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„The Effect of Soil Additions on DOM Dynamics and Biofilm Growth in stream Mesocosms"

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Contents

Abstract

Biofilms are hotspots of microbial diversity in streams and orchestrate biogeochemical fluxes. They contribute to in-stream metabolism and are strongly affected by different organic carbon sources. Streams, especially headwaters are net heterotrophic systems and thus relying on allochthonous input as an energy, carbon and nutrient source. Recent findings have shown that terrestrial organic carbon sources are responsible for large amounts of CO_2 outgassing from aquatic ecosystems. With predicted increases of terrestrial material subsidization to streams due to intense precipitation events, freshwater biofilms and in-stream DOM (dissolved organic matter) responses to those changes are of great importance. In my study I investigate the effect of soil additions on benthic biofilms and DOM dynamics using six streamside mesocosms to simulate natural streams. I measured water DOC and nitrate concentrations as well as biofilm AFDM (ash free dry mass), ChlA (chlorophyll a) and C:N ratio (carbon to nitrogen ratio) over a period of 49 days. In addition, I sampled the biofilm communities twice to determine community composition and diversity using 16S- rRNA genes for microbial fingerprinting. My results suggest that the in-stream microbial community composition as well as diversity measures are altered by the soil additions, indicating allochthonous material as a potential driver for biofilm assemblage. Furthermore, AFDM and nitrate were affected by the treatment, confirming the relevance of terrestrial inputs to freshwater ecosystems.

1 Introduction

Soils store large amounts of organic matter (Jobbágy & Jackson 2000; Schmidt *et al.* 2011) that can be transported to freshwater ecosystems subsidizing stream carbon budgets. Each year, terrestrial exports from soils to rivers are estimated to deliver 1.7 Pg of carbon (IPCC 2013). Those inputs represent an important organic matter source for aquatic ecosystems, as rivers are active, net heterotrophic (Battin *et al.* 2008) components of the global carbon cycle and do not only transport material, but also transform it on its way downstream towards the ocean (Cole *et al.* 2007). The changing climate affects stream ecosystems and alters in-stream carbon dynamics. Predictions assume that heavy rain events will likely increase due to a warming climate (Dore 2005; Lubchenco & Karl 2012). Therefore, an increasing amount of allochthonous dissolved organic matter (DOM) and particulate organic matter (POM) is expected to be washed into streams (Battin *et al.* 2009; Regnier *et al.* 2013; Jones & Lennon 2014). Initially, terrestrial organic carbon (OC) was considered less labile than microbially derived OC (Battin *et al.* 2008). However, numerous recent studies suggest that the impact of allochthonous OC on in-stream DOM dynamics is versatile and may also affect microbial degradation of terrestrial DOM, resulting in increased CO_2 outgassing from streams (Battin *et al.* 2008; Fasching *et al.* 2014). In addition, increasing terrestrial influence implies higher potential for DOC (dissolved organic carbon) degradation (Lapierre *et al.* 2013). Since freshwater and terrestrial environments are tightly coupled through hydrological networks, as the smallest streams are the most abundant (Bishop *et al.* 2008) and receive their water from the surrounding catchment area, microorganisms from catchment soils end up in streams. As a result of biofilm formation, these microbial communities have enhanced metabolic capacities (Battin *et al.* 2008) and contribute to the DOC turnover in freshwater ecosystems. This microbial inoculation from soils is an important driver of in-stream microbial diversity (Crump, Amaral-Zettler & Kling 2012) and therefore affects ecosystem functions such as carbon cycling.

1.1 Biofilms perform ecosystem functions

Biofilms are attached, matrix enclosed, three-dimensional structures that typically dominate microbial life in ecosystems with high sediment-surface-area to water-volume ratios and elevated downstream transport, such as in headwater streams (Battin *et al.* 2008). In comparison to free-living microorganisms, benthic biofilms are capable of increasing biomass as well as activity by forming communities that are well adapted to certain environmental conditions. The three-dimensional structure built by microbes that release extracellular polymeric substances (EPS) is composed of different microhabitats,

each with characteristic abiotic and biotic properties such as oxygen availability and species allocation. Moreover, stream hydrodynamics are influenced by biofilms leading to increased transient storage times (Battin *et al.* 2003). Extracellular enzymes function as an external digestive system and are kept close to the cells in the EPS matrix to maximize metabolic efficiency. Furthermore, channel networks are present to facilitate sorption of organic compounds as well as inorganic ions (Flemming & Wingender 2010). This is especially of importance in high through-flow ecosystems such as rivers and streams where storage of DOC is essential for microbial metabolism.

The importance of coupling in-stream DOC dynamics and biofilms is crucial to get a mechanistic insight on how microbial ecology affects biogeochemical cycles (Hall *et al.* 2011). Numerous studies indicate that biodiversity affects ecosystem functioning (Hooper *et al.* 2005; Cardinale *et al.* 2006; Allison & Martiny 2008) and that diversity of microbes in freshwater ecosystems is highest in headwater streams (Crump *et al.* 2012; Besemer *et al.* 2013). As allochthonous inputs to the upper reaches of streams is increasing with climate change, the metacommunity structure as well as their metabolic activity may pass through significant changes. Metabolism of OC, particularly terrestrial material in headwaters is responsible for a substantial amount of microbial respiration and therefore carbon dioxide outgassing to the atmosphere (Battin *et al.* 2008; Fasching *et al.* 2014). In headwater streams, microbial biofilms are the dominant lifeforms (Battin *et al.* 2008), thus it is of great importance to assess the effect of allochthonous OC inputs to streams and the microbial response to those inputs. Biofilms play an important role in streams since they are major sources of CO_2; however, the amount of carbon dioxide production as well as their assemblage is strongly influenced by allochthonous inputs. Microbial diversity in headwater streams is influenced by soil inoculation, followed by species sorting processes downstream (Crump *et al.* 2007, 2012). Also, altitudinal patterns contribute to diversity gradients driven mainly through elevated specialization in lower altitudes (Wilhelm *et al.* 2015). Hence, biofilm diversity depends on versatile mechanisms including physical heterogeneity, local environment, carbon source, biotic interactions and metacommunity dynamics (Singer *et al.* 2010; Besemer *et al.* 2013).

1.2 In-stream biogeochemistry is influenced by allochthonous material

The dendritic structure of fluvial networks makes them efficient integrators of terrestrial ecosystems. They link multiple components of the landscape, such as soils and groundwater, with the oceans and the atmosphere (Battin *et al.* 2008). Energy and carbon inputs in streams can originate from in-stream primary production, litter fall, lateral contributions from riparian vegetation and soils, DOM in groundwater and upstream inputs (Tank *et al.* 2010). The fifth assessment of the international panel on climate change (IPCC) highlighted that the extent to which freshwater ecosystems contribute to the global

carbon cycle is higher than previously estimated (IPCC 2008, 2013). This change in perception is in part due to the present view of terrestrial OC as an important source of carbon dioxide outgassing from inland waters to the atmosphere (Cole *et al.* 2007; Battin *et al.* 2008; Aufdenkampe *et al.* 2011; Fasching *et al.* 2014). Input of terrestrial OC (DOM and POM (particulate organic matter)) is believed to increase with ongoing climate change (Regnier *et al.* 2013; Jones & Lennon 2014). Energy from terrestrial net ecosystem production (NEP) can enter freshwater ecosystems in the form of DOC and POC (particulate organic carbon). The largest carbon input is commonly identified as DOC (0.25 Pg C y^{-1}), with POC inputs estimated at 0.18 Pg C y^{-1}. Both phases have certain properties and interact or shift from one to the other via processes such as lysis or aggregation (Cauwet 2002; Raymond & Spencer 2015). Terrigenous inputs deliver, amongst other dissolved organic matter molecules, chromophoric dissolved organic matter (CDOM) to aquatic systems causing browning of the water. Browning affects freshwater ecosystems by influencing light regime and therefore primary production (Hansson *et al.* 2012) as well as CO_2 outgassing due to photochemical degradation of CDOM (Fasching & Battin 2012; Lapierre *et al.* 2013). Given the above mentioned implications that terrestrial deliveries to freshwater ecosystems can have, it is crucial to better understand the mechanisms that drive carbon dynamics after soil additions. This holds true especially for headwaters, since low order streams are the most abundant and highly connected to their surrounding terrestrial environment. Those new findings highlight the current lack of knowledge of the stream carbon cycle and the role of biofilm communities in it.

1.3 Research questions and experiment design

The aim of my study is to determine the effect of soil additions on in-stream biogeochemical cycles and benthic biofilm communities.

1. How are in-stream DOM dynamics altered by allochthonous (soil addition) inputs?
2. Is in-stream microbial community assemblage affected by soil additions?
3. To what extend is inoculation with microbes from soils affecting carbon cycling in rivers and streams?

To address these questions work at the interface between biogeochemical cycles and microbiology is needed to better comprehend the mechanisms that drive microbial responses and community composition to changing DOM pools. Since those questions are not easily answered, I used streamside mesocosms that have the advantage of simulating a natural environment but under controlled conditions in order to study effects of soil additions.

2 Methods

2.1 Stream mesocosms experimental setup

I used six streamside mesocosms situated right beside the stream Oberer Seebach in Lunz am See (Lower Austria, Austria). The flumes are 40 m long, 40 cm wide and 40 cm high wooden constructions that are sealed with plastic foil. The water for the flumes was pumped directly from the stream Oberer Seebach into a header tank, situated at the beginning of the mesocosms on a wooden plateau. From there it was distributed equally over all six flumes. The bottom of the stream mesocosms was covered with washed gravel (Ø= 20 – 50 cm) to simulate a natural streambed. To avoid inputs of organic material in the form of falling leaves, nets were placed over the flumes. This experimental setting (Fig. 1) allows to simulate allochthonous inputs to stream ecosystems under controlled conditions.

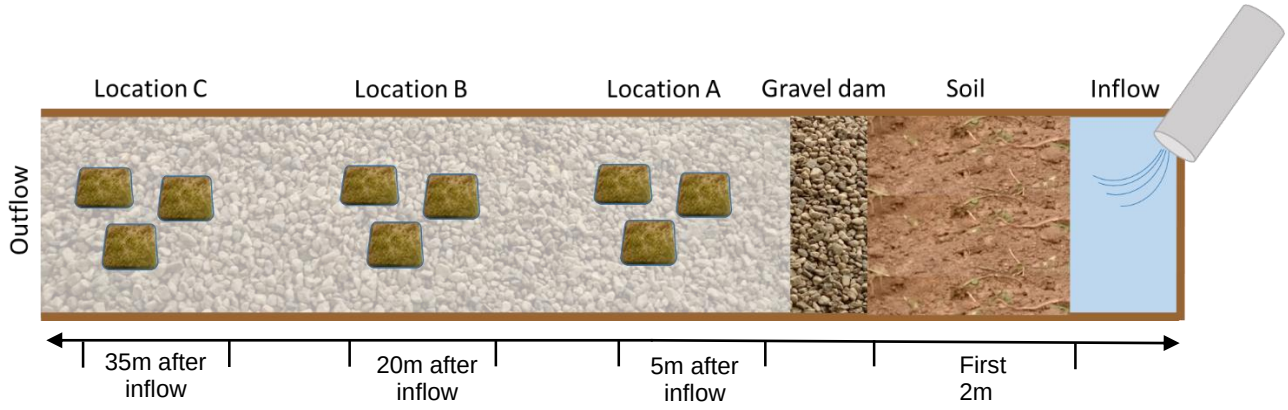

Fig 1. Flume scheme with soil additions (first two meters of the flumes); Gravel dam to avoid flushing of the soil; Location A 5m after the inflow; Location B 20m after the inflow; Location C 35m after the inflow; Tiles for biofilm growth in green;

2.2 Discharge

The amount of water for each single mesocosm could be adjusted by opening or closing a valve. The exact amount of water that was flowing through the pipes was measured with a bucket that was marked at 10 l. The time it took to fill the bucket up to the 10 l mark was measured with a stop watch. This process was repeated until the discharge of every single flume was adjusted to 0.38 – 0.42 l s^{-1} in order to obtain uniform flow. By doing so, homogenous flow conditions were created.

2.3 Soil addition

The treatment consisted in a soil addition of about 200 dm^3 of soil per flume. The soil was added in the first two meters of the flumes number two, four and six. To hinder the soil

from being washed out, a dam like structure with gravel was built (Fig. 1). Since the soil addition took place after the mesocosms were set up and water was already flowing, the gravel downstream of the treated flumes had to be moved thoroughly in order to remove the initial sediment load, especially of POC and TSS (total solved solids). To be able to compare the treated with the control flumes, also the control mesocosms had to be moved thoroughly to create the same disturbance in the system as in the treated flumes.

2.4 Data collection and Sampling

At each water sampling triplicates were taken at the header tank as well as duplicates at each mesocosms outflow. Samples were taken every day for the first week. For the rest of the experiment samples were taken every second day. The last sample was taken with a week distance from the previous one. The first four samples were taken before the soil was added. Each sample was labeled with JS0xx with x being the number of the sampling, starting from one and going up to 25. Temperature (in °C) and light intensity (in Lux) were monitored every 5 minutes using HOBO (Onset Computer Corporation; 470 MacArthur Blvd. Bourne, MA) loggers; one was placed at the outflow of each flume and two in the header tank. To look at the biofilm growth, tiles (5cm x 5 cm) were laid out at three different locations. Location A was situated 5 m after the inflow, location B 20 m after the inflow and location C 35 m after the inflow.

2.4.1 Water samples

The water samples were taken with 60 ml syringes. For DOC quality (optic properties) and for DOC quantity (concentration) two GF/F filters with 2.5 cm diameter were used for each sample. Regarding nutrient and amino acid sampling, filters with a smaller pore size (0.45 µm) and non-peptide binding properties were used. For each DOC sample (optics and concentrations) 25 ml of water were taken. To measure nutrients and the amino acids 13 ml were sampled. The samples were stored in a temperature-isolated box until they were frozen or refrigerated respectively in the laboratory. GF/F filters were muffled at 450 °C for 6 hours to get rid of any kind of organic contamination. Filter holder were washed and put into mQ water to prevent contamination.

2.4.2 Biofilm sampling

Biofilm was collected by sampling tiles that were laid out in the beginning of the experiment. Ash free dry mass (AFDM), chlorophyll A (ChlA) and carbon to nitrogen ratios (C:N ratios) were determined using these biofilm samples. Tiles were sampled during every second sampling. For each flume, nine tiles (three for every location) were taken. Tiles were taken out of the water by removing them very carefully and slowly so that no

part of the biofilm got lost during the process. Once taken out of the water, the tiles were packed into plastic bags and put in a temperature-isolated box until they were frozen at -20°C in the laboratory.

2.5 Sample processing

2.5.1 Water samples

The filtered water samples were analyzed in the lab of the Wassercluster Lunz (WCL) as well as the lab of the Department of Limnology and Bio-Oceanography at the University of Vienna. Nutrient concentrations (NH_4^+; NO_2^-; NO_3^-; PO_4^{3-}) were analyzed in the WCL using a Continuous-Flow Analyzer (Alliance Instruments, Frepillon, France). DOC concentration and optic properties were analyzed in Vienna. DOC concentrations were determined using a Sievers 5210C (GE). DOC quality was assessed spectrofluorometrically using the Aqualog (Horiba, Kyoto, Japan). Due to a malfunction of the Aqualog device this data is not further discussed.

2.5.2 Biofilm samples

Biofilm samples were thawed and scrubbed off the tiles with a sterile razorblade. The material was diluted in 25 ml mQ and transferred into 50 ml Falcon (Greiner) tubes. The mixture was homogenized using a Sonifier (Branson; W-250 D) set at 30 seconds and 50% amplitude. Before and after the sonification, the Falcon tubes were put on ice to prevent the sample from heating up. The homogenized solution was filtered through GF/F filters (25 mm diameter) for ChlA and AFDM using a vacuum pump. The volume that was used depended on the amount of material that was on the tile in the first place. If there was already a strong biofilm developed, 2.5 ml were used for the ChlA as well as for AFDM and 7.5 ml for the C:N ratio. For tiles with little biofilm 5ml were used for the ChlA and the AFDM and 15 ml for the C:N ratio. After the filtration, filters were folded in the middle, packed into labelled aluminum foil and frozen at - 20 °C.

2.5.2.1 Carbon to nitrogen ratio

For the C:N ratios the leftover of the homogenized solution was centrifuged for four minutes at 3000 rotations per minute (rpm) using a Rotanta 460 R by Hettich. Thereby, a separation of the fluid and the solid parts was achieved. Thereafter the water was carefully poured out of the Falcon tube and the remaining pallet of the solid substances was transferred into tin caps. The filled tin caps were put in the drying oven for 36 hours at 60

°C. Once the samples were completely dry, they were wrapped thoroughly into the tin foil and stored. The samples were then analyzed using an Element Analyzer (vario MICRO cube, Elementar GmbH, Hanau, Germany). Given limited resources for C:N analysis, I decided to analyze a selection of three samplings (Sample number: 013,016 and 021).

2.5.2.2 Chlorophyll A

ChlA was determined using a spectrofluorometer (F7000, Hitachi, Tokyo, Japan). Therefore, the filter obtained by the filtration of the homogenized biofilm solution was put into 15 ml Falcon tubes and 5 ml of 90 % acetone (Carl Roth GmbH + Co. Kg, Germany, Karlsruhe) were added. The extraction time ranged between eight and a half and nine hours. During the extraction, the samples were stored in an airtight box at 4 °C. The measurement itself was performed at an excitation wavelength of 440 nm and emission wavelength of 660nm. The first measurement was always a blank (just acetone), that could then be subtracted from the actual value of the sample to minimize the background noise. According to the calibration of the spectrofluorometer, the following equations were used to compute the actual ChlA concentrations. For values ranging from 0 to 400 µg per tile:

f(x)= ((1.0501x)-(1.1728*vol.extract))/vol.filtrate

For values ranging from 400 to 1000 µg per tile:

f(x)= ((1.157x)-(11.078*vol.extract))/vol.filtrate

With x being the measured ChlA value. Thereafter, each value was corrected in order to obtain the ChlA concentration per cm^2 of tile surface area.

2.5.2.3 Ash free dry mass

AFDM was obtained by putting the filters into the drying oven for 15 hours at 60 °C. Afterwards the filters were weighted and the dry weight was noted. Thereafter the filters were put into the muffle-oven at 450 °C for 5 hours to remove all the organic compounds. Subsequently the filters were again weighted and the weight was noted. The AFDM was computed by subtracting the weight of the muffled filters from the weight of the dried filters. After that, the data was corrected for the different dilutions that were used. Thereafter, corrections were computed in order to get the AFDM content per cm^2 of tile surface area.

2.5.2.4 Terminal restriction fragment length polymorphism

Terminal restriction fragment length polymorphism (t-RFLP) is a microbial fingerprinting method that allows to explore differences in the microbial community composition between different habitats. This analysis method can provide reproducible semi-

quantitative results (Bent & Forney 2008; Singer *et al.* 2010). For my study, two time points were chosen for t-RFLP sampling. The first biofilm sampling was 18 days after the experiment begun, so that the biofilm was already well established and the second one at the last day of the experiment in order to see potential community shifts over time. In addition, soil material used for the soil additions was also analyzed via t-RFLP.

Biofilm was scrubbed of the tiles and weighted in order to obtain around 0.5 g of fresh weight. DNA extraction from soils was performed using 0.25 g of wet soil. To extract the DNA the MoBio Power Soil Extraction Kit (MoBio Laboratories, Carlsbad, CA, USA) was used. After the DNA extraction, the samples were frozen or immediately used for the PCR. I used the bacteria specific primers 27F (labelled with HEX) and 519R (table 1) to amplify a part (approximately 500 base-pairs (bp) long) of the 16S rRNA gene. The master mix for the PCR was prepared in the laminar flow according to table 1.

Table 1: Preparation of the master mix for the PCR

	µl	**number**
sample	**3**	**44**
Reaction volume	**50**	
Aqua purificata	27,25	1199
10 x Buffer	5	220
dNTPs	4	176
MgCl	4	176
BSA	2,5	110
Primer 27F HEX	2	88
Primer 519R	2	88
Taq Polymerase	0,25	11
SUM	**47**	**2068**

The mix of sample and master mix was put in the Thermocycler (Eppendorf vapo.protect) and run with the setting according to table 2.

Table 2: PCR settings

PCR conditions		Temperature	Time
Initial denaturation		94°C	3 min
25 cycles	Denaturation	94°C	45 sec
	Annealing	55°C	45 sec
	Elongation	72°C	60 sec
Final elongation		72°C	10 min

Thereafter a gel-electrophoresis was performed to ensure the quality of the PCR product as well as the quantity of the extracted DNA. The gel (1% agarose) was cast with 0.5 g agarose (Fermentas TopVisio^{TM} Agarose) and 50 ml of 1x TAE buffer. The mixture was heated in the microwave until the agarose was fully dissolved. After the mixture has cooled down, 2.5 µl of EtBr (Ethidium bromide) were added and poured into the horizontally set tray. After adding the comb (eight wells), 20 to 30 minutes had to pass so that the gel could solidify. Subsequently the comb was removed, the tray was put into the electrophoresis chamber (BIO-RAD Power Pac Basic) which was filled with 1x TAE buffer so that it covered the gel completely. Five µl of sample were placed on a parafilm foil and mixed with one µl of loading dye (Thermo Scientific; 6X DNA Loading Dye) respectively. The first well was filled with five µl of MassRuler (Thermo Scientific; MassRuler DNA Ladder Mix) and the other wells were filled with the sample - loading dye mixture. The gel-electrophoresis ran for 45 minutes at 90V. Thereafter, the gel was taken out of the chamber and put in the BIO-RAD (Bio-RAD GelDocTM XR+) to detect the bands. The software Image Lab was used to quantify the DNA according to the specifications of the DNA ladder. The used 1x TAE buffer was poured back, since it could be reused. Pictures of the gels for the biofilm and the soil samples can be found in the appendix.

If DNA quantity and quality were satisfying, the frozen PCR product was purified in order to remove nucleotides, primes, salts and other impurities from the samples using QIAquick PCR Purification Kit (Qiagen, Venlo, Netherlands). Purified samples were frozen at -20°C.

The cleaned PCR product was used for the restriction digestion (RD) with Hhal (5' GCG ↓C 3'; 3' C ↑GCG 5' ; Thermo Scientific; Hhal) and Hinfl (5' G↓A (A/T) T C 3'; 3' C T (A/T) A↑G 5';Thermo Scientific; Hinfl) in order to get the terminal DNA Fragments. Therefore, 10 µl of sample were mixed with two µl of 10x buffer as well as with one µl of enzyme. Each sample was processed with Hhal and with Hinfl respectively. The mixture was put into the Thermocycler for 16 hours at 37°C. After the RD the enzymes were deactivated for 20 minutes at 60°C. The digested mixture was frozen at -20°C.

Thereafter, a Sephadex purification was performed to desalinate the samples. 200 µl Sephadex G50 solution were loaded onto the 96 well filter plates. The filter plate was loaded onto a 96 well collection plate and then centrifuged (Eppendorf 5810) at 230 rcf (relative centrifugal force) for 75 seconds. The flow through was discarded and then the step was repeated one more time. Subsequently 20 µl of sample were loaded carefully onto the Sephadex gel to avoid the destruction or puncturing of the gel by penetrating it with the tip. The plates were centrifuged at 420 rcf for 60 seconds. This procedure was repeated twice, once for the enzyme Hhal and once for the enzyme Hinfl. Since some extra buffer from the Sephadex was coming through, the total sample volume was more than the 20 µl that were loaded on the gel. I ended up with about 28 to 33 µl final volume. The filter plates were wrapped in parafilm and frozen at - 20°C.

In order to prepare the samples for the analysis with the capillary sequencer a master mix was prepared. For 16 samples 165 µl HiDi Formamide (Applied Biosystems) as well as 5 µl MapMarker1000 (x-Rhodamine; BioVentures,Inc.) as the size standard were used. In each well 10 µl of master mix and 4 µl of purified sample were loaded. The samples were denatured for 3 minutes at 95°C, thereafter immediately incubated on ice for 5 minutes. Samples were transported in ice to the Capillary Sequencer lab, were they were analyzed. The samples were run on a capillary sequencer (3130 Gene Analyzer, Applied Biosystems, Waltham, Massachusetts), which separates the samples using capillary electrophoresis. Thereafter, a fluorescence detector determined the sizes of the different terminal fragments. The raw sequence data was checked using the software Peak Scanner. OTU matrices were de-noised using Excel macros on the manually checked data.

2.6 Data analysis

Changes of stream water DOC and NO_3^- (nitrate) concentrations were measured at the header tank and at the outflow. In order to increase the representability and minimize the background noise from the stream Oberer Seebach, delta values (difference between header tank and outflow) were used to analyze DOC and NO_3^- changes of stream water over time. NH_4^+, NO_2^- and PO_4^{3-} changes were excluded from the data analysis since nearly all values were below detection limit.

Time series for water (DOC, NO_3^-) and biofilm samples (AFDM, ChlA, C:N) were computed with two-way analysis of variance (ANOVA). To account for possible autocorrelation due to the repeated measurements, permutation based ANOVA designs were employed to check the more robust standard two-way ANOVA results. As mentioned above, the data for DOC and NO_3^- was used as delta values. Absolute numbers (values measured at the outflow) depict trends of the stream Oberer Seebach regarding DOC and NO_3^- dynamics. All statistical analyses were computed using R studio operating on R (ver. 3.2.2).

I used linear regression to predict ΔDOC and ΔNO_3^- concentrations based on empiric AFDM and ChlA values according to the following protocol: First, I established linear models for the relationship of ΔDOC and ΔNO_3^- against AFDM and ChlA using data obtained from the control mesocosms. Then I used the model formula to estimate ΔDOC and ΔNO_3^- based on AFDM and ChlA data from the treated flumes. Thereafter, I compared the model residuals of the empiric vs. modelled data to the model residuals of a f(x)=y model to assess potential divergence from an ideal model system using one-way ANOVA.

T-RFLP data was analyzed using different types of statistical analysis. The OTU (operational taxonomic unit) matrix was transposed. Non-metric multidimensional scaling (nMDS) was performed using the function “metaMDS” in R. Subsequently, nMDS plots were created to display the data. Based on the nMDS plots one and two factor permutation multivariate analysis of variance (perMANOVA) were computed to assess differences between the treatments and the two samplings using the function “adonis”.

To describe diversity patterns, Shannon diversity index (as a proxy for alpha diversity) as well as species richness and evenness (Pielou evenness) using the functions “diversity”, “specnumber” and “diversity/(log(specnumber))” respectively were computed in R. Possible differences between groups were tested using t-tests for paired samples or Wilcoxon-tests (if normal distribution or variance homogeneity could not be achieved) for paired differences. To test differences between soil samples, biofilms from the treated flumes and biofilms from the control flumes at once, I used a one-way ANOVA. The one-way ANOVA was followed by Turkey post-hoc tests (function “TukeyHSD”) to verify which group differs significantly from another.

3 Results

3.1 Water chemistry

3.1.1 Dissolved organic carbon

DOC concentrations over the timespan of the whole experiment at the outflow of the control flumes were in average 2.149 mg l^{-1} (Standard deviation (SD)= 0.071). In the treated flumes concentrations were similar with values of 2.147 mg l^{-1} (SD= 0.054). Outflow DOC concentrations decreased over time ($F = 22.56$, $d.f._1 = 21$, $d.f._2 = 88$, $p<0.001$) in all flumes. There was no evidence that the outflow DOC concentrations in the treated mesocosms were different than those in the control flumes. Figures on DOC concentration at the outflow are supplied in the appendix (Fig.12, appendix).

ΔDOC concentrations in the flumes revealed that DOC concentrations did not change with the treatment (two-way ANOVA; factor 1= treatment, factor 2= time), despite substantial carbon (C) additions (200 dm^3 soil per mesocosms; C content= 8%). Likewise, there was no detectable effect of change in treatment over time. However, ΔDOC concentrations of all flumes increased significantly over time ($F = 4.39$, $d.f._1 = 21$, $d.f._2 = 88$, $p<0.05$). Although ΔDOC values showed a steep increase in the beginning of the experiment, they stagnated in the third week and slightly decreased towards the end (Fig.2a). In addition, the SD was relatively high for some samplings, thus making it hard to draw any conclusions. Overall, trends suggest slight differences in average between treated and control flumes, although they were not significant (Fig.2b). Looking at the first two weeks, ΔDOC values increased over time and were slightly higher in the treated flumes than in the control flumes with median differences up to 0.05 mg l^{-1}. From the third week on, ΔDOC values decreased and differences between the treatments got smaller.

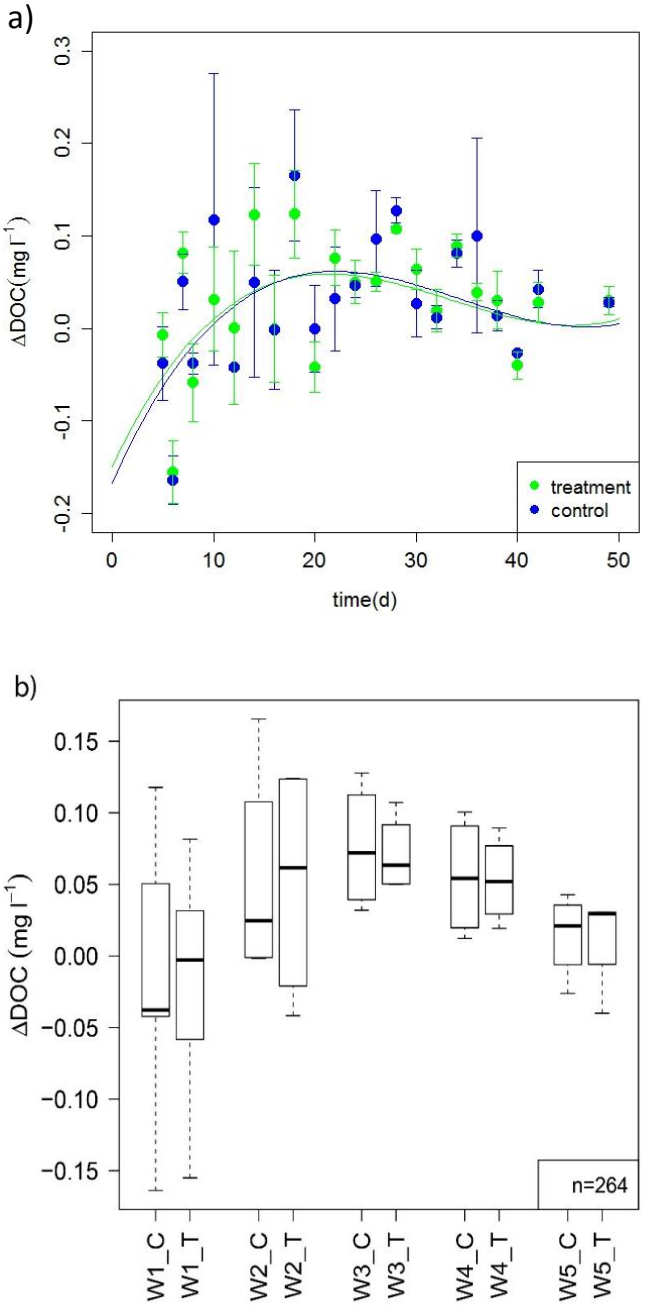

Figure 2. ΔDOC values along the timespan of the whole experiment (n=264). Values obtained by subtracting inflow concentrations from the header tank. Concentrations are increasing over time, especially in the first two weeks of the experiment. (a) Scatter plot; Dots represent the mean and bars the standard deviation (SD). ΔDOC values from the control flumes are displayed in blue, values from the treated flumes in green. (b) Boxplot with weekly pooled data for control (W_C) and treatment (W_T).

3.1.2 Nitrate concentrations

Mean NO_3^- outflow concentrations in the control flumes over the timespan of the whole experiment were in average 718.3 µg l^{-1} (SD= 4.28). In the treated flumes similar concentrations were measured with 725.7 mg l^{-1} (SD= 3.72). NO_3^- values at the outflow (Fig. 13, appendix) showed a slight decrease over time ($F = 2.855$, $d.f._1 = 21$, $d.f._2 = 88$, $p<0.05$) and no significant difference between the treated and the control mesocosms.

Concentrations of NO_3^- were strongly affected by the treatment as well as by time when looking at delta values (Fig. 3). Before the addition, soil had mean nitrate values of 14.6 µg g^{-1} (SD ± 1.58). Nitrate levels in the treated mesocosms were significantly decreased by the soil addition ($F = 9.473$, $d.f._1 = 21$, $d.f._2 = 88$, $p<0.01$). Interestingly, the ΔNO_3^- concentrations were higher in the control flumes than in the treated flumes (Fig. 3b). In addition, time had a positive effect on the ΔNO_3^- concentrations ($F = 79.55$, $d.f._1 = 21$, $d.f._2 = 88$, $p<0.001$), increasing ΔNO_3^- values from the beginning of the experiment up to sixtyfold towards the end of the experiment. Hence, the trends of nitrate dynamics in the flumes (delta values) and in the stream Oberer Seebach (outflow values) are opposing each other. SD was rather high also for ΔNO_3^- values. Nevertheless, distinct patterns could be detected. In contrast to ΔDOC concentrations, differences in ΔNO_3^- between treatment and control flumes were clearly visible (Fig. 3b). As nitrate dynamics look counterintuitive, I have to mention that the gravel for the mesocosms was washed before delivery, to avoid additional input of nutrients or other chemical components.

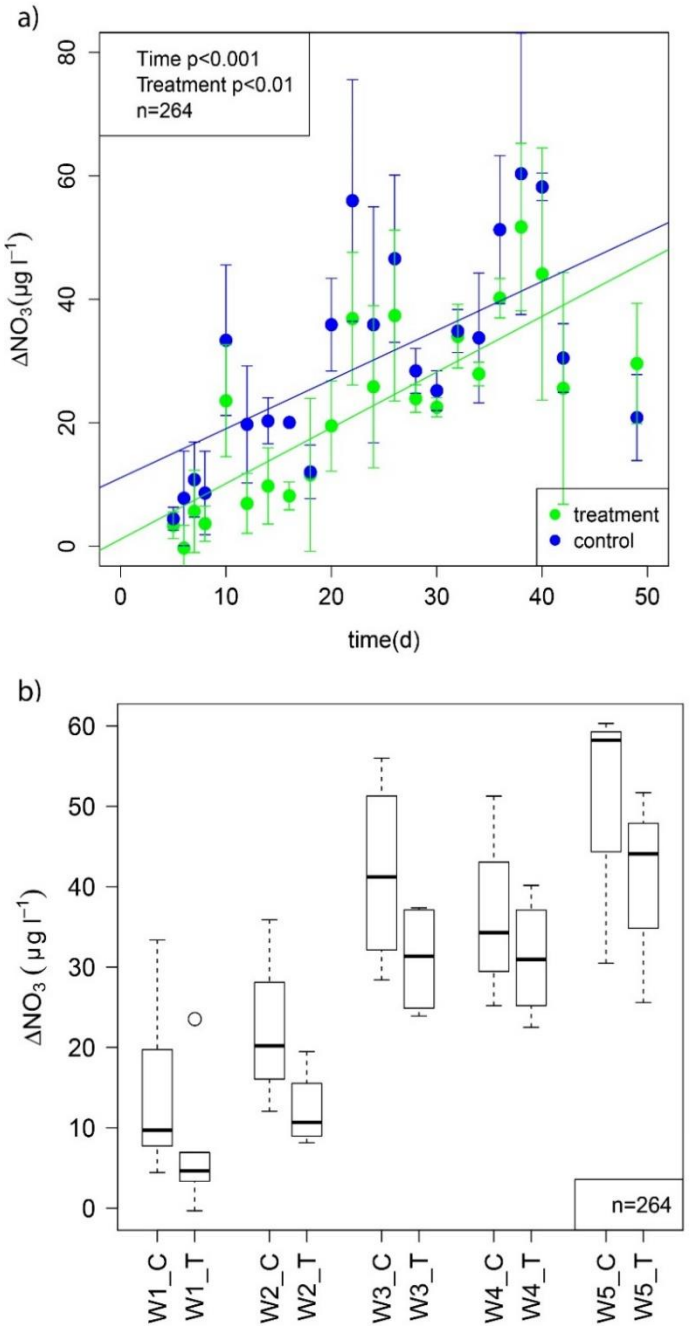

Figure 3. ΔNO_3^- values along the timespan of the whole experiment (n=264). Values obtained by subtracting inflow concentrations from the header tank. (a) Scatter plot; Dots represent the mean and bars the SD. (b) Boxplot with weekly pooled data for control (W_C) and treatment (W_T). Dots represent outliers

3.2 Biofilm properties

3.2.1 Biofilm ash free dry mass

AFDM was collected over ten time points, starting from the 10th day after the beginning of the experiment until the last sampling (day 49). Trends depicting AFDM over time indicate a divergence between treatment and control values (Fig. 4), with AFDM in the treated mesocosms gaining faster than in the control mesocosms. Thus, the soil addition had a significant effect on AFDM development ($F = 11.54$, $d.f._1 = 1$, $d.f._2 = 56$, $p<0.01$), resulting in a higher AFDM (integrated over all locations) content in the treated flumes than in the control flumes. Furthermore, AFDM increased significantly with time ($F = 878.83$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$). Similarly to a strong treatment effect on AFDM as shown above, AFDM also showed largest gains at the location situated closest to the inflow ($F = 17.77$, $d.f._1 = 1$, $d.f._2 = 56$, $p<0.001$). The same holds true for time ($F = 1185.38$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$). AFDM at the intermediate situated location B was highly affected by time ($F = 358.41$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$). Also, the soil addition led to a significant increase of AFDM in location B of the treated mesocosms ($F = 5.098$, $d.f._1 = 1$, $d.f._2 = 56$, $p<0.05$). Location C, situated 5m upstream of the outflow, was affected similarly to location B. Over time the AFDM content increased ($F = 514.02$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$). Regarding the treatment, the flumes with soil additions had significantly higher values as those in the control flumes ($F = 4.264$, $d.f._1 = 1$, $d.f._2 = 56$, $p<0.05$). For all locations, the treatment effect consisted in an increase of AFDM (Fig. 5). Location A had clearly the fastest gain in AFDM in comparison to the others flumes. Location B and C show analogous patterns. However they are more alike regarding AFDM gains between control and treatment as well as AFDM development over time.

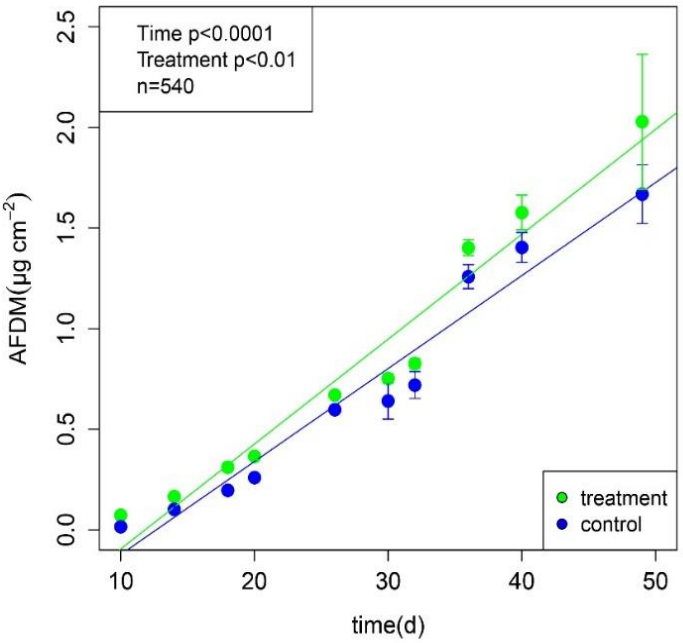

Figure 4. AFDM values over time. Dots represent means and bars SD (n=540). If SD is not shown, SD values were too small.

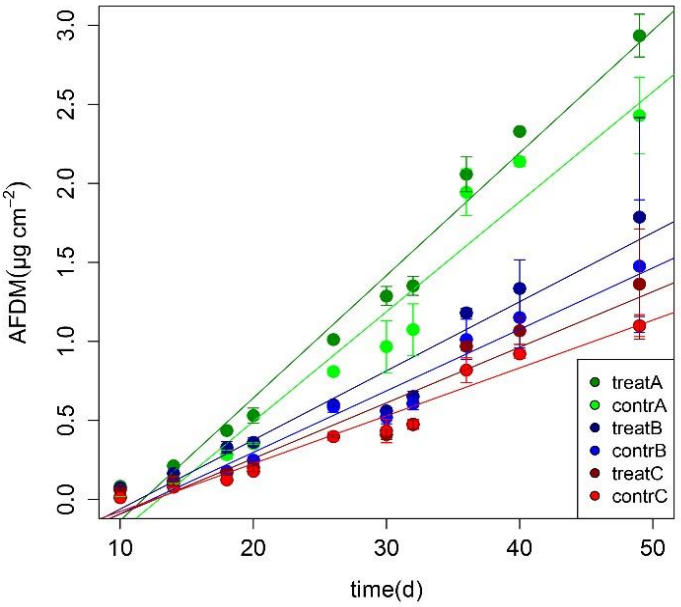

Figure 5. AFDM values per location over time. Dots represent the means and bars SD (n=540).

3.2.2 Biofilm chlorophyll a

The treatment had no significant effect on ChlA concentrations. ChlA values changed over time ($F = 22.698$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$) and were driven by light intensity ($F = 17.11$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.01$). However, no clear pattern of ChlA development over the whole experiment was seen. In the first three weeks of the experiment ChlA values showed a steep increase (Fig. 6). In addition, values from the treated flumes were consistently lower than those in the control flumes. From day 32 on, ChlA dynamics changed. Despite day 32, were ChlA concentrations suddenly dropped, the remaining duration of the experiment was characterized by slightly decreasing concentrations. ChlA concentrations did not show a clear pattern regarding the single locations. I tested whether ChlA concentrations were driven by treatment, time and light intensity. Time and light intensity were identified as important drivers of ChlA. This was also true for single locations. Near the inflow (location A) time affected ChlA ($F = 22.95$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$) as well as in the intermediate located location B ($F = 73.18$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$). Also, ChlA concentrations in location C were significantly changed over time ($F = 167.54$, $d.f._1 = 9$, $d.f._2 = 56$, $p<0.001$). Interestingly, no evidence for a treatment effect on ChlA was found, not even in location A were AFDM showed the strongest treatment effect.

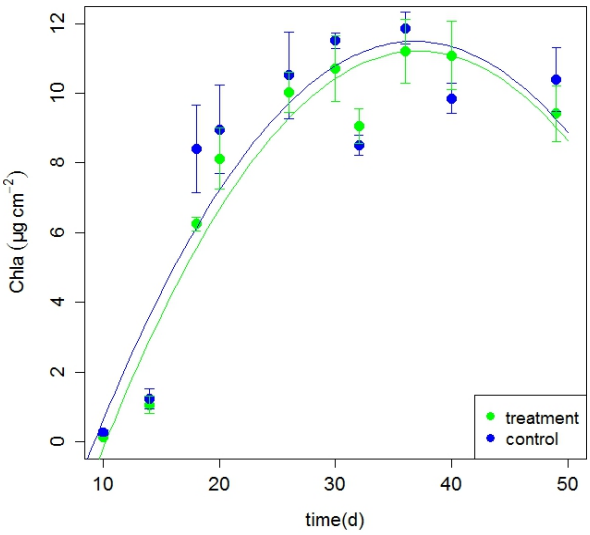

Figure 6. ChlA values over time. Dots represent means and bars SD. n=540

3.2.3 Biofilms carbon to nitrogen ratio

C:N ratios of biofilm samples showed no significant difference between the treated mesocosms and the control mesocosms. Looking at C:N ratios for all the flumes (all locations together), time increased the C:N ratio significantly ($F = 17.91$, $d.f._1 = 2$, $d.f._2 = 12$, $p<0.001$). As for ChlA, the SD of the single samplings was high (Fig. 7) in comparison to AFDM. The same pattern holds true when looking at single locations. For neither one of the locations A, B or C a treatment effect could be detected. However, a significant effect of time for the C:N ratio at locations A ($F = 5.65$, $d.f._1 = 2$, $d.f._2 = 12$, $p<0.05$), B ($F = 24.91$, $d.f._1 = 2$, $d.f._2 = 12$, $p<0.001$) and C ($F = 12.12$, $d.f._1 = 2$, $d.f._2 = 12$, $p<0.01$), was found. Also, the last sampling showed a clearly higher C:N ratio in the treated flumes.

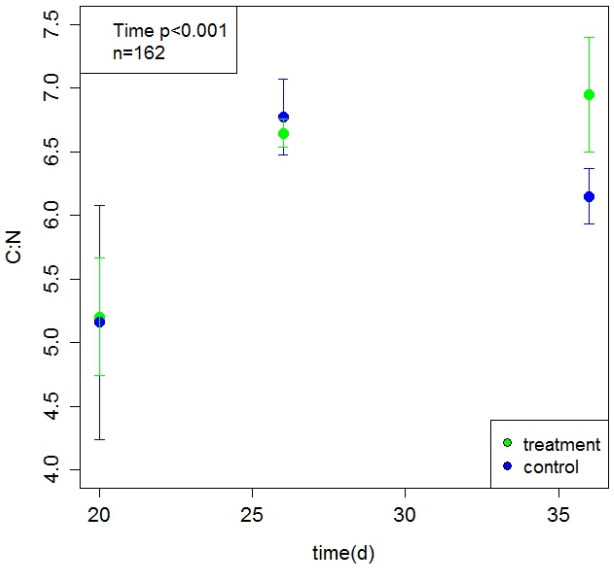

Figure 7. C:N values over time. Dots represent means and bars SD. n=162

3.2.4 Predictor models for dissolved organic carbon and nitrate

Based on ΔDOC, ΔNO_3^-, AFDM and ChlA results I computed prediction models to assess whether AFDM or ChlA can be used as a predictor variable for ΔDOC or ΔNO_3^- respectively. I found that AFDM as well as ChlA could not be used as predictors for neither ΔDOC nor ΔNO_3^-. All the modelled values are clearly different from the one to one line (f(x)= y) (Fig. 8). Statistically, I could prove that there is a significant difference between the measured values and a normally distributed data frame representing f(x)= y. AFDM as a predictor for ΔDOC ($F = 10.5$, $d.f._1 = 1$, $d.f._2 = 58$, $p<0.01$) and ΔNO_3^- ($F = 6.85$, $d.f._1 = 1$, $d.f._2 = 58$, $p<0.05$) as well as ChlA as a predictor for ΔDOC ($F = 10.05$, $d.f._1 = 1$, $d.f._2 = 58$, $p<0.01$) and ΔNO_3^- ($F = 5.31$, $d.f._1 = 1$, $d.f._2 = 58$, $p<0.05$) showed that the modelled values do not describe the one to one line, which represents a perfect prediction model.

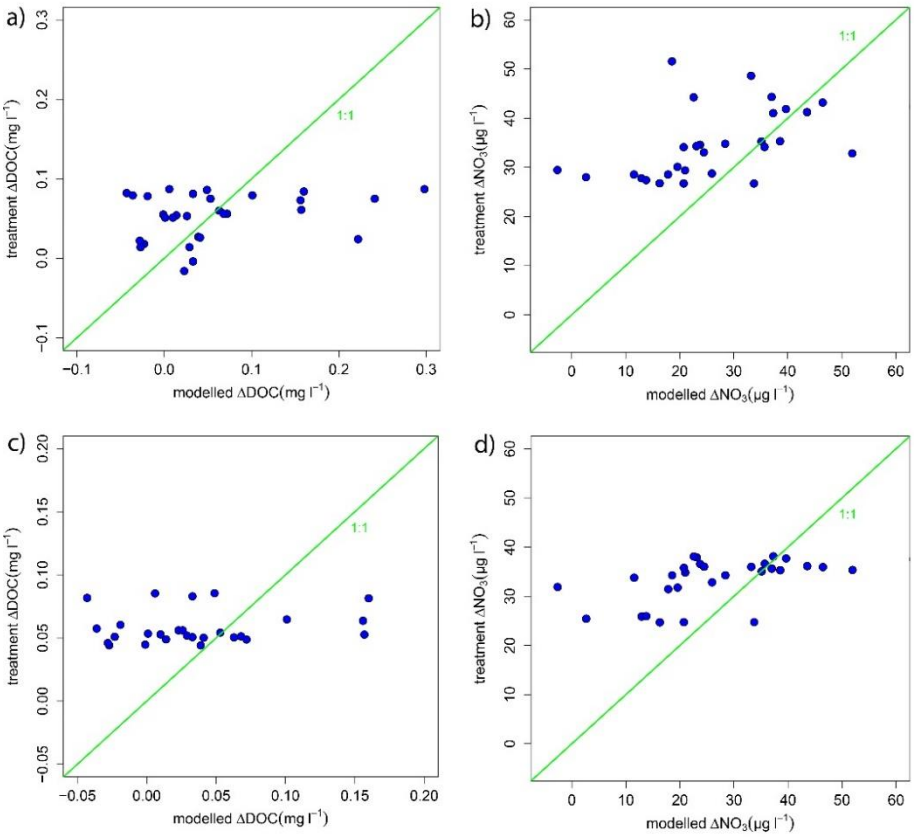

Figure 8. Modelled and measured values for ΔDOC and ΔNO_3^- as predictors for AFDM and ChlA. a) ΔDOC as predictor for AFDM; b) ΔNO_3^- as predictor for AFDM; c) ΔDOC as predictor for ChlA; d) ΔNO_3^- as predictor for ChlA; n= 30;

3.2.5 Terminal restriction fragment length polymorphism

3.2.5.1 Community composition according to the enzyme HinfI

A total of 168 bacterial OTUs with an average of 46 ± 12 (mean ± SD) per sample regarding the enzyme HinfI were detected. Focusing exclusively on the in-stream and on the soil community, I found on average 44 ± 12 and 53 ± 7 OTUs respectively. Taking into account one factor at the time (one-way perMANOVA, factor: time or treatment, two levels each), the soil microbial community was distinguishable from the combined in-stream communities ($F = 12.75$, $d.f._1 = 1$, $d.f._2 = 40$, $p<0.001$). Moreover the in-stream biofilm communities shifted over time resulting in a significant composition change ($F = 13.17$, $d.f._1 = 1$, $d.f._2 = 34$, $p<0.001$) between the two samplings (Fig. 9b). However, 18 days after the beginning of the experiment, in-stream microbial communities were significantly altered by the treatment ($F = 2.73$, $d.f._1 = 1$, $d.f._2 = 16$, $p<0.05$).

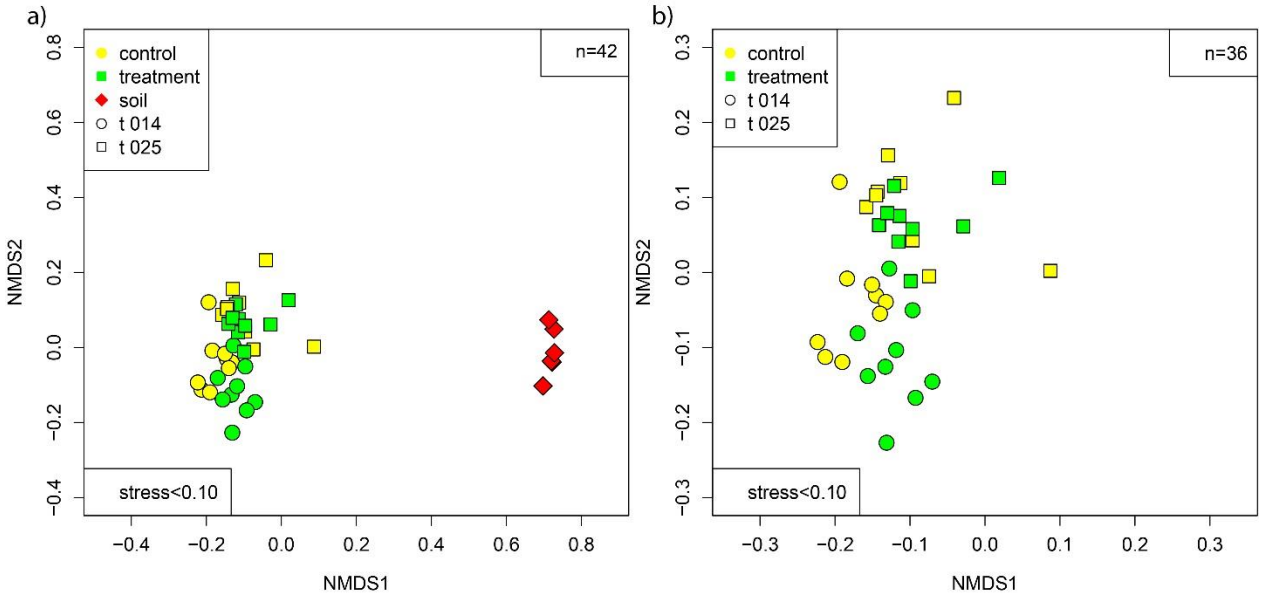

Figure 9. NMDS plot based on the digestion with the enzyme HinfI, showing dissimilarities between microbial communities from the treated flumes (green), control flumes (yellow) and the soil (red). Circles represent samples taken 18 days after experiment start (t014), squares samples taken at the end of the experiment (t025), rhomb soil communities. a) Total microbial communities (n= 42). b) In-stream communities (n=36).

This treatment effect was no longer significant when looking at communities at the end of the experiment (49 days after the beginning of the experiment). However, considering two factors at once (factor 1: treatment; factor 2: time, 2 levels each) the p-value for overall treatment effect improved in comparison to the one-way perMANOVA resulting in a significant difference between control and treated flumes ($F = 2.29$, $d.f._1 = 1$, $d.f._2 = 32$, $p<0.05$). It also confirmed the different results obtained for the two different sampling times ($F = 14$, $d.f._1 = 1$, $d.f._2 = 32$, $p<0.001$).

Alpha diversity, expressed by the Shannon diversity index was highest in the soil (Fig. 10a-d). In-stream microbial communities were significantly less diverse than the soil community ($F = 17.66$, $d.f._1 = 1$, $d.f._2 = 40$, $p<0.001$). The average Shannon Index at the first sampling was higher in the treated flumes, although no significant difference between the flumes regarding biofilm diversity could be detected (Fig. 10a). However, average OTU richness at the first sampling (Fig. 10e) was significantly increased by the treatment (t-test, $t=4.57$, $d.f.=8$, $p<0.01$); evenness measures did not differ significantly between control and treatment mesocosms (Fig. 10i). Shannon diversity of in-stream microbial communities at the second sampling (Fig. 10b) was not significantly different from those in the control. Analogous patterns were found when looking at species richness (Fig. 10h) and evenness (Fig. 10i). Comparing total treated and control biofilm communities, no evidence for a significant treatment effect on diversity, species richness and evenness was found. Over time, diversity of in-stream communities decreased (Fig. 10d) significantly (t-test, $t=4.12$, $d.f.=17$, $p<0.001$). Species richness and Pielou evenness (Fig. 10h,l) showed the same pattern with significantly lower values for in-stream communities

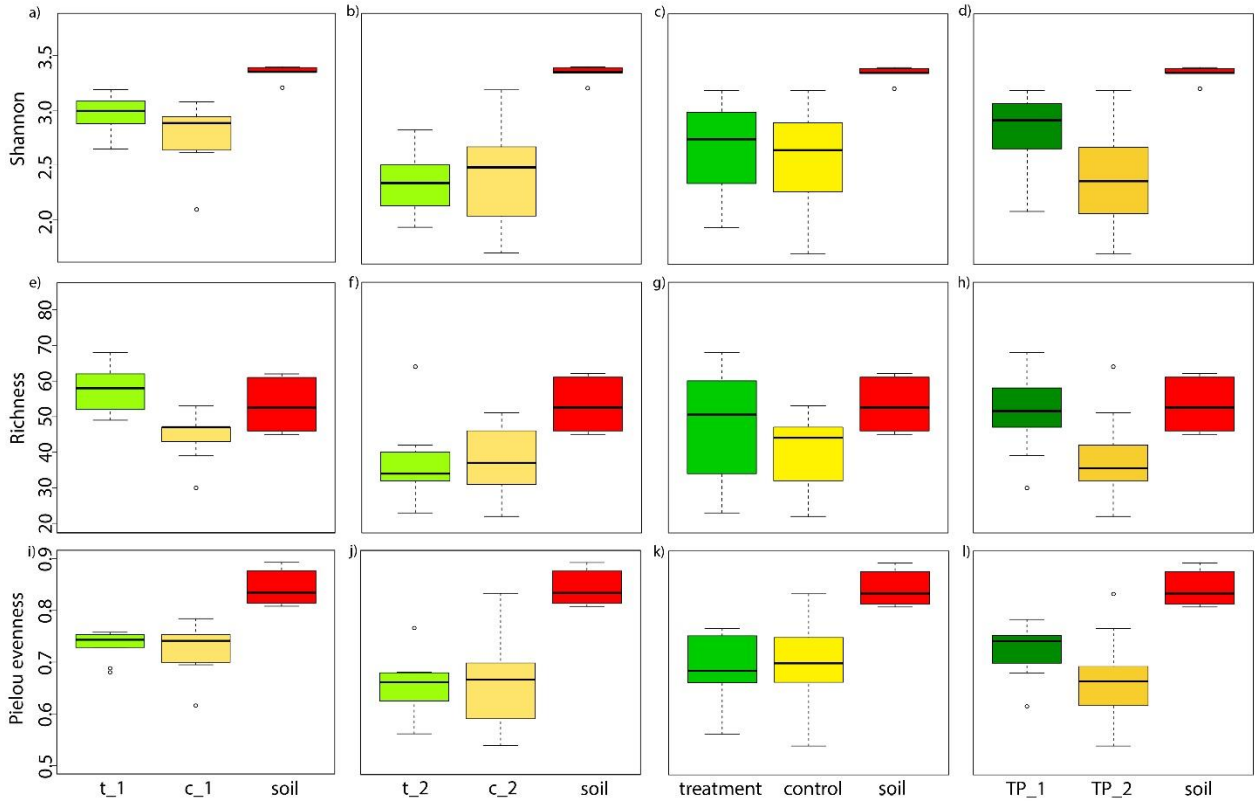

Figure 10. Biofilm and soil diversity. (a)-(d) Shannon Index, (e)-(h) Species richness, (i)-(l) Pielou evenness. Biofilm from the treated (light green, n=18) and control flumes (light yellow, n=18) at time point one (t_1; c_1) and time point two (t_2; c_2). Combined biofilm communities of treated flumes from TP_1 and TP_2 (green, n=36) and control flumes (yellow, n=36). Combined biofilm communities from the treated and the control flumes at time point one (dark green, TP_1, n=36) and time point two (dark yellow, TP_2, n=36). Soil community (red, n=6).

over time (t-test, t=3.97, d.f.=17, p<0.001; Wilcoxon test, n=72, p<0.01). Interestingly, a significant decrease in Shannon diversity comparing t_1 (treatment at the first sampling) and t_2 (treatment at the second sampling) (t-test, t=6.03, d.f.=8, p<0.001) was found. No significant difference between the control biofilm communities from the first sampling and the second sampling could be detected. Similar dynamics were found also when looking at species richness and evenness. Regarding species richness, a significant decrease comparing t_1 and t_2 (t-test, t=4.3, d.f.=8, p<0.01) was detected. C_1 (control at the first sampling) and c_2 (control at the second sampling) did not differ significantly from each other. The same pattern holds true for evenness, were t_1 was significantly higher than t_2 (t-test, t=4.01, d.f.=8, p<0.01) and no difference between c_1 and c_2 was found.

3.2.5.2 Community composition according to the enzyme Hhal

I identified altogether 170 OTUs with an average of 46 ± 12 using the enzyme Hhal. Looking solely at the communities in the flumes 44 ± 10 OTUs were detected. Within the soil community 61 ± 10 OTUs could be found. Hence, considering both enzymes the number of total OTUs was highest in the soil samples. Considering all OTU's, the same pattern as for the enzyme Hinfl can be observed (Fig. 11). I tested the treatment effect for

the first sampling (t_014) in a one-way perMANOVA (factor: treatment, 2 levels) and found a significant difference between the community composition of the treated and the controlled mesocosms ($F = 2.58$, $d.f._1 = 1$, $d.f._2 = 16$, $p<0.05$).

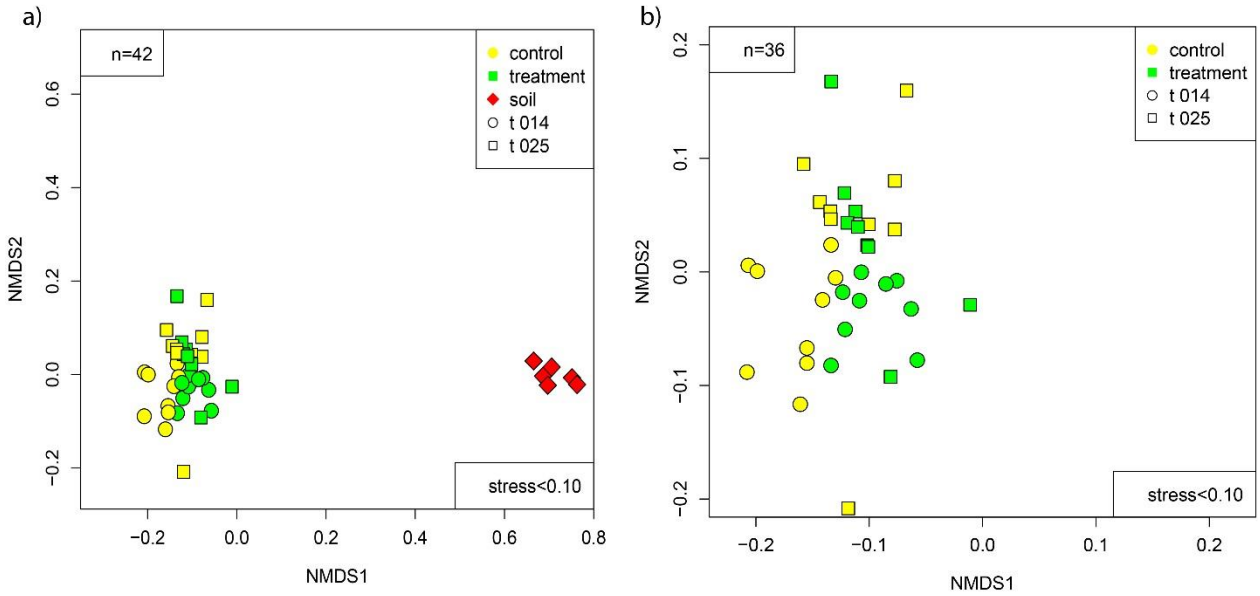

Figure 11. NMDS plot based on the digestion with the enzyme Hhal, showing dissimilarities between microbial communities from the treated flumes (green), control flumes (yellow) and the soil (red). Circles represent samples taken 18 days after experiment start (t014), squares samples taken at the end of the experiment (t025), rhomb soil communities. a) Total microbial communities (n= 42). b) In-stream communities (n= 36).

The same test design was used for the second sampling (t 025), which resulted (as for the enzyme Hinfl) in a not significant difference between the communities from treated and control flumes. A difference between the soil communities and total in-stream biofilm communities could be detected ($F = 13.91$, $d.f._1 = 1$, $d.f._2 = 40$, $p<0.001$) as well as a difference in community composition over time ($F = 9.46$, $d.f._1 = 1$, $d.f._2 = 34$, $p<0.001$). Considering the similar patterns between the two enzymes, I employed a two-way perMANOVA (factor 1: treatment; factor 2: time, 2 levels each) and observed that there was no significant total treatment effect ($F = 1.37$, $d.f._1 = 1$, $d.f._2 = 32$, $p=0.196$). I could confirm the result from the one-way perMANOVA, stating a difference among first and second sampling ($F = 9.67$, $d.f._1 = 1$, $d.f._2 = 32$, $p<0.001$).

The Shannon diversity Index was significantly higher in the soil (Fig. 12a-d) in comparison to the total in-stream communities (Wilcoxon test, n=42, $p<0.001$). Focusing on the first sampling, I found a significantly higher alpha diversity in the treatment than in the control communities (Wilcoxon test, n=36, $p<0.01$). Pielou's evenness was the strongest driver of diversity at the first sampling (Fig 12i), were I detected significantly higher evenness in the treated biofilms (Wilcoxon test, n=36, $p<0.01$). However, I found no significant difference regarding species richness between the treated and control communities (Fig. 12e). At the second sampling I found no significant difference between treated and control communities in terms of diversity, richness and evenness (Fig 12b,f,j). In addition, for total

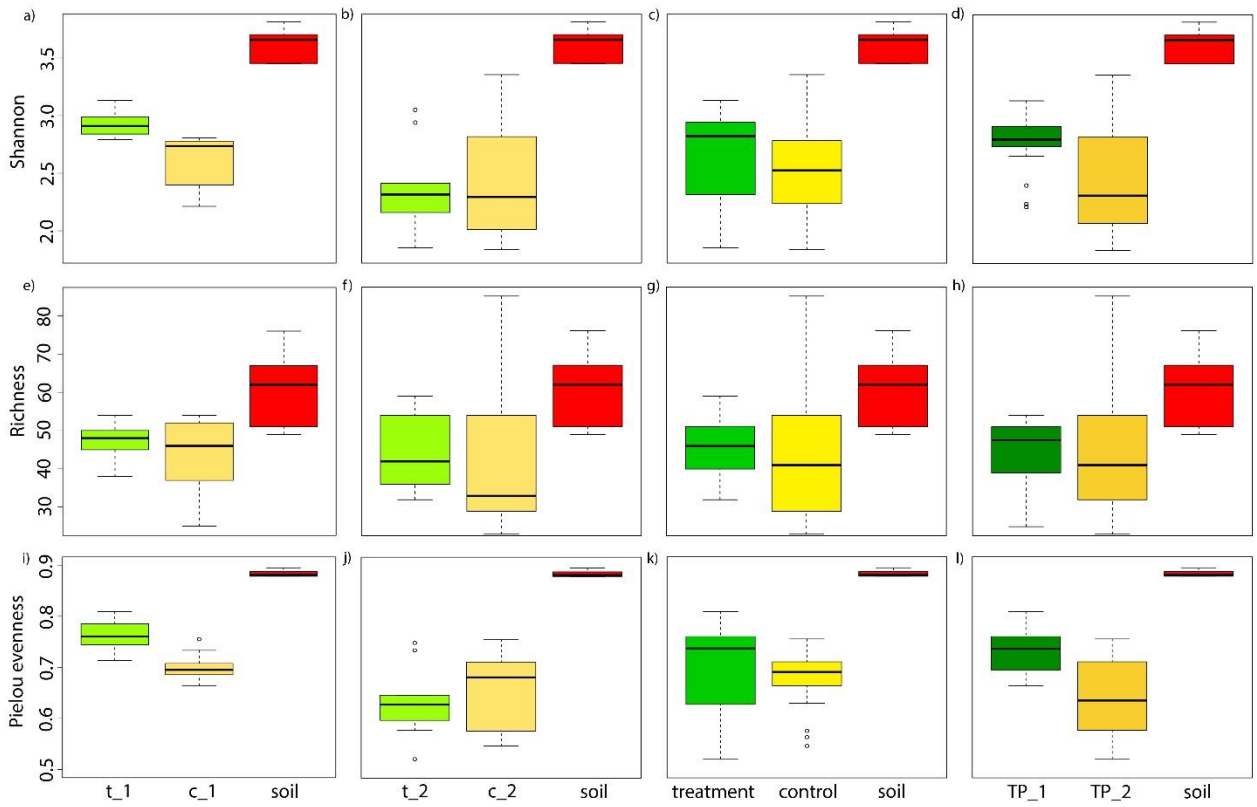

Figure 12. Biofilm and soil diversity. (a)-(d) Shannon Index, (e)-(h) Species richness, (i)-(l) Pielou evenness. Biofilm from the treated (light green, n=18) and control flumes (light yellow, n=18) at time point one (t_1; c_1) and time point two (t_2; c_2). Combined biofilm communities of treated flumes from TP_1 and TP_2 (green, n=36) and control flumes (yellow, n=36). Combined biofilm communities from the treated and the control flumes at time point one (dark green, TP_1, n=36) and time point two (dark yellow, TP_2, n=36). Soil community (red, n=6).

treatment and control comparisons no significant difference could be detected (Fig. 12c,g,k). Shannon diversity was significantly higher at the first sampling in comparison to the second (t-test, t=2.87, d.f.=17, $p<0.05$). Looking at evenness I also found significantly higher values in the first sampling in comparison to the second (t-test, t=3.94, d.f.=17, $p<0.01$); species richness could not be proven significantly different (Fig. 11h) regarding the first and the second sampling. Comparing the treated community at the first sampling (t_1) and the second sampling (t_2) I could show a significantly higher diversity at the first sampling (t-test, t=3.77, d.f.=8, $p<0.01$). By applying the same test criteria for the control communities no significant difference could be detected. This pattern is found also when comparing first and second sampling of the treated communities as well as first and second sampling of the control communities in terms of evenness. Only when comparing treated communities, significantly higher evenness in the first sampling could be found (t-test, t=4.31, d.f.=8, $p<0.01$). However, looking at non-treated communities no differences could be found. For species richness no differences in neither the treatment nor the control communities over time could be shown.

4 Discussion

4.1 Biogeochemistry of streamside mesocosms

Past work has shown that soil and vegetation in the catchment area of headwater streams (Thurman 1985), as well as distinct landscape formations such as hillslopes (McGlynn & McDonnell 2003; Inamdar & Mitchell 2006) are important sources of allochthonous DOC inputs to streams. Mei *et al.* showed that the contribution of DOC from hillslope soils is an important source of organic matter to headwater streams. They found subsurface DOC fluxes of 6600 kg a^{-1} in the White Clay Creek watershed located in southern Pennsylvania. Hence, subsurface DOC fluxes are important contributors to in-stream DOC budgets. However, during high intensity rainfall events, which result in fully saturated soils, DOC as well as POC inputs peak. Regarding POC contributions, material from the soil is mixed with the water and transported towards the river, therefore flushing soil into the stream (Dhillon & Inamdar 2014). Considering that heavy precipitation events will likely increase in intensity and frequency with ongoing climate change (Dore 2005; Lubchenco & Karl 2012), allochthonous input patterns due to storm events are likely to increase as well (Inamdar & Mitchell 2006; Dhillon & Inamdar 2014).

My thesis focused on short term allochthonous inputs as they would occur after heavy precipitation events. One core expectation of the soil addition was that the treatment would result in a higher DOC concentrations in the treated flumes in comparison to the control flumes. Soil DOC leachate was expected to show a decreasing DOC release over time due to dilution processes and since the soil addition took place only once at the beginning of the experiment. But despite substantial soil additions, I was not able to show an increase in DOC concentrations in the treated mesocosms in comparison to the controls. However, biofilm AFDM was significantly increased by the treatment suggesting that POC sedimentation (low discharge favors POC sedimentation, see Minshall & Thomas 2000), rather than DOC drove AFDM development in this experimental setting. Another explanation for the AFDM increase may be that the biofilm community took up the DOC provided by the soil additions immediately, so that no DOC increase could be detected at the outflow.

Assuming an in-situ removal of DOC in the treated flumes by the microbial community, the above mentioned mechanism may explain biofilm biomass gains. Earlier work by Teissier *et al.* (2007) used AFDM as a proxy for bacterial biomass in a benthic chamber experiment with unmodified pebbles taken directly from the stream. However, making this assumption is questionable for my experimental setting, as AFDM reflects all organic substances including not just bacterial biomass, but also algal biomass and all other organic compounds such as POC or extracellular polymeric substances (EPS). Nevertheless, AFDM augmentation in the treated flumes may also be driven partially by bacterial biomass gains. This hypothesis could be tested, if data on cell counts and/or lipid phosphate quantifications were available. However, as such data was not collected, more

specific statements regarding AFDM cannot be made. Considering the above-mentioned explanations it remains unresolved whether DOC uptake by the microbial community, POC sedimentation on the biofilm and possible faster developing bacterial biomass may have driven AFDM patterns. One aspect that may be excluded as a factor leading to higher AFDM in the treated flumes are algal contributions, since no difference regarding ChlA values between the treated and the control flumes could be detected. In addition, ΔNO_3^- concentrations in the treated flumes were significantly lower than those in the control flumes. This supports the hypothesis, that bacterial biomass increased in the treated flumes, since nitrate is a commonly used nitrogen source (Ghosh & Leff 2013). Other explanations for the observed nitrate pattern may be that the soil acted as a filter and reduced nitrate concentrations in the treated flumes. However, since I had slow flow conditions, most of the water did not pass through the soil, but rather floated on top of it. Hence, I consider the first argumentation more likely.

C:N ratios did not differ significantly between treatment and control flumes. As for AFDM, changes in the algal communities as drivers for C:N can be left aside since no difference in the ChlA values between the treatments was found. Given that C:N ratios are believed to be higher in allochthonous than in autochthonous material (Hein *et al.* 2003), I supposed that biofilms in the treated flumes would have higher C:N ratios. The only sampling were the SD of treatment and control C:N ratio did not overlap is the last one. In addition, at the last C:N sampling, treatment C:N ratios are higher than C:N ratios in the control mesocosms. This may suggest that the effect of the treatment on the C:N dynamics is becoming more pronounced with time. Especially since the flow velocity in the mesocosms was very low, FPOM from the soil may have sedimented on the biofilms (Jones & Smock 1991; Cushing, Minshall & Newbold 1993), therefore leading to an increasing gap between treatment C:N ratios and control C:N ratios at the last sampling (Fig. 7).

4.2 Biofilm community composition and diversity

In my study, I present evidence that benthic biofilm community composition is altered by soil additions to streamside mesocosms. Although assemblage mechanisms of in-stream biofilms have been subject of numerous studies (Besemer *et al.* 2007, 2012; Singer *et al.* 2010; Peter *et al.* 2011; Wang *et al.* 2013; Wagner *et al.* 2015), biofilm responses to allochthonous inputs are still largely unknown.

I found that the microbial communities in the treated flumes were significantly different from those in the control flumes at the first sampling that is, two weeks after the soil addition. This pattern is consistent for both applied t-RFLP enzymes (Fig.9, Fig. 11). Thus, the results of my study point out that in-stream microbial community assemblage is affected by soil additions. I argue that inputs of terrestrial material in freshwater ecosystems are not just a nutrient and carbon source, but contribute to the bulk in-stream

microbial community. Crump *et al.* (2012) showed that soil inoculation is an important driver of microbial diversity in streams in the arctic tundra, although their study reflects general patterns and is not explicitly researching effects of soil inputs to streams.

However, my study is the first I know of, attempting to explain local and short-term (five weeks) soil inoculation patterns on in-stream microbial communities after substantial terrestrial inputs. As mentioned earlier, the amount of allochthonous material washed into streams is increasing globally (Regnier *et al.* 2013; Jones & Lennon 2014). Therefore, short term in-stream biofilm responses to ecosystem disturbances such as storm events or landslides, also leading to additional terrestrial inputs, may become more important.

Soil subsidization induced an increase of diversity and evenness in the treated communities at the first sampling when looking at data from the enzyme HhaI (Fig. 11). This supports my hypothesis that the treatment had an effect on benthic biofilms. In addition, data form the enzyme HinfI supports this pattern showing a significant increase in species richness (Fig. 10). Interestingly, over time a clear decrease in diversity, richness and evenness can be observed only in the treated communities, providing additional support for the idea that changing community structures can be attributed to the treatment. These results are in concordance with Crump *et al.* (2012) who suggests initial soil inoculation as a driver for microbial diversity structures followed by downstream species sorting. Indeed, in my case the initial community composition, thus the differences in the biofilm communities between the treated and the control flumes, are most likely driven by the soil addition. The community shift from the first to the second sampling in the treated flumes may be driven by species sorting, since the influence of the soil addition most likely decreased with time. Furthermore, studies have shown that light availability and thereby the ratio of available allochthonous and autochthonous material are important drivers for microbial community composition in headwater streams (Wagner *et al.* 2015). Overall the findings of this thesis agree with the general notion that terrestrial inputs affect in-stream biofilm communities (Battin *et al.* 2008; Crump *et al.* 2012; Fasching *et al.* 2014; Wagner *et al.* 2015).

Studies have indicated changing microbial community compositions along environmental gradients (e.g. Van der Gucht *et al.* 2007; Ask *et al.* 2009; Wagner *et al.* 2014). However, not only bacterial community composition but also biofilm functioning is of great concern, especially regarding the link between microbial and ecosystem ecology (Hall *et al.* 2011). I showed that epilithic microbial biofilm communities in streamside mesocosms respond to soil additions leading to a significant change in community composition and diversity measures, namely Shannon diversity index, species richness and Pielou's evenness. In addition, lower nitrate concentrations in the flumes with the soil addition suggest that biofilm communities in the treated flumes took up more nitrate maybe leading to a higher microbial activity, as nitrate is an important nitrogen source for bacteria. Therefore, future research to unravel the functional effects of those biofilm community changes is necessary to get better insights in the implications for biogeochemical cycling.

5 Conclusions

Evidence in this study indicates that future changes regarding terrestrial inputs to stream ecosystems may alter in-stream microbial communities and thereby, ecosystem functions provided by epilithic biofilms may shift. Significantly different microbial communities in the treated mesocosms in comparison to the ones acting as control, as well as lower nitrate concentrations and elevated AFDM in the treated flumes support this hypothesis, suggesting soil addition mediated implications for biofilm metabolism.

In-stream microbial community assemblage was altered by the soil addition 18 days after the experiment start. However, 49 days after experiment begun biofilm communities did not differ significantly between the treated mesocosms and the control flumes, suggesting that the effect of the microbial inoculation form soils may have decreased over time. Shannon index, species richness as well as Pielou's evenness support this conclusion. In addition, in-stream microbial communities may have influenced DOC concentrations in the flumes with soil additions.

Although stream mesocosm DOC dynamics over the whole experiment showed no evidence for a treatment effect, median DOC concentrations for the first two weeks were higher in the flumes with soil additions. Hypothesized DOC uptake by biofilms as well as scarce soil additions (200 dm^3) may have masked DOC concentration increase in the treated mesocosms.

Nitrate dynamics may also be driven by changes in the benthic biofilm community composition. At first glance, nitrate patterns with lower concentrations in the treatment flumes seem counterintuitive, as the soil addition was believed to add nutrients to the mesocosms. I argue that nitrate decrease in the flumes with soil additions may be a result of higher nitrate uptake and therefore possibly higher activity by the biofilms of the treated flumes.

5.1 Outlook

To properly assess the biogeochemical implications of soil additions and of the thereby altered biofilm communities, more detailed analyses are needed in order to quantify carbon and nitrogen dynamics. Labelled $\delta^{15}N$ and $\delta^{13}C$ could be used to trace whether different carbon and nutrient sources are used up by biofilms or not combined with parallel factor analysis (PARAFAC) to determine the origin of DOC. In addition, deeper sequencing techniques like Illumina or 454 pyrosequencing to address bacterial biofilms on a species level, combined with metatranscriptomics to look at the active functions of the biofilm community could result in a deeper mechanistic insight on how in-stream biogeochemistry is coupled to the microbial community.

6 Attachment

Zusammenfassung

Biofilme sind Hotspots mikrobieller Diversität in Fließgewässern und tragen wesentlich zur Abwicklung von Stoffkreisläufen bei. Sie werden stark von unterschiedlichen organischen Kohlenstoffverbindungen sowie deren Herkunft beeinflusst und tragen maßgeblich zum Metabolismus von aquatischen Ökosystemen bei. Flüsse, vor allem Oberläufe gelten als netto heterotrophe Systeme und sind daher auf allochthonem Material als Energie-, Kohlenstoff- und Nährstoffquelle angewiesen. Kürzlich veröffentlichte Studien zeigen, dass terrestrischer organischer Kohlenstoff für eine erhöhte CO_2 Ausgasung aus Flüssen verantwortlich ist. Aufgrund der Klimaveränderung werden vermehrt Starkregenereignisse eintreten, welche zur Folge haben, dass mehr allochthones Material in Fließgewässer eingetragen wird. Daher ist es wichtig die Reaktion von Biofilmen und gelöstem organischem Material (DOM) auf diese Veränderungen hin zu untersuchen, um mögliche Implikationen des Klimawandels abzuschätzen. In unserem Versuch erforschen wir die Auswirkungen von Bodenzugaben auf benthische Biofilme sowie gelöstem organischem Material in sechs flussnahen Mesokosmen. Diese haben den Vorteil, dass sie einerseits naturnahe Bedingungen wiedergeben und andererseits Möglichkeiten bieten, um gewisse Aspekte von Fließgewässern genauer zu untersuchen. Dies wird dadurch ermöglicht, dass gewisse Parameter, welche für Flüsse relevant sind gezielt beeinflusst werden können. In unserem Fall wurden Neigung der Mesokosmen sowie Durchfluss so eingestellt, dass eine lange Verweilzeit des Wassers in den Rinnen erzielt wurde. Gelöster organischer Kohlenstoff (DOC) und Nitrat Werte wurden im Wasser erhoben sowie aschefreie Trockenmasse, Chlorophyll A und C:N Verhältnis von Biofilmen über eine Zeitspanne von 49 Tagen. Zusätzlich wurden die bakteriellen Biofilmgemeinschaften zweimal beprobt, um die Zusammensetzung der Gemeinschaften sowie deren Diversität zu erheben. Dies wurde mittels mikrobiellen Fingerprinting Methoden mit 16S rRNA Genen verifiziert. Die Ergebnisse deuten darauf hin, dass sowohl die Zusammensetzung der mikrobiellen Gemeinschaft als auch deren Diversität von der Bodenzugabe beeinflusst werden. Daraus ergibt sich, dass allochthones Material ein möglicher Treiber für die Biofilmzusammensetzung ist. Diese Aussage kann getroffen werden, da die mikrobielle Gemeinschaft der ersten Probenahme (18 Tage nach Experiment Beginn) signifikante Unterschiede zwischen Rinnen mit und ohne Bodenzugabe aufweisen. Des Weiteren wurden aschefreie Trockenmasse und Nitrat von der Bodenzugabe beeinflusst, was die Relevanz von Zugaben terrestrischen Materials für aquatische Ökosysteme bestätigt.

Curriculum Vitae

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9 Appendix

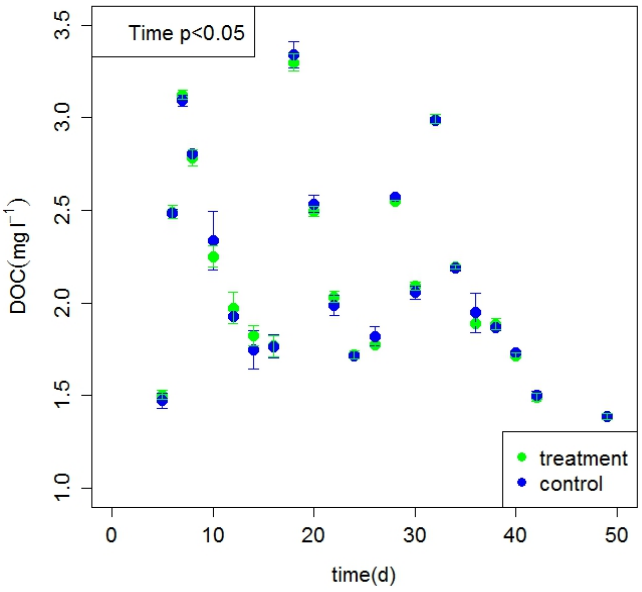

Figure 13. DOC concentrations at the outflow. Dots represent the mean and bars the standard deviation (SD). DOC values from the control flumes are displayed in blue, values from the treated flumes in green.

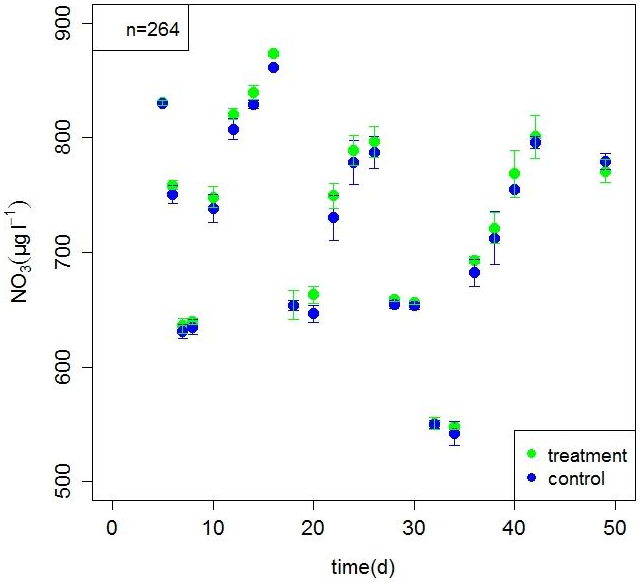

Figure 14. NO_3^- concentrations at the outflow. Dots represent the mean and bars the standard deviation (SD). NO_3^- values from the control flumes are displayed in blue, values from the treated flumes in green.

A1. DOC mean values (in µg l^{-1}) and SD for each flume and time point.

Time (d)		R1	R2	R3	R4	R5	R6	H
5	mean	1505	1477	1487	1517	1428	1520	1511
	SD	25.9	14.1	0.0	0.0	68.4	47.1	100.1
6	mean	2467	2452	2467	2507	2512	2513	2646
	SD	78.1	35.4	78.1	14.1	7.1	37.7	82.6
7	mean	3067	3123	3125	3102	3087	3147	3042
	SD	0.0	4.7	7.1	2.4	14.1	0.0	155.1
8	mean	2812	2765	2792	2755	2810	2832	2842
	SD	21.2	96.6	40.1	110.8	4.7	7.1	33.4
10	mean	2225	2187	2517	2292	2268	2273	2219
	SD	7.1	56.6	377.1	134.4	68.4	117.9	53.0
12	mean	1927	2068	1932	1935	1932	1917	1972
	SD	42.4	294.6	91.9	96.6	40.1	56.6	49.1
14	mean	1733	1787	1657	1797	1860	1887	1700
	SD	56.6	56.6	9.4	51.9	103.7	221.6	173.6
16	mean	1745	1825	1715	1710	1838	1768	1768
	SD	313.5	49.5	195.6	183.8	30.6	81.3	80.0
18	mean	3415	3345	3275	3250	3330	3300	3174
	SD	134.4	40.1	25.9	80.1	14.1	23.6	117.4
20	mean	2517	2463	2588	2505	2500	2513	2536
	SD	4.2	18.9	115.5	21.2	28.3	18.9	51.9
22	mean	1988	2062	1932	2032	2043	2002	1956
	SD	2.8	54.2	143.8	7.1	33.0	7.1	13.9
24	mean	1703	1743	1717	1697	1730	1720	1670
	SD	47.1	9.4	14.1	42.4	9.4	11.3	25.2
26	mean	1880	1778	1787	1782	1795	1762	1723
	SD	75.4	25.9	42.4	7.1	16.5	21.2	27.3
28	mean	2583	2550	2570	2545	2557	2553	2442
	SD	108.4	4.7	5.7	11.8	4.7	18.9	22.2
30	mean	2043	2108	2028	2068	2097	2103	2029
	SD	9.4	11.8	87.2	54.2	33.0	51.9	15.8
32	mean	2984	2976	2975	2984	3000	3019	2974
	SD	4.7	1.3	7.4	9.1	18.9	1.2	3.7
34	mean	2172	2197	2187	2182	2202	2207	2106
	SD	21.2	4.7	80.1	30.6	63.6	70.7	22.7
36	mean	1887	1878	2070	1897	1888	1887	1848
	SD	14.1	16.5	226.3	4.7	49.5	28.3	31.0
38	mean	1853	1922	1870	1873	1885	1863	1856
	SD	47.1	63.6	0.0	80.1	7.1	4.7	27.1
40	mean	1733	1732	1730	1702	1728	1717	1757
	SD	75.4	49.5	4.7	7.1	25.9	4.2	3.3
42	mean	1487	1497	1527	1467	1502	1508	1462
	SD	4.7	51.9	18.9	9.4	25.9	54.2	43.5
49	mean	1392	1400	1382	1372	1388	1397	1359
	SD	11.8	4.7	7.1	7.1	2.4	2.8	13.5

A2. NO_3^- mean values (in µg l^{-1}) and SD for each flume and time point.

Time (d)		R1	R2	R3	R4	R5	R6	H
5	mean	827.45	832.35	830.55	831.40	830.80	828.25	834.03
	SD	0.78	0.92	0.92	3.82	2.40	5.16	1.42
6	mean	757.15	759.45	752.35	754.60	742.15	761.80	758.30
	SD	7.28	0.64	24.25	3.25	0.49	20.65	4.86
7	mean	624.25	630.75	636.20	633.85	632.20	643.50	641.67
	SD	0.21	0.07	3.54	3.04	6.08	1.70	2.80
8	mean	631.70	637.40	630.30	643.00	642.65	639.15	643.50
	SD	1.27	6.36	0.71	2.83	2.47	3.18	3.41
10	mean	724.65	737.95	742.15	750.95	748.10	755.40	771.67
	SD	5.44	1.77	6.29	3.46	1.70	3.39	1.04
12	mean	797.95	816.50	816.90	818.80	807.85	825.80	827.30
	SD	9.40	5.66	15.41	0.00	1.34	17.11	2.23
14	mean	830.60	846.75	831.80	836.50	824.85	835.65	849.40
	SD	0.85	1.34	6.22	0.57	0.35	1.06	0.10
16	mean	861.30	870.70	861.60	875.05	861.00	873.90	881.37
	SD	55.58	9.19	12.73	6.58	2.97	1.13	2.32
18	mean	658.55	668.30	651.75	647.80	650.45	646.05	665.63
	SD	0.92	6.08	10.96	2.55	0.07	6.58	12.99
20	mean	637.95	656.40	649.20	661.35	652.20	670.80	682.33
	SD	10.96	9.19	1.98	0.78	0.71	1.70	9.95
22	mean	708.25	737.05	736.45	753.85	745.90	757.10	786.20
	SD	5.73	10.54	6.29	1.20	0.14	1.41	9.57
24	mean	757.25	773.50	784.30	794.85	794.15	797.40	814.43
	SD	4.31	9.05	4.10	4.74	1.34	1.98	2.50
26	mean	771.55	781.75	793.90	809.25	795.95	798.00	833.70
	SD	6.86	6.15	5.09	4.03	7.28	0.57	1.97
28	mean	651.35	657.05	654.20	661.50	658.55	659.00	683.10
	SD	0.35	0.21	2.83	1.98	1.34	0.85	4.45
30	mean	652.10	654.95	651.15	655.60	657.15	657.95	678.67
	SD	4.67	1.06	2.62	2.97	1.63	0.49	3.69
32	mean	546.00	546.25	551.30	549.70	552.55	556.40	584.80
	SD	0.00	0.49	7.92	2.55	1.34	1.84	2.52
34	mean	531.20	545.70	542.50	549.40	552.20	548.30	575.70
	SD	2.55	0.14	0.42	5.23	0.71	1.56	2.17
36	mean	668.40	696.05	687.05	693.70	690.75	689.75	733.33
	SD	7.78	0.49	0.35	4.81	3.75	0.78	3.72
38	mean	688.65	705.70	714.85	726.65	734.05	731.05	772.83
	SD	2.05	1.56	1.91	1.20	1.06	4.45	1.36
40	mean	757.00	766.20	753.45	790.10	752.90	749.45	812.67
	SD	4.24	3.68	0.64	2.97	0.14	2.05	6.64
42	mean	792.65	792.55	802.20	822.15	792.50	787.35	826.27
	SD	6.01	2.90	1.27	0.78	0.28	6.86	6.83
49	mean	785.70	767.30	772.05	781.95	781.25	763.50	800.50
	SD	8.06	2.69	1.63	1.63	2.90	1.56	0.98

A3. AFDM mean values (in µg cm^{-2}) and SD for each flume location and time point.

Time (d)		R1A	R1B	R1C	R2A	R2B	R2C	R3A	R3B	R3C	R4A	R4B	R4C	R5A	R5B	R5C	R6A	R6B	R6C
10	mean	0.0191	0.0148	0.0111	0.0840	0.0787	0.0580	0.0194	0.0146	0.0110	0.0847	0.0780	0.0587	0.0187	0.0147	0.0111	0.0837	0.0800	0.0650
	SD	0.0020	0.0014	0.0019	0.0111	0.0042	0.0069	0.0011	0.0017	0.0040	0.0168	0.0100	0.0129	0.0009	0.0013	0.0011	0.0146	0.0046	0.0087
14	mean	0.1260	0.0913	0.0827	0.2087	0.1660	0.1040	0.1487	0.1353	0.0733	0.2127	0.1520	0.1000	0.1013	0.0847	0.0727	0.2193	0.1793	0.1547
	SD	0.0251	0.0110	0.0050	0.0151	0.0211	0.0053	0.0234	0.0258	0.0090	0.0186	0.0125	0.0053	0.0133	0.0090	0.0095	0.0189	0.0061	0.0042
18	mean	0.2700	0.1813	0.1240	0.4353	0.3027	0.1540	0.3173	0.1940	0.1027	0.4347	0.3133	0.1680	0.2667	0.1627	0.1487	0.4433	0.3707	0.1847
	SD	0.0223	0.0095	0.0131	0.0295	0.0147	0.0212	0.0117	0.0212	0.0083	0.0437	0.0175	0.0125	0.0241	0.0133	0.0214	0.0151	0.0042	0.0101
20	mean	0.3513	0.2633	0.1753	0.4773	0.3920	0.1800	0.3480	0.2600	0.1767	0.5567	0.3620	0.2333	0.3593	0.2227	0.1813	0.5607	0.3347	0.1960
	SD	0.0333	0.0234	0.0234	0.0371	0.0442	0.0151	0.0236	0.0069	0.0227	0.0870	0.0280	0.0117	0.0186	0.0241	0.0190	0.0717	0.0277	0.0197
26	mean	0.7907	0.6240	0.3987	1.0120	0.6027	0.3947	0.8147	0.5587	0.3733	1.0027	0.5880	0.4093	0.8267	0.5773	0.4253	1.0213	0.6107	0.3947
	SD	0.0855	0.0386	0.0241	0.1122	0.0424	0.0620	0.1109	0.0180	0.0648	0.1443	0.0240	0.0424	0.0607	0.0521	0.0621	0.0869	0.0227	0.0345
30	mean	1.1573	0.5613	0.5147	1.3556	0.5703	0.4023	0.8840	0.4800	0.3653	1.2649	0.5436	0.4436	0.8613	0.5187	0.4227	1.2423	0.5689	0.3849
	SD	0.4958	0.1293	0.3106	0.5390	0.1461	0.0690	0.1224	0.0779	0.0546	0.0690	0.1805	0.0799	0.0281	0.1029	0.1097	0.0572	0.0833	0.0606
32	mean	0.9720	0.5680	0.4533	1.3293	0.6880	0.5080	1.2627	0.6493	0.4693	1.4200	0.6347	0.4667	0.9893	0.6067	0.5107	1.3067	0.6333	0.4493
	SD	0.1224	0.0779	0.0546	0.0690	0.0855	0.0799	0.1689	0.1293	0.0780	0.1548	0.1461	0.0690	0.0455	0.1029	0.1097	0.0572	0.0833	0.0606
36	mean	2.0680	0.9307	0.8827	1.9387	1.1840	0.9907	1.7813	0.9480	0.8400	2.1560	1.2067	0.9760	1.9840	1.1600	0.7320	2.0760	1.1533	0.9413
	SD	0.5868	0.1466	0.1765	0.2170	0.0525	0.0464	0.5277	0.1218	0.0394	0.1526	0.0983	0.0183	0.1352	0.1803	0.0734	0.3539	0.1263	0.1052
40	mean	2.1027	1.0200	0.8893	2.3293	1.3187	1.0640	2.1587	1.0640	0.9427	2.3213	1.5240	1.1547	2.1507	1.3707	0.9307	2.3360	1.1627	0.9827
	SD	0.6460	0.0866	0.3580	0.2682	0.2505	0.2130	0.0926	0.3153	0.1354	0.2890	0.1308	0.3528	0.1791	0.1894	0.3640	0.8205	0.1191	0.2948
49	mean	2.2480	1.2933	1.0573	3.0880	1.7320	1.3893	2.7053	1.1787	1.0627	2.8827	2.4413	1.6987	2.3320	1.9560	1.1800	2.8333	1.1867	1.0013
	SD	0.3598	0.2092	0.1992	0.4675	0.3659	0.1860	0.4627	0.1436	0.2543	0.5436	0.1785	0.2681	0.6231	0.2346	0.2289	0.4669	0.1405	0.4294

A4. ChlA mean values (in µg cm^{-2}) and SD for each flume location and time point.

Time (d)		R1A	R1B	R1C	R2A	R2B	R2C	R3A	R3B	R3C	R4A	R4B	R4C	R5A	R5B	R5C	R6A	R6B	R6C
10	mean	0.429	0.288	0.17	0.154	0.075	0.138	0.232	0.158	0.159	0.195	0.141	0.083	0.587	0.3	0.148	0.133	0.141	0.056
	SD	0.052	0.111	0.041	0.054	0.034	0.03	0.126	0.037	0.067	0.038	0.027	0.011	0.11	0.156	0.061	0.045	0.019	0.025
14	mean	1.388	0.684	0.623	1.032	0.747	0.567	1.797	1.421	0.82	1.516	1.234	0.743	2.247	1.334	0.782	1.741	1.116	0.852
	SD	0.426	0.172	0.012	0.117	0.206	0.259	0.553	0.092	0.468	0.14	0.131	0.104	0.129	0.632	0.334	0.13	0.305	0.113
18	mean	10.08	6.352	4.985	9.058	5.318	4.883	10.82	8.596	5.837	7.954	5.215	4.932	12.89	10.08	6.03	9.148	6.328	3.451
	SD	2.146	1.372	0.468	1.438	1.238	1.695	1.536	2.052	2.731	0.437	0.385	0.418	2.21	4.403	1.345	2.345	1.961	0.505
20	mean	8.167	7.955	7.516	9.07	6.637	6.084	9.867	8.998	7.076	11.61	8.922	6.522	13.06	11.07	6.935	11.31	6.401	6.544
	SD	1.76	0.655	1.742	3.393	0.756	2.551	0.554	0.595	1.355	2.059	0.362	0.506	1.038	2.37	0.454	1.537	0.183	0.979
26	mean	13.88	10.35	8.581	13.66	8.273	6.405	13.84	7.751	5.743	13.86	9.478	6.87	15.02	10.85	8.63	13.63	11.68	6.455
	SD	1.271	2.249	4.027	0.094	2.957	1.493	0.699	1.006	0.484	1.08	1.354	2.125	0.364	1.086	2.565	0.397	1.302	0.638
30	mean	13.58	12.63	7.611	12.86	10.7	8.077	14.3	12.4	8.379	14.12	11.15	9.882	14.19	11.97	8.482	13.29	9.041	7.239
	SD	0.794	1.46	1.808	0.916	1.293	1.057	0.491	0.658	0.867	0.897	1.579	0.757	0.819	1.419	2.138	0.526	2.141	1.415
32	mean	12.34	6.903	6.615	13.22	8.632	6.468	11.64	9.258	5.291	13.17	8.378	6.127	10.8	8.393	5.384	12.21	8.141	5.177
	SD	3.417	1.48	1.002	0.752	3.5	0.963	1.188	3.399	0.672	1.105	1.596	0.893	2.117	1.828	0.543	0.896	1.481	2.041
36	mean	11.25	12.55	12.78	12.12	11.78	11.81	10.42	13.09	12.71	11.9	12.07	10.65	11.02	11.94	11.07	10.93	9.63	9.912
	SD	2.326	0.182	1.024	1.084	0.949	1.734	2.697	0.491	0.632	0.436	0.075	1.87	0.991	0.13	1.605	0.882	1.483	2.5
40	mean	11.79	8.696	7.698	12.85	9.416	7.658	11.29	10.86	8.613	13.15	10.81	11.68	12.21	10.27	7.176	13.11	10.85	10.22
	SD	1.352	1.362	1.797	0.209	1.132	1.271	2.17	1.86	2.54	0.304	0.661	0.623	1.427	1.345	0.999	0.577	0.884	0.403
49	mean	9.884	12.3	11.76	7.751	10.68	11.66	6.885	12.09	12.25	8.942	6.844	9.748	8.431	8.622	11.32	6.348	10.85	11.9
	SD	1.14	0.342	1.286	1.198	2.344	0.155	1.287	0.356	0.663	0.777	0.711	0.733	3.147	1.285	0.644	1.209	0.489	0.196

A5. C:N mean values and SD for each flume location and time point.

Time (d)		R1A	R1B	R1C	R2A	R2B	R2C	R3A	R3B	R3C	R4A	R4B	R4C	R5A	R5B	R5C	R6A	R6B	R6C
20	mean	5.07	3.60	3.71	5.61	5.30	3.17	6.22	5.09	5.07	7.58	4.74	3.62	6.72	4.83	6.13	6.53	5.51	4.77
	SD	0.93	0.95	0.45	0.67	0.32	1.33	0.31	0.19	0.37	0.38	0.79	0.52	0.29	0.89	0.58	0.41	0.38	0.32
26	mean	7.20	7.20	6.86	7.42	6.51	5.77	7.72	6.33	5.43	7.90	6.45	5.96	7.26	6.71	6.23	6.90	6.66	6.23
	SD	0.22	0.52	0.07	0.26	0.40	0.53	0.16	0.32	0.47	0.18	0.22	0.54	0.35	0.25	0.64	0.28	0.29	0.36
36	mean	6.35	5.95	5.48	7.19	7.61	7.53	6.35	6.63	5.48	6.67	6.45	7.35	6.22	6.45	6.43	6.83	6.56	6.31
	SD	0.66	0.67	0.08	0.55	0.18	0.48	0.66	0.64	0.08	0.13	1.28	0.02	0.36	0.32	0.82	0.20	0.42	0.32

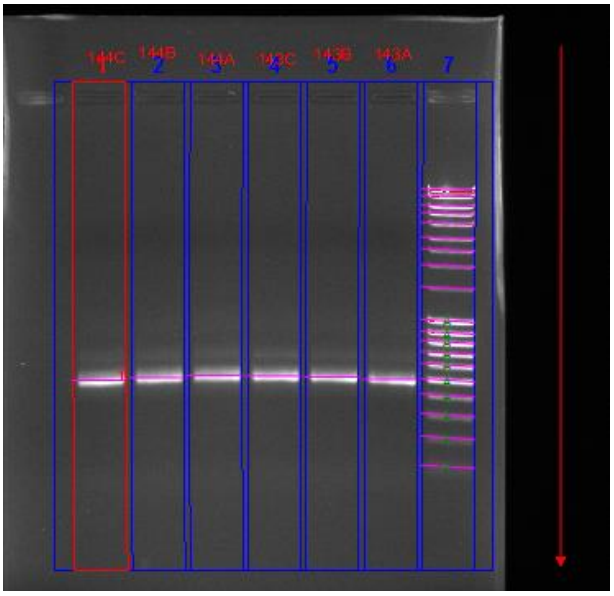

Figure 15. Gel of the first biofilm sampling; flumes one and two.

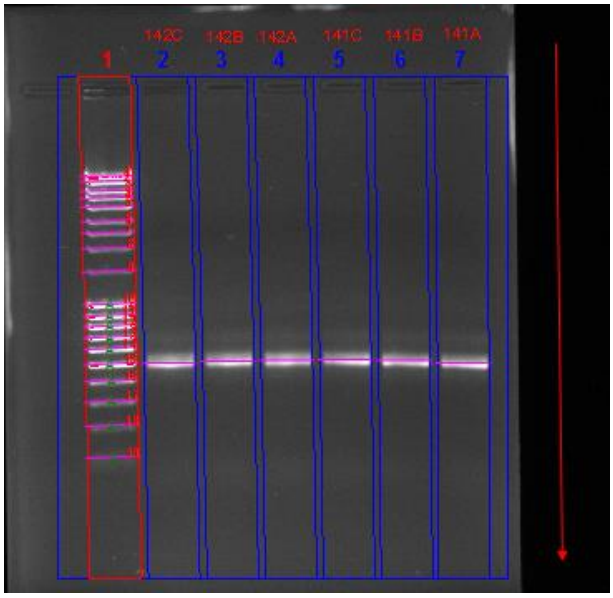

Figure 16. Gel of the first biofilm sampling; flumes three and four.

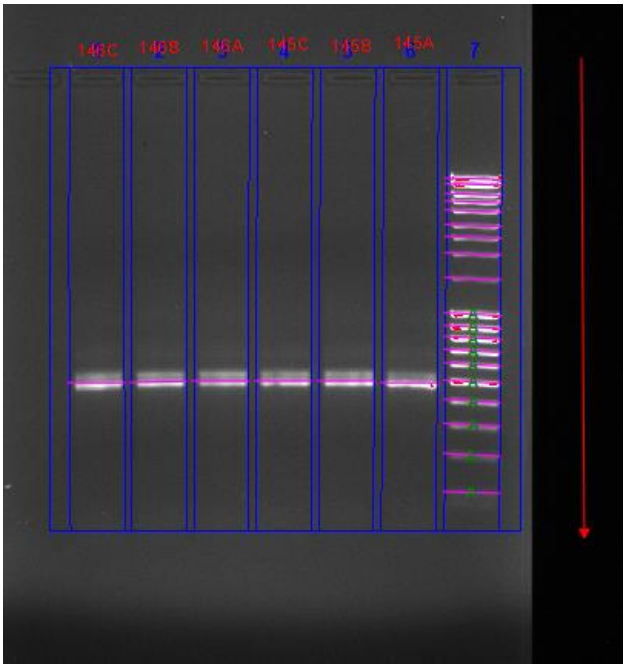

Figure 17. Gel of the first biofilm sampling; flumes five and six.

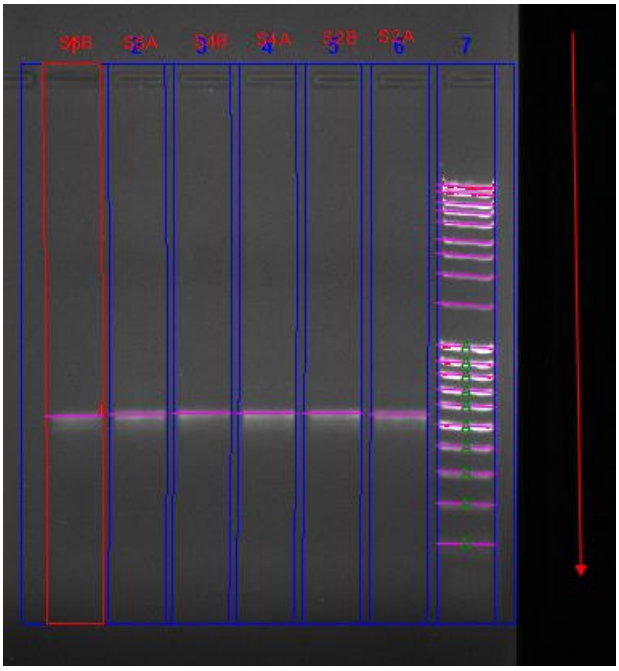

Figure 18. Gel of the first soil sampling.

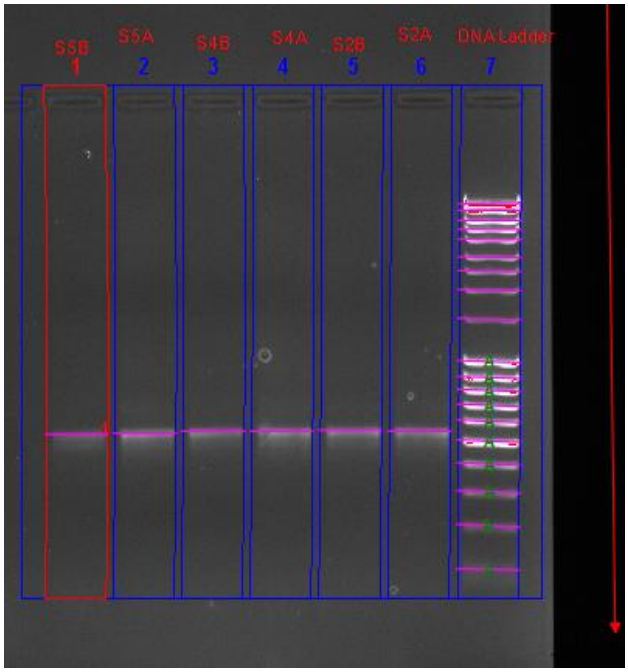

Figure 19. Gel of the second soil sampling.

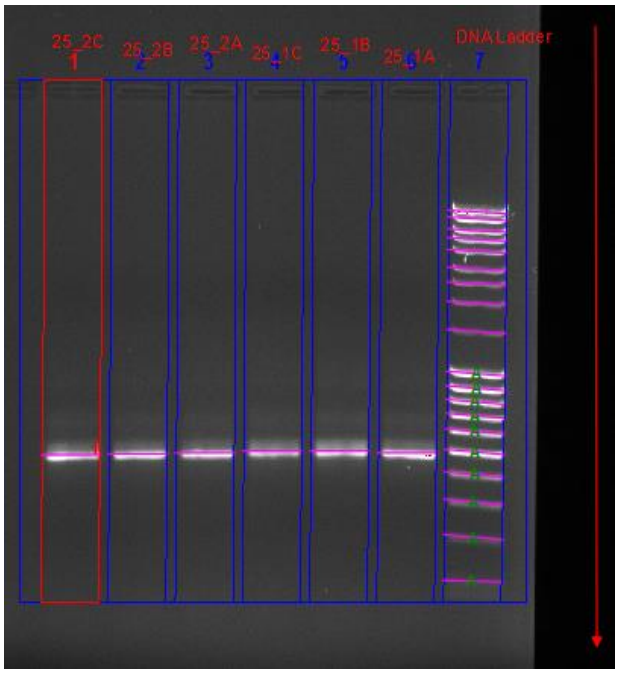

Figure 20. Gel of the second biofilm sampling; flume one and two.

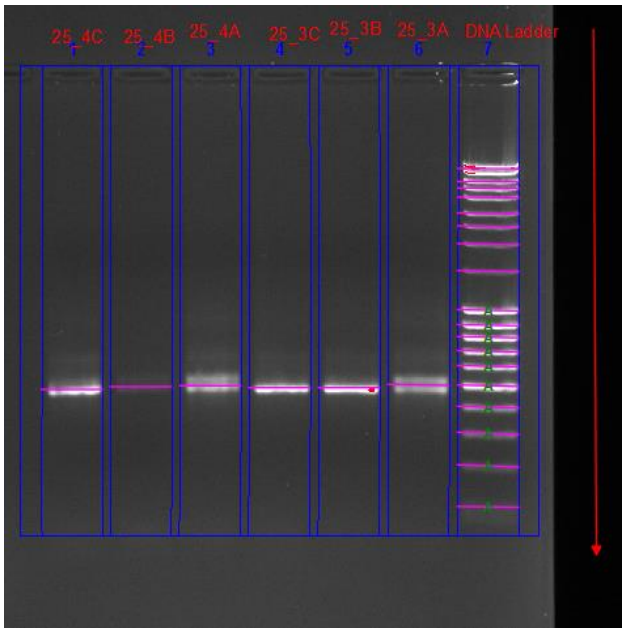

Figure 21. Gel of the second biofilm sampling; flume three and four.

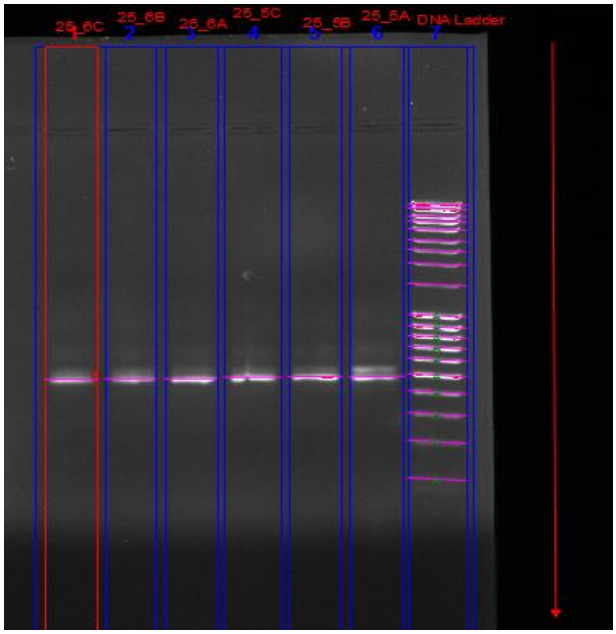

Figure 22. Gel of the second biofilm sampling; flume five and six.