart-Vaihingen, 2006, ISBN 3-933761-53-0

151 Niessner, Jennifer: Multi-Scale Modeling of Multi-Phase - Multi-Component Processes in Heterogeneous Porous Media, 2006, ISBN 3 933761 54 9

152 Fischer, Markus: Beanspruchung eingeerdeter Rohrleitungen infolge Austrocknung bindiger Böden, 2006, ISBN 3-933761-55-7

153 Schneck, Alexander: Optimierung der Grundwasserbewirtschaftung unter Berücksichtigung der Belange der Wasserversorgung, der Landwirtschaft und des Naturschutzes, 2006, ISBN 3-933761-56-5

154 Das, Tapash: The Impact of Spatial Variability of Precipitation on the Predictive Uncertainty of Hydrological Models, 2006, ISBN 3-33761-57-3

155 Bielinski, Andreas: Numerical Simulation of CO2 sequestration in geological formations, 2007, ISBN 3-933761-58-1

156 Mödinger, Jens: Entwicklung eines Bewertungs- und Entscheidungsunterstützungs¬systems für eine nachhaltige regionale Grundwasserbewirtschaftung, 2006, ISBN 3-933761-60-3

157 Manthey, Sabine: Two-phase flow processes with dynamic effects in porous media - parameter estimation and simulation, 2007, ISBN 3-933761-61-1

158 Pozos Estrada, Oscar: Investigation on the Effects of Entrained Air in Pipelines, 2007, ISBN 3-933761-62-X

159 Ochs, Steffen Oliver: Steam injection into saturated porous media – process analysis including experimental and numerical investigations, 2007, ISBN 3-933761-63-8

160 Marx, Andreas: Einsatz gekoppelter Modelle und Wetterradar zur Abschätzung von Niederschlagsintensitäten und zur Abflussvorhersage, 2007, ISBN 3-933761-64-6

161 Hartmann, Gabriele Maria: Investigation of Evapotranspiration Concepts in Hydrological Modelling for Climate Change Impact Assessment, 2007, ISBN 3-933761-65-4

162 Kebede Gurmessa, Tesfaye: Numerical Investigation on Flow and Transport Characteristics to Improve Long-Term Simulation of Reservoir Sedimentation, 2007, ISBN 3-933761-66-2

163 Trifković, Aleksandar: Multi-objective and Risk-based Modelling Methodology for Planning,

Design and Operation of Water Supply Systems, 2007, ISBN 3-933761-67-0

164 Götzinger, Jens: Distributed Conceptual Hydrological Modelling - Simulation of Climate, Land Use Change Impact and Uncertainty Analysis, 2007, ISBN 3-933761-68-9

165 Hrsg.: Braun, Jürgen; Koschitzky, Hans-Peter; Stuhrmann, Matthias: VEGAS – Kolloquium 2007, Tagungsband zur Veranstaltung am 26. September 2007 an der Universität Stuttgart, Campus Stuttgart-Vaihingen, 2007, ISBN 3-933761-69-7

166 Freeman, Beau: Modernization Criteria Assessment for Water Resources Planning; Klamath Irrigation Project, U.S., 2008, ISBN 3-933761-70-0

167 Dreher, Thomas: Selektive Sedimentation von Feinstschwebstoffen in Wechselwirkung mit wandnahen turbulenten Strömungsbedingungen, 2008, ISBN 3-933761-71-9

168 Yang, Wei: Discrete-Continuous Downscaling Model for Generating Daily Precipitation Time Series, 2008, ISBN 3-933761-72-7

169 Kopecki, Ianina: Calculational Approach to FST-Hemispheres for Multiparametrical Benthos Habitat Modelling, 2008, ISBN 3-933761-73-5

170 Brommundt, Jürgen: Stochastische Generierung räumlich zusammenhängender Niederschlagszeitreihen, 2008, ISBN 3-933761-74-3

171 Papafotiou, Alexandros: Numerical Investigations of the Role of Hysteresis in Heterogeneous Two-Phase Flow Systems, 2008, ISBN 3-933761-75-1

172 He, Yi: Application of a Non-Parametric Classification Scheme to Catchment Hydrology, 2008, ISBN 978-3-933761-76-7

173 Wagner, Sven: Water Balance in a Poorly Gauged Basin in West Africa Using Atmospheric Modelling and Remote Sensing Information, 2008, ISBN 978-3-933761-77-4

174 Hrsg.: Braun, Jürgen; Koschitzky, Hans-Peter; Stuhrmann, Matthias; Schrenk, Volker: VEGAS-Kolloquium 2008 Ressource Fläche III, Tagungsband zur Veranstaltung am 01. Oktober 2008 an der Universität Stuttgart, Campus Stuttgart-Vaihingen, 2008, ISBN 978-3-933761-78-1

175 Patil, Sachin: Regionalization of an Event Based Nash Cascade Model for Flood Predictions in Ungauged Basins, 2008, ISBN 978-3-933761-79-8

176 Assteerawatt, Anongnart: Flow and Transport Modelling of Fractured Aquifers based on a Geostatistical Approach, 2008, ISBN 978-3-933761-80-4

177 Karnahl, Joachim Alexander: 2D numerische Modellierung von multifraktionalem Schwebstoff- und Schadstofftransport in Flüssen, 2008, ISBN 978-3-933761-81-1

178 Hiester, Uwe: Technologieentwicklung zur In-situ-Sanierung der ungesättigten Bodenzone mit festen Wärmequellen, 2009, ISBN 978-3-933761-82-8

179 Laux, Patrick: Statistical Modeling of Precipitation for Agricultural Planning in the Volta Basin of West Africa, 2009, ISBN 978-3-933761-83-5

180 Ehsan, Saqib: Evaluation of Life Safety Risks Related to Severe Flooding, 2009, ISBN 978-3-933761-84-2

181 Prohaska, Sandra: Development and Application of a 1D Multi-Strip Fine Sediment Transport Model for Regulated Rivers, 2009, ISBN 978-3-933761-85-9

182 Kopp, Andreas: Evaluation of CO2 Injection Processes in Geological Formations for Site Screening, 2009, ISBN 978-3-933761-86-6

183 Ebigbo, Anozie: Modelling of biofilm growth and its influence on CO2 and water (two-phase) flow in porous media, 2009, ISBN 978-3-933761-87-3

184 Freiboth, Sandra: A phenomenological model for the numerical simulation of multiphase multicomponent processes considering structural alterations of porous media, 2009, ISBN 978-3-933761-88-0

185 Zöllner, Frank: Implementierung und Anwendung netzfreier Methoden im Konstruktiven Wasserbau und in der Hydromechanik, 2009, ISBN 978-3-933761-89-7

186 Vasin, Milos: Influence of the soil structure and property contrast on flow and transport in the

unsaturated zone, 2010, ISBN 978-3-933761-90-3

187 Li, Jing: Application of Copulas as a New Geostatistical Tool, 2010, ISBN 978-3-933761-91-0

188 AghaKouchak, Amir: Simulation of Remotely Sensed Rainfall Fields Using Copulas, 2010, ISBN 978-3-933761-92-7

189 Thapa, Pawan Kumar: Physically-based spatially distributed rainfall runoff modelling for soil erosion estimation, 2010, ISBN 978-3-933761-93-4

190 Wurms, Sven: Numerische Modellierung der Sedimentationsprozesse in Retentionsanlagen zur Steuerung von Stoffströmen bei extremen Hochwasserabflussereignissen, 2011, ISBN 978-3-933761-94-1

191 Merkel, Uwe: Unsicherheitsanalyse hydraulischer Einwirkungen auf Hochwasserschutzdeiche und Steigerung der Leistungsfähigkeit durch adaptive Strömungsmodellierung, 2011, ISBN 978-3-933761-95-8

192 Fritz, Jochen: A Decoupled Model for Compositional Non-Isothermal Multiphase Flow in Porous Media and Multiphysics Approaches for Two-Phase Flow, 2010, ISBN 978-3-933761-96-5

193 Weber, Karolin (Hrsg.): 12. Treffen junger WissenschaftlerInnen an Wasserbauinstituten, 2010, ISBN 978-3-933761-97-2

194 Bliefernicht, Jan-Geert: Probability Forecasts of Daily Areal Precipitation for Small River Basins, 2011, ISBN 978-3-933761-98-9

195 Hrsg.: Koschitzky, Hans-Peter; Braun, Jürgen: VEGAS-Kolloquium 2010 In-situ-Sanierung - Stand und Entwicklung Nano und ISCO -, Tagungsband zur Veranstaltung am 07. Oktober 2010 an der Universität Stuttgart, Campus Stuttgart-Vaihingen, 2010, ISBN 978-3-933761-99-6

196 Gafurov, Abror: Water Balance Modeling Using Remote Sensing Information - Focus on Central Asia, 2010, ISBN 978-3-942036-00-9

197 Mackenberg, Sylvia: Die Quellstärke in der Sickerwasserprognose: Möglichkeiten und Grenzen von Labor- und Freilanduntersuchungen, 2010, ISBN 978-3-942036-01-6

198 Singh, Shailesh Kumar: Robust Parameter Estimation in Gauged and Ungauged Basins, 2010, ISBN 978-3-942036-02-3

199 Doğan, Mehmet Onur: Coupling of porous media flow with pipe flow, 2011, ISBN 978-3-942036-03-0

200 Liu, Min: Study of Topographic Effects on Hydrological Patterns and the Implication on Hydrological Modeling and Data Interpolation, 2011, ISBN 978-3-942036-04-7

201 Geleta, Habtamu Itefa: Watershed Sediment Yield Modeling for Data Scarce Areas, 2011, ISBN 978-3-942036-05-4

202 Franke, Jörg: Einfluss der Überwachung auf die Versagenswahrscheinlichkeit von Staustufen, 2011, ISBN 978-3-942036-06-1

203 Bakimchandra, Oinam: Integrated Fuzzy-GIS approach for assessing regional soil erosion risks, 2011, ISBN 978-3-942036-07-8

204 Alam, Muhammad Mahboob: Statistical Downscaling of Extremes of Precipitation in Mesoscale Catchments from Different RCMs and Their Effects on Local Hydrology, 2011, ISBN 978-3-942036-08-5

205 Hrsg.: Koschitzky, Hans-Peter; Braun, Jürgen: VEGAS-Kolloquium 2011 Flache Geothermie - Perspektiven und Risiken, Tagungsband zur Veranstaltung am 06. Oktober 2011 an der Universität Stuttgart, Campus Stuttgart-Vaihingen, 2011, ISBN 978-3-933761-09-2

206 Haslauer, Claus: Analysis of Real-World Spatial Dependence of Subsurface Hydraulic Properties Using Copulas with a Focus on Solute Transport Behaviour, 2011, ISBN 978-3-942036-10-8

207 Dung, Nguyen Viet: Multi-objective automatic calibration of hydrodynamic models – development of the concept and an application in the Mekong Delta, 2011, ISBN 978-3-942036-11-5

208 Hung, Nguyen Nghia: Sediment dynamics in the floodplain of the Mekong Delta, Vietnam,

2011, ISBN 978-3-942036-12-2

209 Kuhlmann, Anna: Influence of soil structure and root water uptake on flow in the unsaturated zone, 2012, ISBN 978-3-942036-13-9

210 Tuhtan, Jeffrey Andrew: Including the Second Law Inequality in Aquatic Ecodynamics: A Modeling Approach for Alpine Rivers Impacted by Hydropeaking, 2012, ISBN 978-3-942036-14-6

211 Tolossa, Habtamu: Sediment Transport Computation Using a Data-Driven Adaptive Neuro-Fuzzy Modelling Approach, 2012, ISBN 978-3-942036-15-3

212 Tatomir, Alexandru-Bodgan: From Discrete to Continuum Concepts of Flow in Fractured Porous Media, 2012, ISBN 978-3-942036-16-0

213 Erbertseder, Karin: A Multi-Scale Model for Describing Cancer-Therapeutic Transport in the Human Lung, 2012, ISBN 978-3-942036-17-7

214 Noack, Markus: Modelling Approach for Interstitial Sediment Dynamics and Reproduction of Gravel Spawning Fish, 2012, ISBN 978-3-942036-18-4

215 De Boer, Cjestmir Volkert: Transport of Nano Sized Zero Valent Iron Colloids during Injection into the Subsurface, 2012, ISBN 978-3-942036-19-1

216 Pfaff, Thomas: Processing and Analysis of Weather Radar Data for Use in Hydrology, 2013, ISBN 978-3-942036-20-7

217 Lebrenz, Hans-Henning: Addressing the Input Uncertainty for Hydrological Modeling by a New Geostatistical Method, 2013, ISBN 978-3-942036-21-4

218 Darcis, Melanie Yvonne: Coupling Models of Different Complexity for the Simulation of CO2 Storage in Deep Saline Aquifers, 2013, ISBN 978-3-942036-22-1

219 Beck, Ferdinand: Generation of Spatially Correlated Synthetic Rainfall Time Series in High Temporal Resolution - A Data Driven Approach, 2013, ISBN 978-3-942036-23-8

220 Guthke, Philipp: Non-multi-Gaussian spatial structures: Process-driven natural genesis, manifestation, modeling approaches, and influences on dependent processes, 2013, ISBN 978-3-942036-24-5

221 Walter, Lena: Uncertainty studies and risk assessment for CO2 storage in geological formations, 2013, ISBN 978-3-942036-25-2

222 Wolff, Markus: Multi-scale modeling of two-phase flow in porous media including capillary pressure effects, 2013, ISBN 978-3-942036-26-9

223 Mosthaf, Klaus Roland: Modeling and analysis of coupled porous-medium and free flow with application to evaporation processes, 2014, ISBN 978-3-942036-27-6

224 Leube, Philipp Christoph: Methods for Physically-Based Model Reduction in Time: Analysis, Comparison of Methods and Application, 2013, ISBN 978-3-942036-28-3

225 Rodríguez Fernández, Jhan Ignacio: High Order Interactions among environmental variables: Diagnostics and initial steps towards modeling, 2013, ISBN 978-3-942036-29-0

226 Eder, Maria Magdalena: Climate Sensitivity of a Large Lake, 2013, ISBN 978-3-942036-30-6

227 Greiner, Philipp: Alkoholinjektion zur In-situ-Sanierung von CKW Schadensherden in Grundwasserleitern: Charakterisierung der relevanten Prozesse auf unterschiedlichen Skalen, 2014, ISBN 978-3-942036-31-3

228 Lauser, Andreas: Theory and Numerical Applications of Compositional Multi-Phase Flow in Porous Media, 2014, ISBN 978-3-942036-32-0

229 Enzenhöfer, Rainer: Risk Quantification and Management in Water Production and Supply Systems, 2014, ISBN 978-3-942036-33-7

230 Faigle, Benjamin: Adaptive modelling of compositional multi-phase flow with capillary pressure, 2014, ISBN 978-3-942036-34-4

231 Oladyshkin, Sergey: Efficient modeling of environmental systems in the face of complexity and uncertainty, 2014, ISBN 978-3-942036-35-1

232 Sugimoto, Takayuki: Copula based Stochastic Analysis of Discharge Time Series, 2014, ISBN

978-3-942036-36-8

233 Koch, Jonas: Simulation, Identification and Characterization of Contaminant Source Architectures in the Subsurface, 2014, ISBN 978-3-942036-37-5

234 Zhang, Jin: Investigations on Urban River Regulation and Ecological Rehabilitation Measures, Case of Shenzhen in China, 2014, ISBN 978-3-942036-38-2

235 Siebel, Rüdiger: Experimentelle Untersuchungen zur hydrodynamischen Belastung und Standsicherheit von Deckwerken an überströmbaren Erddämmen, 2014, ISBN 978-3-942036-39-9

236 Baber, Katherina: Coupling free flow and flow in porous media in biological and technical applications: From a simple to a complex interface description, 2014, ISBN 978-3-942036-40-5

237 Nuske, Klaus Philipp: Beyond Local Equilibrium — Relaxing local equilibrium assumptions in multiphase flow in porous media, 2014, ISBN 978-3-942036-41-2

238 Geiges, Andreas: Efficient concepts for optimal experimental design in nonlinear environmental systems, 2014, ISBN 978-3-942036-42-9

239 Schwenck, Nicolas: An XFEM-Based Model for Fluid Flow in Fractured Porous Media, 2014, ISBN 978-3-942036-43-6

240 Chamorro Chávez, Alejandro: Stochastic and hydrological modelling for climate change prediction in the Lima region, Peru, 2015, ISBN 978-3-942036-44-3

241 Yulizar: Investigation of Changes in Hydro-Meteorological Time Series Using a Depth-Based Approach, 2015, ISBN 978-3-942036-45-0

242 Kretschmer, Nicole: Impacts of the existing water allocation scheme on the Limarí watershed – Chile, an integrative approach, 2015, ISBN 978-3-942036-46-7

243 Kramer, Matthias: Luftbedarf von Freistrahlturbinen im Gegendruckbetrieb, 2015, ISBN 978-3-942036-47-4

244 Hommel, Johannes: Modeling biogeochemical and mass transport processes in the subsurface: Investigation of microbially induced calcite precipitation, 2016, ISBN 978-3-942036-48-1

245 Germer, Kai: Wasserinfiltration in die ungesättigte Zone eines makroporösen Hanges und deren Einfluss auf die Hangstabilität, 2016, ISBN 978-3-942036-49-8

246 Hörning, Sebastian: Process-oriented modeling of spatial random fields using copulas, 2016, ISBN 978-3-942036-50-4

247 Jambhekar, Vishal: Numerical modeling and analysis of evaporative salinization in a coupled free-flow porous-media system, 2016, ISBN 978-3-942036-51-1

248 Huang, Yingchun: Study on the spatial and temporal transferability of conceptual hydrological models, 2016, ISBN 978-3-942036-52-8

249 Kleinknecht, Simon Matthias: Migration and retention of a heavy NAPL vapor and remediation of the unsaturated zone, 2016, ISBN 978-3-942036-53-5

250 Kwakye, Stephen Oppong: Study on the effects of climate change on the hydrology of the West African sub-region, 2016, ISBN 978-3-942036-54-2

251 Kissinger, Alexander: Basin-Scale Site Screening and Investigation of Possible Impacts of CO2 Storage on Subsurface Hydrosystems, 2016, ISBN 978-3-942036-55-9

252 Müller, Thomas: Generation of a Realistic Temporal Structure of Synthetic Precipitation Time Series for Sewer Applications, 2017, ISBN 978-3-942036-56-6

253 Grüninger, Christoph: Numerical Coupling of Navier-Stokes and Darcy Flow for Soil-Water Evaporation, 2017, ISBN 978-3-942036-57-3

254 Suroso: Asymmetric Dependence Based Spatial Copula Models: Empirical Investigations and Consequences on Precipitation Fields, 2017, ISBN 978-3-942036-58-0

255 Müller, Thomas; Mosthaf, Tobias; Gunzenhauser, Sarah; Seidel, Jochen; Bárdossy, András: Grundlagenbericht Niederschlags-Simulator (NiedSim3), 2017, ISBN 978-3-942036-59-7

256 Mosthaf, Tobias: New Concepts for Regionalizing Temporal Distributions of Precipitation and

for its Application in Spatial Rainfall Simulation, 2017, ISBN 978-3-942036-60-3

257 Fenrich, Eva Katrin: Entwicklung eines ökologisch-ökonomischen Vernetzungsmodells für Wasserkraftanlagen und Mehrzweckspeicher, 2018, ISBN 978-3-942036-61-0

258 Schmidt, Holger: Microbial stabilization of lotic fine sediments, 2018, ISBN 978-3-942036-62-7

Die Mitteilungshefte ab der Nr. 134 (Jg. 2005) stehen als pdf-Datei über die Homepage des Instituts: www.iws.uni-stuttgart.de zur Verfügung