universität
wien

DIPLOMARBEIT

Titel der Diplomarbeit

„Assessing dissolved organic carbon properties and dynamics in a microcosm experiment simulating the hyporheic zone"

Verfasserin

Judith Andrea Rosentreter

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2013

TABLE OF CONTENTS

Abstract

Terrestrially derived organic carbon entering fluvial networks is stored, transformed and respired to carbon dioxide by microbial metabolism, occurring in particular in benthic and hyporheic sediments. In the hyporheic zone, where ground water mixes with surface water, high microbial abundance and metabolism of biofilm communities may influence carbon fluxes to higher trophic levels. In this microcosm experiment, bioreactors with established heterotrophic biofilms simulated microbial activity of the hyporheic zone and additions of bioavailable dissolved organic carbon (recalcitrant and/or labile DOC) were used to assess DOC properties and dynamics linked to biofilm metabolism. Absorption and fluorescence spectroscopy provided insights into chemical composition of the carbon pool. The bulk DOC was dominated by ~80 % humic-like components and ~20 % protein-like components and six different fluorescence components were identified by parallel factor analysis (PARAFAC) modelling. BDOC was not related to the abundance of any of the fluorescence components, inversely related to the aromaticity ($SUVA_{254}$) and further not related to oxygen consumption. I infer that bioavailable carbon was incorporated into microbial biomass and extracellular polymeric substances (EPS) of the biofilm matrix. The main finding of the thesis highlight the different dynamics of protein- and humic-like DOC utilization and production in the biofilms. The functional complexity of hyporheic sediments due to biofilm processes emphasizes the ecological relevance of this transitional zone and their potential impact on the global carbon balance.

Introduction

Global carbon budget

Human-induced climate change associated with increasing greenhouse gases makes it essential to understand and assess the hydrological cycle and the functions of freshwater ecosystems with regard to the global carbon budget. Previously, fluvial ecosystems were considered to have mainly transport functions of organic and inorganic material and to discharge the material unchanged from the continent to the ocean. Recent studies showed that large amounts of carbon are stored and respired by fluvial ecosystems (Cole et al. 2007). For example Cole et al. (2007) showed that only 0.9 Pg C y^{-1} of the 1.9 Pg C y^{-1} terrestrially derived carbon entering fluvial systems is transported to the ocean. Another study found that only 0.4 Pg C y^{-1} globally is discharged via rivers and streams (Kim et al. 2010). However, approximately ~2 Pg C y^{-1} of the terrestrial organic carbon (OC) is stored and transformed in fluvial networks (Battin et al. 2008) and ~0.75 Pg C y^{-1} is respired in the form of CO_2 to the atmosphere (Cole et al. 2007). Since microbial processes transform and oxidize organic carbon (OC), heterotrophic microbial communities are of particular importance for global carbon fluxes on a large scale as they control CO_2 outgassing and net heterotrophy in fluvial networks (Battin et al. 2008).

Today it is of particular importance to assess and predict global carbon fluxes, because increasingly human impacts such as nutrient runoff from agriculture to surface waters have a large potential not only to alter dissolved organic carbon (DOC) composition and amount in surface waters (Ogle et al. 2005) but also to affect metabolism and health in these ecosystems (Young et al. 2008) and therefore may have strong consequences for both humans and nature. Preserving freshwater ecosystems and water quality is another matter of deep concern to many aquatic scientists and increasingly also to the wider public. Because of the growing human population and associated increasing water use, water availability and water quality will be of growing importance for humankind in the future.

Dissolved organic carbon constitutes a substantial part of the organic carbon pool in fluvial ecosystems (Wetzel 1992) but the huge number of organic compounds and many possible pathways of degradation make it difficult to characterise the large carbon pool. DOC can be operationally divided into two classes - humic and non-humic compounds. Humic compounds are polymers of high molecular weight such as humic acids, fulvic acids or humins, and are the major contribution of the DOC pool in stream water ecosystems (Thurman 1985). Humified substances cannot be assimilated directly by microorganisms, hence heterotrophic microorganisms need to hydrolyse the large macromolecules into smaller monomers before uptake (Confer & Logan 1998). The non-humic compounds present in DOC are more often of low molecular weight and consist of free amino acids, lipids, sugars, urea, but also proteins and other carbohydrates, deriving from enzymatic degradation of macromolecules, as exudates by photoautotrophic organisms or other processes, such as sloppy feeding or viral lysis (Findlay & Sinsabaugh 2003). In contrast to complex humified compounds, monomer substances are supposed to be highly biodegradable but have relatively low concentrations in stream water ecosystems. However, some studies have found that degradation of humic substances induced by stream microorganisms is responsible for alteration of the terrestrially derived DOC pool (e.g. Kim et al. 2006); but there are still large gaps in understanding how DOC supply, biodegradability and microbial metabolism are linked together in fluvial networks and how interactions influence carbon fluxes on a global scale.

Ecological Concepts

Rivers and streams are lateral habitats and some general approaches are useful to describe the dynamics of organic material fluxes in these fluvial ecosystems.

First, production and degradation processes in river and stream waters depend on organic material inputs originating either from outside the system (allochthonous) or from inside the system via autotrophic organisms (autochthonous) (Dodds & Whiles 2010). The relative contribution of

autochthonous primary production and external sources of organic carbon are crucial, as the ratio may influence metabolic processes and energy fluxes.

Second, the organic material in a stream is moving unidirectionally, but it is also cycling through the water column, where it is removed, incorporated into biota and released back to the water (Elwood et al. 1983). In terms of the concept of material spiralling, a spiral is explained as the average distance of a nutrient molecule that travels downstream (Newbold et al. 1981) and the nutrient turnover time in the water column can be described as the spiralling length.

Depending on the balance between external OC inputs and primary production processes within the system, which affects the ratio of production and respiration processes (P:R) (Dodds & Whiles 2010), the net ecosystem production may vary greatly from headwater streams to larger rivers. The river continuum concept (RCC; Vannote et al. 1980) views flowing waters as a continuum from small forested headwater streams to large rivers with different P:R ratios, specific abiotic and riparian characteristics, and predictions with regard to the biological community (Dodds & Whiles 2010). The concept describes fluvial ecosystems as continuous gradients, where heterotrophic activity dominates in small forested headwater streams (P:R << 1), autotrophic activity prevails with increasing stream width (P:R > 1) and heterotrophic activity dominates again in large rivers (P:R << 1). In a more recent approach Battin et al. (2008) suggests fluvial networks as meta-ecosystems, where each ecosystem (headwaters, streams, estuaries) functions as a sink or source of material spirals with different net heterotrophy impacts (river = 0.12 Pg C y^{-1}, stream = 0.07 Pg C y^{-1}, estuary = 0.13 Pg C y^{-1}). The connected energy and material flow between the individual ecosystems implies a continuum, where downstream ecosystem processes depend on upstream metabolic performance (Battin et al. 2008).

These selected concepts outline the importance of understanding how OC and nutrients travel downstream in rivers and streams and demonstrate the imperative to determine the quality and quantity of the DOC pool, estimate uptake rates and metabolic processes, and assess the dynamics within fluvial ecosystems and their impacts on adjacent systems.

Microbial Biofilms

Microbial attachment on surfaces such as biofilms on streambeds or sediments enhance metabolic capacity (Battin et al. 2008) and are therefore of special interest in terms of net ecosystem productivity in rivers and streams.

It is well established that microorganisms accumulate as mats, films or flocs at solid-liquid interfaces (Flemming & Wingender 2010, Hall-Stoodley et al. 2004) and are distinct from microorganisms growing in the planktonic state in the water column (Hall-Stoodley et al. 2004). Multispecies biofilms produce extracellular polymeric substances (EPS) that are responsible for adhesion at the surface and form the three-dimensional architectural structure of the biofilm (Flemming & Wingender 2010). Polysaccharides are the major fraction of EPS (Frølund et al. 1996) but the matrix also consists of other chemical groups such as proteins, lipids, nucleic acids and even larger biopolymers such as humic substances (Flemming & Wingender 2010). Further functions of the EPS matrix are suggested, for instance protection against antibiotics, ultraviolet radiation or oxidization (Flemming & Wingender 2010). The matrix also allows close interactions between the cells within the biofilm, and can serve as a nutrient source during starvation (Flemming & Wingender 2010). However, the composition and structure of the EPS matrix can vary greatly between biofilms (Flemming & Wingender 2010).

In an ecological context much heterotrophic processing of terrestrially derived carbon occurs in stream biofilms (Lane et al. 2012). Biofilms located on the solid-liquid interface in benthic and hyporheic zones may have a considerable influence to the rate of carbon and inorganic nutrient cycling (Stewart & Franklin 2008).

The hyporheic zone

The hyporheic zone is a dynamic transition zone, influenced by both surface water and groundwater and characterised by bi-directional water exchange (Triska et al. 1993). This interface, where reduced ground water mixes with surface water, may imply an effective exchange of nutrients and food resources and provides a habitat for not only aerobic and anaerobic microorganisms but

also for complex macrozoobenthos communities. Furthermore the hyporheic zone is used as a refuge during storm events for many riverine invertebrates (Bretschko 1981).

The hyporheic zone is a transitional ecotone and can vary over time and space (Dodds & Whiles 2010). Extensive interactions between groundwater and surface water make the hyporheic zone an area of biological and biogeochemical importance. Inorganic and organic material flux quality and quantity, oxygen concentrations, temperature mixture, light and hydrological conditions (Dodds & Whiles 2010) are responsible for gradients of specialised communities.

The supply of DOC from both ground water and surface water provides a potential but highly variable carbon and energy source for hyporheic microbes (Sobczak & Findlay 2002). However, the bioavailability of the carbon pool determines how much DOC is removed by microbial metabolism. Carbon limitation and DOC quality are often supposed to be the controlling factors for microbial processes (Kaplan & Newbold 2000) and a study in 2002, Sobczak & Findlay showed high variations of microbial metabolism among different hyporheic zones in New York State, USA.

Heterotrophic microorganisms utilize DOC, convert it into particulate fractions and make it available for higher trophic levels (Meyer 1994), thus the microbial loop directs material and energy flux back to the food web.

Depending on (e.g.) bedrock geology and topography, the storage and exchange of the hyporheic zone can varies greatly among streams, but in almost all streams microbial processes are an important control on downstream carbon fluxes (Sobczak & Findlay 2002). Sobczak & Findlay (2002) found pathways in hyporheic sediments could remove ~50% of the bulk DOC and left a recalcitran fraction to further metabolism, thus describing the hyporheic flowpath as a filter that alters DOC composition to downstream communities.

The Priming Effect, a phenomenon first described in soil science, posits microbially induced changes in mineralization rates of recalcitrant organic material in the presence of labile organic matter, such as glucose (Guenet et al. 2010). Since the composition of DOC supply influences its bioavailability and turnover rates in fluvial networks, links between recalcitrant and labile organic matter should be considered more in carbon balance studies.

This diploma thesis was undertaken in the framework of a larger project, which aims to address the mechanisms of the priming effect in aquatic ecosystems with the overall hypothesis that stream biofilms are hot spots for priming.

To estimate the occurrence of the priming effect mediated by heterotrophic stream biofilms, a microcosm experiment was performed in the laboratory and consisted of bioreactors with growing heterotrophic biofilms, fed with stream water and recalcitrant or/and labile organic matter (e.g. glucose, algal extract). This was to assess whether heterotrophic bacterial degradation of recalcitrant DOC is primed by the presence of labile carbon sources.

The aim of this diploma thesis is to understand how microbial heterotrophic communities may change the chemical composition of DOC en route through streambeds. The main questions are 1) how is organic matter transformed by heterotrophic biofilms during advective flow, 2) does the quality of DOC affect microbial activity in hyporheic sediments, and if so 3) how do different fractions of DOC influence microbial activity?

First, UV absorption and fluorescence spectroscopy were used to describe the quality and quantity of DOC (Scully et al. 2004) during the microcosm experiment. Combining fluorescence excitation-emission matrix (EEM) spectroscopy with parallel factor analysis (PARAFAC) allows characterization of DOC in different fractions that serve as proxies for DOC source and relative lability and are referred to as (e.g.) humic-like or protein-like fractions (Stedmon et al. 2003). Subsequently, spectroscopic data was combined with oxygen consumption and biodegraded dissolved organic carbon (BDOC) to assess metabolic processes in heterotrophic biofilms and predict their effects on higher trophic levels.

This thesis addresses the hypotheses that 1) the protein-like pool represents the more biodegradable fraction of the DOC, while 2) the humic-like fraction is more recalcitrant and less respired by microorganisms. Overall, this thesis aims to characterize and trace DOC into its different compositional fractions, which provide more knowledge of the complex mixture of DOC.

Material and Methods

For the microcosm experiment bioreactors with growing heterotrophic biofilms were used to simulate the hyporheic zone. The biofilms were grown from stream microbial communities in flumes and fed directly with raw stream water of the Oberer Seebach (OSB), Austria. All water that was used for the experiment was taken from the OSB.

Figure 1Flumes where heterotrophic biofilms were grown and fed with stream water from the Oberer Seebach, Austria

Oberer Seebach, Austria

Stream water used for the experiment was taken from the Oberer Seebach (OSB), which is located in Upper Austria, in the karstic pre-Alps and has a catchment area up to 20 km² at an altitude ranging from 600 to 1000 m above sea level. The climate is temperate with an annual precipitation average of 1608 mm, annual air temperature ranging from -25° to 33°C and stream water annual temperature average of 12.4°C. DOC is mainly terrestrial in origin and the concentration ranges from 0.73 to 3.5 mg L^{-1} and 0.88 to 5.7 mg L^{-1} in OSB and the hyporheic sediment, respectively (EcoCatchLunz, report 2006-2009). In the hyporheic sediment the grain size is characterised by coarse sand and gravel and it is calcareous with calcite and dolomite moieties (Battin et al. 1999).

Biofilms

Biofilms were directly grown on sintered glass beads, gathered in mesh bags and put in microcosm flumes for 26 days in Oct./Nov. 2011. The flumes were fed with raw stream water from the OSB (figure 1) and covered with black plastic sheeting to avoid growth of phototrophs on the glass beads. Once in the laboratory, the beads were partitioned into bioreactors and fed again with stream water for another three weeks to acclimatise the biofilms.

Allochthonous DOC

Willow cuttings (*Salix fragilis*) were received from the botanical garden in Vienna and used as source of recalcitrant allochthonous DOC (RDO^{12}C). One-third of the willow cuttings used in the course of the experiment were grown in a controlled ^{13}C-enriched atmosphere (at 100% $^{13}CO_2$, Isolife, Netherlands). Both ^{12}C and ^{13}C willow material was dried, ground and macerated in hot water overnight at 60°C. Subsequently the ^{13}C-enriched organic material was inoculated with native biofilm communities, cycled through bioreactors and fed with raw stream water from the OSB for two weeks. The remaining degraded willow extract was assumed to be ^{13}C labelled recalcitrant dissolved organic carbon (RDO^{13}C). Both RDO^{12}C and RDO^{13}C willow extracts were 0.45 μm L^{-1} filtered (Whatman GF/F) and stored at 4°C.

Autochthonous DOC

Three different treatments were used to simulate labile autochthonous DOC sources (LDOC). The bioreactors were amended either with glucose (GLUC), with glucose plus nitrate (NaNO$_3$) and phosphate (KH$_2$PO$_4$) (GLUCNP), with algal extract (ALG), or not at all (CON). The algal extract was obtained from a culture of the green algae *Monoraphidium contortum*. The algal cells were concentrated by centrifugation, washed twice in stream water and sonicated to disrupt the cell walls. The extract was sterile filtered and stored at 4°C before use. The added NaNO$_3$ and KH$_2$PO$_4$ to the GLUCNP treatment matched the levels of total dissolved nitrate and phosphate present in the algal extract.

Table 1 input medium mixtures

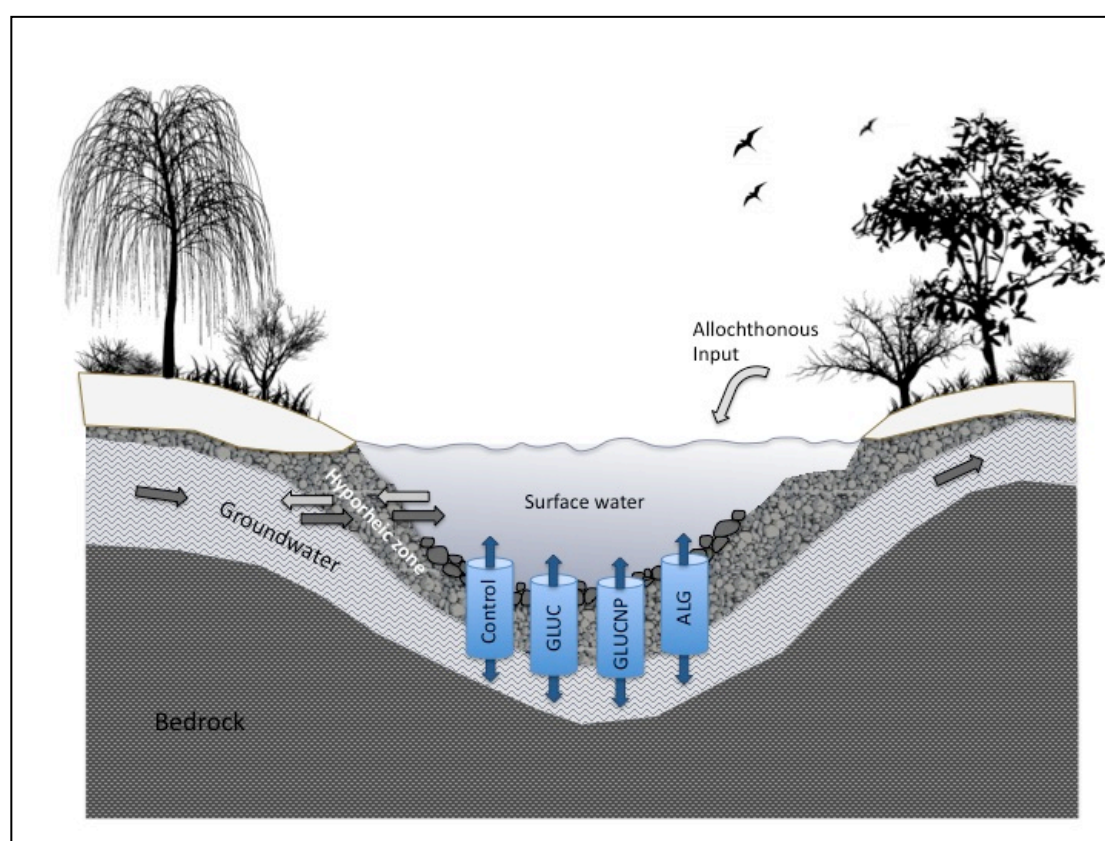|	RDOC [mg L^{-1}]	LDOC [mg L^{-1}]	N_{total} [µg L^{-1}]	PO_4 [µg L^{-1}]
GLUC	0.8	0.4	16.7	13.1
GLUCNP	0.8	0.4	2203.9	19.9
ALG	0.8	0.4	2203.9	19.9
Control	0.8	0	16.7	13.1

Figure 2 Diagram showing the hyporheic zone and bioreactor treatments used in this laboratory microcosm experiment. Bioreactors with grown heterotrophic biofilms simulate the hyporheic zone where ground water mixes with surface water. RDOC derives via degradation processes either from allochthonous input or ground water. LDOC derives via autochthonous processes

Experimental design

The microcosm experiment was carried out in Nov./Dec. 2011 and the setup included 20 plug-flow bioreactors (five replicates for each treatment), 4L bottles for treatment solutions and tubing connections (figure 3). The bioreactors were continuously fed with 0.45 µm L^{-1} filtered (Whatman GF/F) stream water from the OSB. The four experimental phases included (1) ten days when the bioreactors were fed with pure stream water, (2) two days stream water with

amended $RDO^{12}C$, (3) six days stream water amended with $RDO^{13}C$ and LDOC treatments (GLUC, GLUCNP, ALG and CON) and finally (4) six days only $RDO^{13}C$. A peristaltic pump was used to maintain a flow rate of 1.2 ml min^{-1}. The 4 L media bottles were sterilised every two days. During the experimental duration of three weeks, samples for nutrients, DOC and optical analyses were taken with syringes from the input and output tubing (figure 3). DO was measured directly from the input and output tubing using flow-through sensor cells, at least once per day. In the course of my diploma thesis I will focus only at the six days when $RDO^{13}C$ and LDOC were added because the main target is to find out how the different quality and quantity of variable DOC fractions may alter the DOC composition and influence the microbial activity in hyporheic sediments.
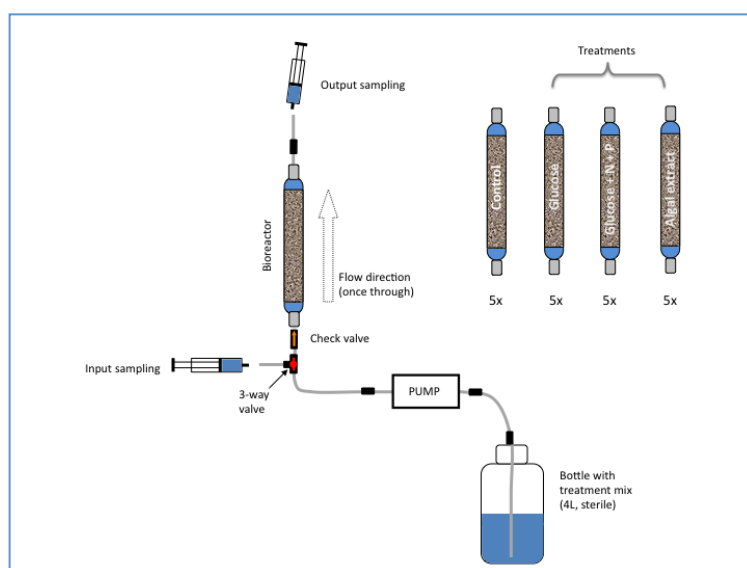

Figure 3 Experimental setup of the microcosm bioreactors in the laboratory. Filtered OSB stream water was pumped once trough and samples were taken before and after passing the bioreactors

Laboratory analyses

Samples were regularly taken and filtered to 0.45 µm (Whatman GF/F) for DOC, oxygen, ammonium, nitrate, nitrite and phosphate measurements. DOC concentration was analysed using persulphate oxidation on a Sievers 5310c laboratory TOC analyser (GE Analytical Instruments). Dissolved oxygen (DO) concentrations were measured with a flow-through cell O_2 mini-sensor (PreSens, Precision Sensing GmbH) and nitrate, nitrite, ammonium and phosphate

concentrations were determined with a continuous flow nutrient analyser (Alliance Instruments). Ammonium was under the detection limit (< 4 ug L^{-1}). UV-vis absorption spectra were measured over a 10 cm path length with a UV-vis Spectrophotometer (UV-1700 PharamaSpec; Shimadzu) between 200 and 700 nm (0.5 nm intervals) and the fluorescence spectra were determined with a Fluorescence Spectrophotometer (F-7000; Hitachi). The fluorescence intensity was recorded at emission wavelengths 250 nm to 600 nm (2 nm intervals) and excitation wavelengths 200 to 450 nm (5 nm intervals), with a scan speed of 12,000 nm s^{-1}.

PARAFAC modelling

To assess the composition of DOM independent fluorophores, the EEMs were corrected for Raman peaks and performed with a PARAFAC model. The PARAFAC analysis was carried out in MATLAB software (R2010b, MathWorks) using the "N-way toolbox for MATLAB". For PARAFAC modelling, EEMs with emission wavelength from 250 nm to 550 nm and excitation wavelength from 240 nm to 450 nm were used. Potential sources and functions of the components were assigned by comparing them to components reported in the literature.

Spectroscopic parameters

From absorption spectra two indices were calculated: (1) the specific UV absorption at emission wavelength 254 nm normalized to the DOC concentration (SUVA$_{254}$, mgC L^{-1} m^{-1}), which is strongly correlated with aromaticity (Weishaar et al. 2003); and (2) the slope ratio of the emission wavelength S$_{275-295}$ to S$_{350-400}$ (SR$_{Helms}$), which is related to molecular weight (HMW > 1000 Da; LMW < 1000 Da; Helms et al. 2008).

From fluorescence spectra the fluorescence index (FI) was calculated using the emission wavelength from 450 nm to 500 nm, obtained with an excitation wavelength of 370 nm (McKnight et al. 2001). McKnight et al. (2001) found that FI values ~1.4 suggest terrestrially derived dissolved organic matter (DOM) and values ~1.9 suggest microbially derived DOM. Hence, the FI is a proxy for the source of DOM.

15

Data analysis and statistics

Spectroscopic parameters and stream chemistry were calculated as effects:

$$Effect = (Input\text{-}Output)/\ Input \qquad (1)$$

Dissolved inorganic nitrogen (DIN) was calculated as:

$$DIN = NH_4^+ + NO_3^- + NO_2^- \qquad (2)$$

Oxygen consumption was calculated as:

$$O_2\ consumption\ [mg\ L^{-1}] = O_{2(Input)} - O_{2(Output)} \qquad (3)$$

Analysis of Variance (ANOVA) and Tukey (HSD) pairwise multiple comparison post-hoc tests were used to test the significances between the treatment bioreactors and the control bioreactors concerning the spectroscopic indices or the stream chemistry. Variables in the multiple linear regression models were standardised and selected based on backward selection, adjusted coefficient of multivariate regressions ($_{adj}R^2$), the Akaike Information Criterion (AIC), and the significance of the variables ($p < 0.5$).

All statistical analyses were performed with the free software ©RStudio (Version 0.97.314; R Development Core Team; 2012) and all graphical presentations were made in ©Sigmaplot (Version 10.0; Systat Software, San Jose, CA; 2006) or ©RStudio (Version 0.97.314; R Development Core Team; 2012).

Results

DOC and nutrient dynamics

DOC concentration ranged from 1.3 to 2.1 mg L^{-1} during the whole experiment and was always higher before passing through the bioreactors. To assess the biodegradable fraction of DOC (BDOC), the DOC input minus DOC output was calculated and this ranged from 0.25 to 0.65 mg L^{-1} in all bioreactors. Total oxygen consumption, measured as the removal of dissolved oxygen with one pass through the bioreactors, amounted to 0.42 ± 0.18 mg L^{-1} in GLUC, 0.41 ± 0.20 mg L^{-1} in GLUCNP, 0.43 ± 0.17 mg L^{-1} in ALG, and 0.41 ± 0.19 mg L^{-1} in CON, and hence did not differ between the bioreactors with treatments and the control. $SUVA_{254}$ serves as a proxy for aromaticity (Weishaar et al. 2003), and generally ranged from 4.1-6.5 mg C L^{-1} m^{-1} in all bioreactors and was higher in the output (5.8 ± 0.6 mg C L^{-1} m^{-1}) than the input (4.5 ± 0.18 mg C L^{-1} m^{-1}), which implies higher concentrations of aromatic compounds after passing through the bioreactors. SR_{Helms} values ranged from 0.93 to 1.21 in all three treatments and the control bioreactors, thus the molecular weight did not vary greatly during the experiment. Nitrate concentrations in both input and output were higher ($\sim$3.1 mg L^{-1}) in the GLUCNP and ALG bioreactors than in the GLUC and control bioreactors ($\sim$1.1 mg L^{-1}), and phosphate concentrations were equal (8.32 ± 0.84 mg L^{-1}) in the input and output in all bioreactors. Hence phosphate and nitrate were likely not the limiting factors of metabolic activitiy in any of the bioreactors. In contrast nitrite ranged from 0.8 to 33.3 µg L^{-1} and was about three times higher in the input than in the output, which suggests nitrification processes during the experiment in all bioreactors (table 2). Furthermore, all treatments had a similar fluorescence index (FI) with values ranging from 1.33 to 1.43 (table 2) and therefore DOC in all bioreactors is supposed to be of primarily terrestrially originated organic material (willow extract).

DOC optical properties

Six different fluorescence components (C1-C6) were identified by the PARAFAC model (figure 4). The modelled EEMs are indicative of different families of

17

fluorescent compounds in the complex mixture of DOC (Stedmon & Markager 2005). Fluorophore names and characteristics, for instance the sources and sinks of the components, and their appearance in the literature, are reported in table 4. Components C1-C4 are associated with humic-like DOC material while C5 and C6 are associated with protein-like material.

C1, C4, C5 and C6 are components with fluorescence peaks well known in the literature. C1 is referred to as peak "A", a humic-like component with a probable sink by photodegradation (Stedmon & Markager 2005; Osburn et al. 2011). C4 is similar to the fluorescence peak "C" (Coble 1996; Stedmon & Markager 2005), a humic-like component, which is described as representing photolabile material (Osburn et al. 2011) and both C1 and C4 are likely derived from microbial degradation (Stedmon & Markager 2005). C5 has been characterized as the protein-like fluorescence component peak "T" (Coble 1996) and is thought to represent tyrosine-like fluorescence with the origin from autochthonous material, for instance during algal blooms (Osburn et al. 2011). C6 is also assumed to derive from autochthonous material but displays more tryptophan-like fluorescence (Coble 1996).

C2 was documented e.g. in Cory and McKnight (2005) and described there as an aromatic-like fraction of DOC, more precisely oxidized quinone derivates. Stedmon & Markager (2005) identified a fluorescence peak, which resembles C3. They described it as a humic-like fluorescence component, which is rapidly removed and therefore has rarely been found in environmental samples in the past.

In all bioreactors the maximum fluorescence values (F_{max}, nm) of the components C2-C6 were almost equal in the input, however, Fmax of C1 in the input were twice as high (0.20 ± 0.02) as all other components, which ranged from 0.05 ± 0.005 to 0.9 ± 0.05 (table 3).

In the output, the protein-like components C5 and C6 were slightly decreasing in the bioreactors while the humic-like components (C1, C2 and C4) did not differ remarkably between the input and the output.

C3 maximum fluorescence was particularly high in the output (0.20 ± 0.18) and therefore had the highest effect during the experiment. C3 Fmax in the input was

highest in the ALG (0.11 ± 0.05), followed by GLUCNP (0.10 ± 0.05), GLUC (0.09 ± 0.03) and CON (0.05 ± 0.03). The same pattern was seen in the output; C3 Fmax was highest in ALG (0.26 ± 0.22), followed by GLUCNP (0.22 ± 0.18), GLUC (0.18 ± 0.16) and CON (0.11 ± 0.10).

Table 5 shows the percentage of C1-C6 found for each treatment and the control in the bioreactors. Although the variation between the treatments and/or the control is not very high, it is obvious that C3 (∼34 %) was the most dynamic component in the bioreactors during the experiment. All other components ranged from 7.3 % to 16.7%.

Temporal dynamics

The experiment was conducted for about three weeks (23 days). In this thesis the focus is on the time period from hour 60.75 (sampling point 3) to hour 181 (sampling point 8), which was about 5 days (120.25 hours) when RDOC and LDOC treatments were added simultaneously.

In this context, dynamics refers to changes in either the magnitude or direction of DOC and nutrient fluxes over time. The temporal dynamics of the variables are considered with regard to their effects (see formula 1), this means that a positive effect reveals a reduction while a negative effect reveals a production or increase. The temporal dynamic effects of BDOC, SR_{Helms}, $SUVA_{254}$ are shown in figure 5-7 and the fluorescence components C1-C6 are shown in figure 9-11. Only the oxygen consumption is calculated as the input O_2-concentration minus the output O_2-concentration and not represented as an effect.

Control

In the control DOC was always biodegraded by more than 20% and increasing up to ∼28% from hours 60 to 70, before decreasing for the rest of the experiment. Further, in control the oxygen consumption generally increased during the ∼120 days with only a small decrease between hour 70 and 80. The molecular weight (SR_{Helms}) effect was around zero with a slight increase during the first 20 hours, a decrease during the next 30 hours and again an increase during the last 50 hours. It should be mentioned that SR_{Helms} effect values above zero mean an

19

increase in molecular weight across the bioreactors from input to output, while values smaller than zero mean a decrease. In terms of the aromaticity effect values ($SUVA_{254}$), the control bioreactors were always above zero with dynamics between -0.2 and -0.4 during the whole time period (e.g. figure 5), thus aromaticity was increasing over time.

In contrast to the protein-like components C5 and C6 and also to C3, the humic-like components C1, C2 and C4 have had no strong effects during the experiment and therefore only low dynamics around the zero line (e.g. figure 9). Stronger effects but not high dynamics were found in C5 and C6. However, the greatest change over time was observed in the fluorescence component C3, where negative effects imply production processes.

Glucose treatments

BDOC did not significantly differ ($p = 0.94$) between the control bioreactors and the glucose bioreactors, but during the last 40 hours there was more DOC bioavailable in the glucose treatments than in the control. Also, oxygen consumption continuously increased in the glucose treatment relative to the control. The effect of molecular weight (SR_{Helms}) was slightly higher in glucose treatments than in the control, meaning a larger average molecular weight in the glucose treatments but not significantly different ($p = 0.11$). $SUVA_{254}$ showed little more variation in the glucose treatments.

In the fluorescence component C1 and C4 there were even less dynamics than in the control. The protein-like components C5 and C6 did not differ remarkably from the control with relatively high variation. C3 in glucose treatments was almost equal to the control (figure 5 and 9).

Glucose plus nutrients treatments

BDOC and oxygen consumption in the GLUCNP treatments were almost equal to the control ($p = 0.90$ and $p = 0.99$, respectively). During the hours 70 to 110 the GLUCNP treatments contained smaller effects with regard to the molecular weight and aromaticity but for both variables only significant differences along

time were found ($p < 0.001$) and no significant difference between the GLUCNP treatments and the control ($p = 0.29$ SR_{Helms}; $p = 0.99$ $SUVA_{254}$).

The humic-like components C1, C2 and C4 in the GLUCNP treatments were varying (relative to control) around zero. A significant difference ($p = 0.02$) was found between the GLUCNP treatments and the control concerning the protein-like component C6 (tyrosine), especially for the first 40 hours. In contrast there was no significant difference ($p = 0.72$) observed for the second protein-like component C5 (tryptophan). Furthermore, a significant difference ($p = 0.01$) was found between the GLUCNP treatments and the control with regard to the humic-like component C3 in the last 40 hours during the experiment (figure 6 and 10).

Algal extract treatments

As in the other two treatments no significant differences were found between the ALG treatments and the control with regard to the biodegradation of DOC or oxygen consumption over time ($p = 0.99$ and $p = 0.95$, respectively). The average molecular weight values in the ALG treatments were slightly higher during the first 20 hours and slightly lower during the remaining 100 hours (figure 7). Aromaticity ($SUVA_{254}$) was increasing but did not differ significantly different from the control ($p = 0.99$).

However, three fluorescence components (C2, C3, C4) were significantly different between the ALG treatments and the control (figure 11). The humic-like component C2 was always higher than the control ($p < 0.001$) and in contrast the humic-like component C4 was always lower than the control ($p < 0.001$). Further, the C3 component was lower within the ALG treatments ($p = 0.002$), particularly during the last 50 hours and the C1 component was remarkably lower between hour 80 and 130 but overall not significantly different from the control ($p = 0.13$). In the protein-like components the dynamics were opposite to the control but again were not significantly different for C5 nor C6 ($p = 0.44$ and $p = 0.93$, respectively).

Correlations between BDOC and DOC optical properties

To assess metabolic processes of heterotrophic biofilms during the microcosm experiment, BDOC and oxygen consumption were correlated with spectroscopic data using least square regressions and multiple linear regressions. C1, C2 and C4 were combined as "humic-like" and C5 and C6 were combined as "protein-like" components. Since the effect of C3 was much higher than the effect of any of the other components, and C3 was also found rarely in literature, it was decided to focus on this fluorescence component separately, although it is thought to be humic-like. The multiple linear regressions of the effects are reported in table 6.

$SUVA_{254}$

The least square regressions between BDOC or oxygen consumption and the aromaticity parameter $SUVA_{254}$ are shown in figure 12.

First, the relationship between BDOC and $SUVA_{254}$ was significant ($p \leq 0.003)$ for each of the three treatments. In the glucose and the algal extract treatments the slopes of the regressions were almost similar to those of the control (*slope = -0.25*). Only in the GLUCNP treatments the slope was higher (*slope = -0.46*) for the BDOC and $SUVA_{254}$ relationship. In the GLUCNP treatments 63 % of the variation could be explained by the regression line, in the glucose treatments 48 %, in the algal extract treatments 24 % and in the control 34 % .

No significant relationship between $SUVA_{254}$ and oxygen consumption was observed and the described regression line did not fit the data set well ($_{adj}R^2$ ranged from -0.03 to 0.08) in the treatment bioreactors and the control.

SR_{Helms}

The relationship between BDOC, oxygen consumption and molecular weight was not significant in any bioreactors (*p > 0.05*) and again the regression line did not describe the data set sufficiently ($_{adj}R^2$ ranged from -0.03 to 0.03) (figure 13).

Component C3

BDOC and oxygen consumption were not significantly correlated to the fluorescence component C3 ($p > 0.05$), neither in the treatment bioreactors nor in the control bioreactors. The adjusted coefficient of determination ($_{adj}R^2$) was almost zero in all bioreactors (figure 17).

Humic-like components

Although the amount of humic-like components (C1, C2 and C4) was not significantly related to BDOC or oxygen consumption ($p > 0.05$), it was conspicuous that in the glucose treatments and control there was much lower variation than in the algal extract treatments. In the GLUCNP treatments the variation was somewhere in between. However, the regression lines could not fit the data very well concerning BDOC and oxygen consumption relationship (see $_{adj}R^2$ in figure 14).

Protein-like components

A significant negative relationship was found between the protein-like components (C5, C6) and BDOC in the glucose treatments ($adjR^2 = -0.12$, $p = 0.03$). Additionally, in the algal extract treatments there was a slight relationship between the summed protein-like components and BDOC ($adjR^2 = -0.08$, $p = 0.07$), but in the GLUCNP treatments no significant relationships were observed ($adjR^2 = -0.04$, $p = 0.83$). The oxygen consumption data were not significantly correlated to the protein-like components ($p > 0.05$, figure 15).

Multiple Linear Regressions

Although there were few correlations between the spectroscopic data and the BDOC or the oxygen consumption, it was of interest to find out what predicts the biodegradation of DOC and which DOC is most likely to be respired. Therefore a multiple linear regression was computed (results are reported in 6). The *AIC* and *p-values* were used to find the best model for each treatment bioreactor and the control.

BDOC

The aromaticity parameter $SUVA_{254}$ was the variable that explained most variation in the multiple linear regression models concerning all treatments and the control, followed by the SR_{Helms} parameter, C3 and the protein-like components. However, C3 and the protein-like components were poor predictors of BDOC. The best-fitted model was found with regard to the GLUCNP treatments (*adjR² = 0.87*, *p < 0.001*) and included $SUVA_{254}$ (*slope = -0.55*), followed by SR_{Helms} (*slope = 0.33*) and C3 (*slope = -0.01*).

Oxygen consumption

The best fitting model was found in the algal extract treatments, but the model explained only 27 % of the variation (*p = 0.008*). The model included the humic-like components (*slope = 2.0*) and a small effect of C3 (*slope = -0.09*). In terms of the glucose treatmentss even the best model was not very good (*adjR² = 0.13*, *p = 0.06*) but included SR_{Helms} and C3. No fitted models could be computed concerning the GLUCNP treatments and the control.

Table 2 Overview DOC and nutrient concentration

	DOC [mg L⁻¹]	SUVA [mgC L⁻¹ m⁻¹]	SR$_{Helms}$	NO$_3^-$ [mg L⁻¹]	NO$_2^-$ [µg L⁻¹]	PO$_4^-$ [µg L⁻¹]	DOC:DIN [mg L⁻¹]
Input							
GLUC	1.91 (0.06)	4.58 (0.22)	1.08 (0.03)	1.02 (0.06)	17.6 (5.9)	76.2 (4.6)	1.86 (0.17)
GLUCNP	1.90 (0.38)	4.58 (0.12)	0.97 (0.02)	3.14 (0.12)	17.0 (6.5)	82.5 (4.7)	0.62 (0.03)
ALG	1.90 (0.03)	4.70 (0.20)	1.01 (0.04)	3.11 (0.10)	10.4 (5.7)	88.5 (5.3)	0.62 (0.02)
Control	1.96 (0.07)	4.60 (0.15)	1.06 (0.03)	1.07 (0.06)	10.3 (5.4)	85.6 (3.6)	1.76 (0.34)
Output							
GLUC	1.43 (0.06)	5.82 (0.30)	1.13 (0.08)	0.99 (0.06)	3.1 (2.4)	77.8 (6.3)	1.39 (0.60)
GLUCNP	1.45 (0.06)	5.81 (0.29)	1.00 (0.06)	3.11 (0.15)	5.1 (1.9)	83.5 (5.8)	0.47 (0.03)
ALG	1.44 (0.06)	5.82 (0.23)	1.03 (0.07)	3.01 (0.16)	6.9 (4.2)	88.8 (4.5)	0.48 (0.03)
Control	1.43 (0.07)	5.83 (0.32)	1.08 (0.06)	1.01 (0.05)	2.1 (1.3)	82.7 (4.0)	1.37 (0.60)

Table 3 Overview fluorescence components

	C1	C2	C3	C4	C5	C6	FI
Input							
GLUC	0.20 (0.01)	0.06 (0.003)	0.09 (0.04)	0.05 (0.002)	0.06 (0.01)	0.07 (0.004)	1.33 (0.03)
GLUCNP	0.19 (0.01)	0.06 (0.003)	0.10 (0.05)	0.05 (0.002)	0.05 (0.01)	0.07 (0.01)	1.35 (0.03)
ALG	0.19 (0.01)	0.06 (0.002)	0.11 (0.05)	0.05 (0.002)	0.06 (0.01)	0.07 (0.01)	1.36 (0.04)
Control	0.20 (0.01)	0.06 (0.002)	0.05 (0.03)	0.05 (0.001)	0.05 (0.01)	0.06 (0.01)	1.33 (0.03)
Output							
GLUC	0.19 (0.01)	0.06 (0.003)	0.18 (0.16)	0.05 (0.002)	0.03 (0.004)	0.03 (0.01)	1.36 (0.04)
GLUCNP	0.19 (0.01)	0.06 (0.004)	0.22 (0.18)	0.05 (0.003)	0.03 (0.01)	0.03 (0.01)	1.39 (0.03)
ALG	0.19 (0.01)	0.06 (0.006)	0.26 (0.22)	0.05 (0.005)	0.03 (0.01)	0.03 (0.01)	1.43 (0.07)
Control	0.19 (0.02)	0.06 (0.002)	0.11 (0.10)	0.05 (0.005)	0.04 (0.01)	0.03 (0.01)	1.34 (0.05)

Table 4 Characterisation of fluorescence components

Component	Fluorophore Name	Emission maximum [nm]	Excitation maximum [nm]	Characterisation
C1	Peak A	418	<245	Humic-like, source: microbial degradation, sink: photodegradation (Stedmon & Markager, 2005), terrestrial origin, photolabile (Osburn et al.2011)
C2	–	504	<250	Humic-like, oxidized quinone-like (Cory & McKnight, 2005)
C3	–	462	<240 (405)	Humic-like, rapid removal, source: microbial degradation, sink: photodegradation (Stedmon & Markager, 2005)
C4	Peak C	438	250 (360)	Humic-like, source: microbial degradation, sink: photodegradation (Stedmon & Markager, 2005), terrestrial origin, refractory (Osburn et al.2011)
C5	Peak T	334	275	Protein-like, tyrosine, source: algal bloom, sink: not identified (Stedmon & Markager, 2005), autochthonous, protein-like (Osburn et al., 2011)
C6	Peak B	306	275	Protein-like, tryptophan in peptides, source: algal bloom, sink: photodegradation (Stedmon & Markager, 2005), autochthonous, protein-like (Osburn et al.,2011)

Table 5 Percentages of fluorescence components

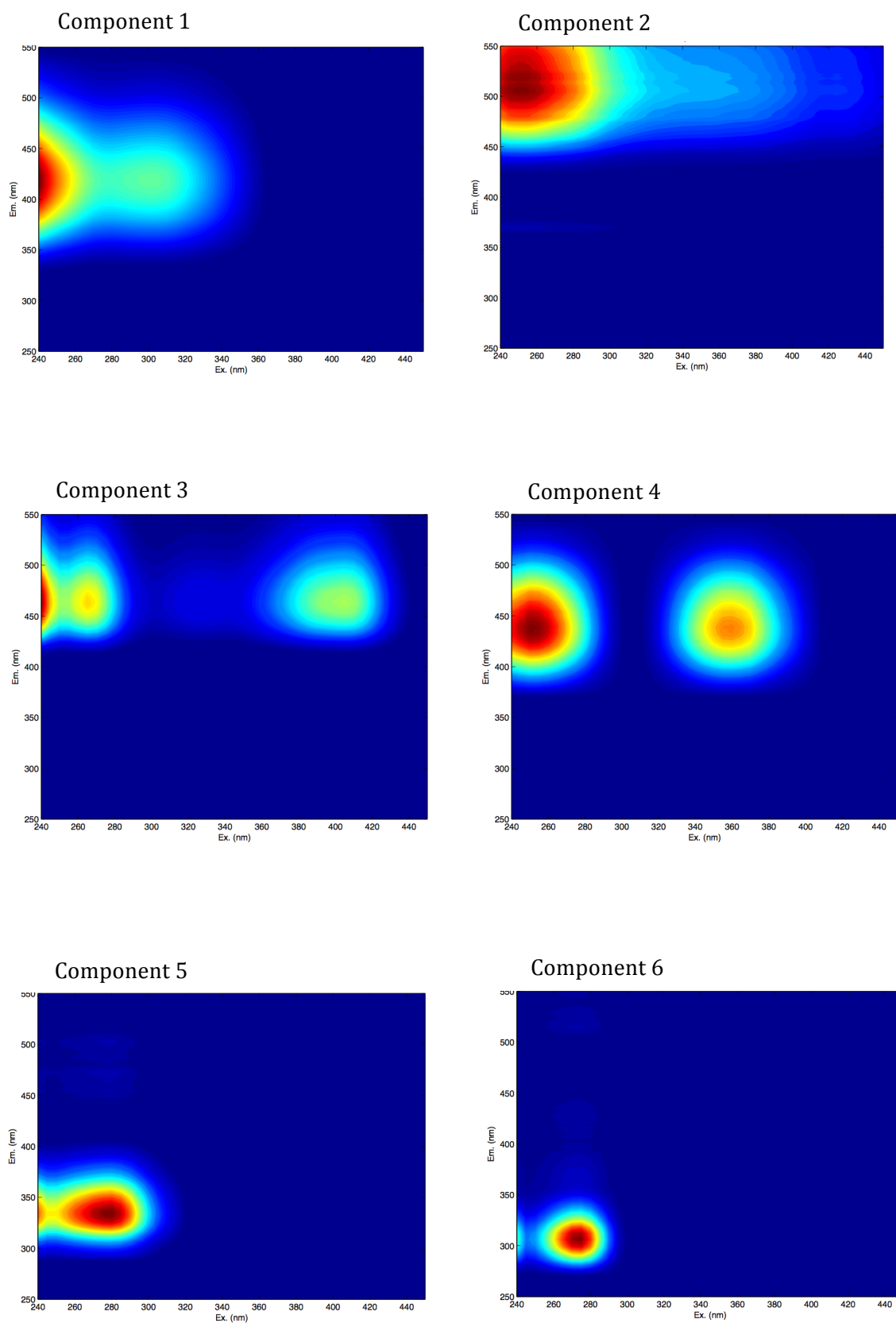|	C1 [%]	C2 [%]	C3 [%]	C4 [%]	C5 [%]	C6 [%]
GLUC	15.7 (0.03)	16.4 (0.04)	34.4 (0.9)	16.3 (0.02)	9.7 (0.09)	7.5 (0.09)
GLUCNP	15.6 (0.04)	16.2 (0.03)	35.3 (0.9)	16.2 (0.04)	9.5 (0.14)	7.3 (0.17)
ALG	15.1 (0.07)	14.7 (0.04)	34.8 (1.1)	16.3 (0.09)	8.9 (0.14)	7.9 (0.18)
Control	16.3 (0.09)	16.7 (0.03)	31.1 (0.8)	15.9 (0.03)	9.9 (0.09)	8.8 (0.13)

Figure 4 Emission-excitation matrices identified by PARAFAC modelling. Component 1-4 are humic-like and component 5 -6 are protein-like
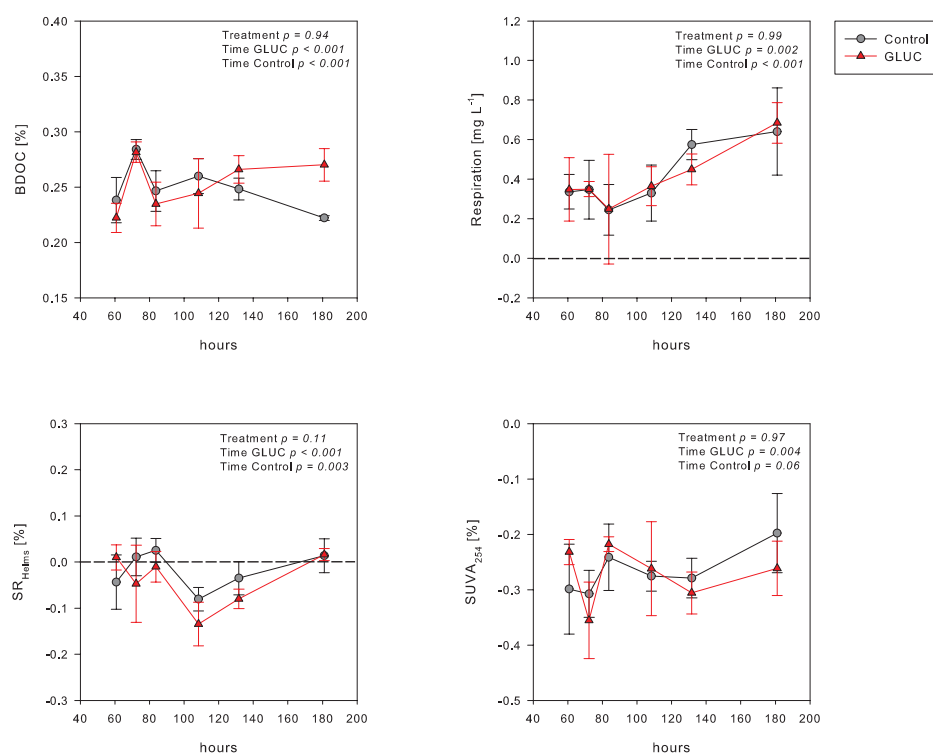
Figure 5 Temporal dynamics of BDOC, oxygen consumption, SR_{Helms} and $SUVA_{254}$ in GLUC and control bioreactors
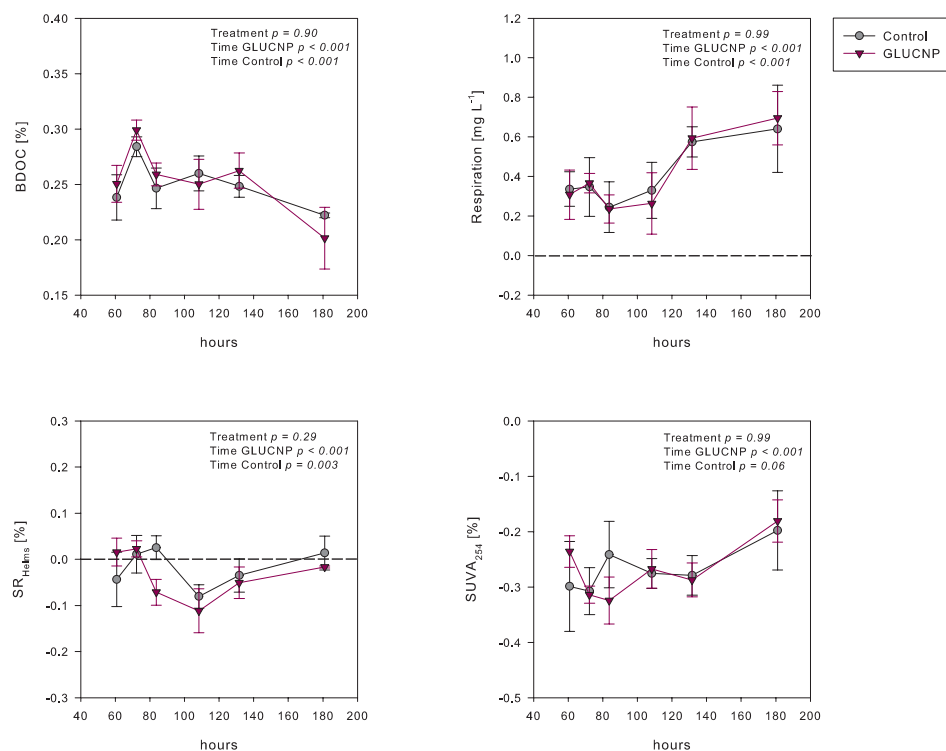

Figure 6 Temporal dynamics of BDOC, oxygen consumption, SR_{Helms} and $SUVA_{254}$ in GLUCNP and control bioreactors
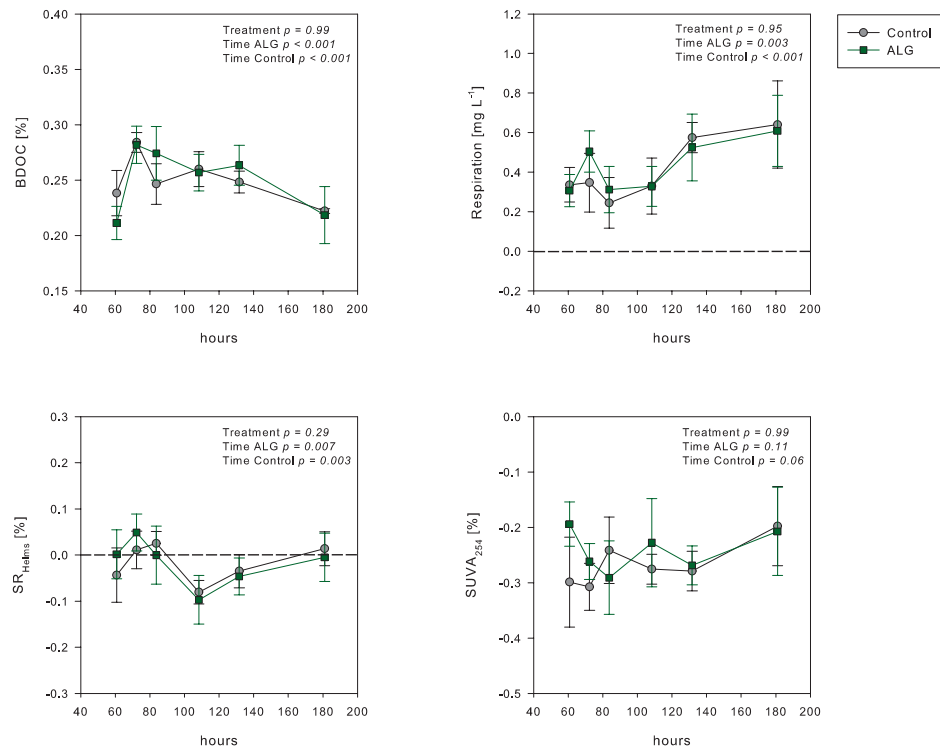
28

Figure 7 Temporal dynamics of BDOC, oxygen consumption, SRHelms and SUVA254 in ALG and control bioreactors
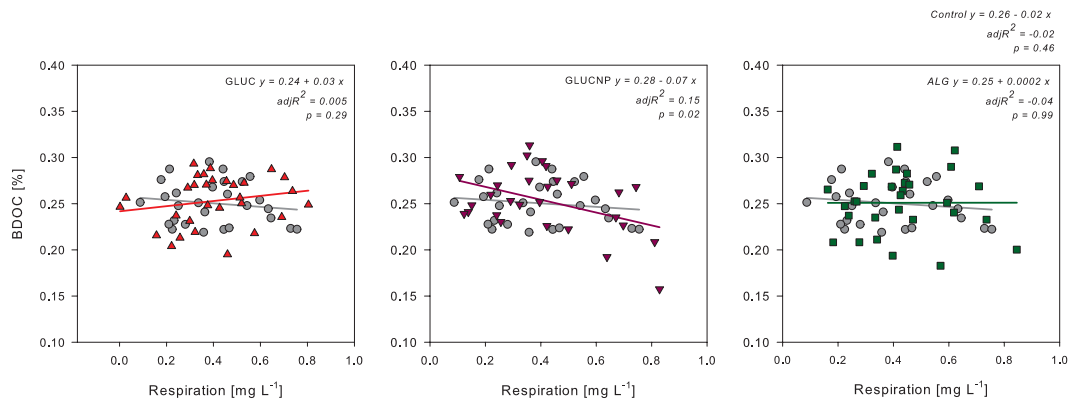

Figure 8 Linear Regression of BDOC and oxygen consumption in GLUC (red), GLUCNP (violet), ALG (green) and control (gray) bioreactors
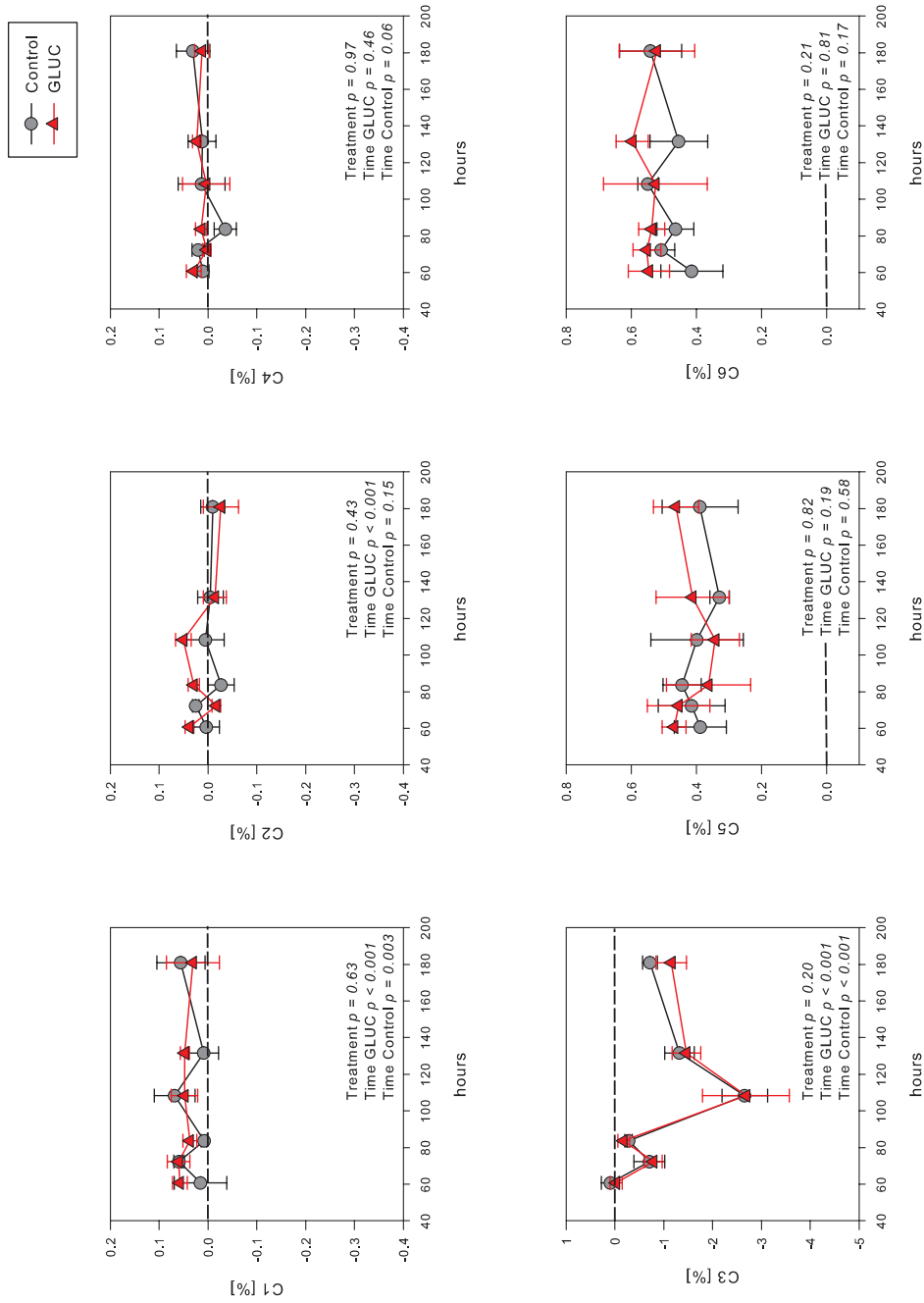
Figure 9 Temporal dynamics of fluorescence components in GLUC and control bioreactors
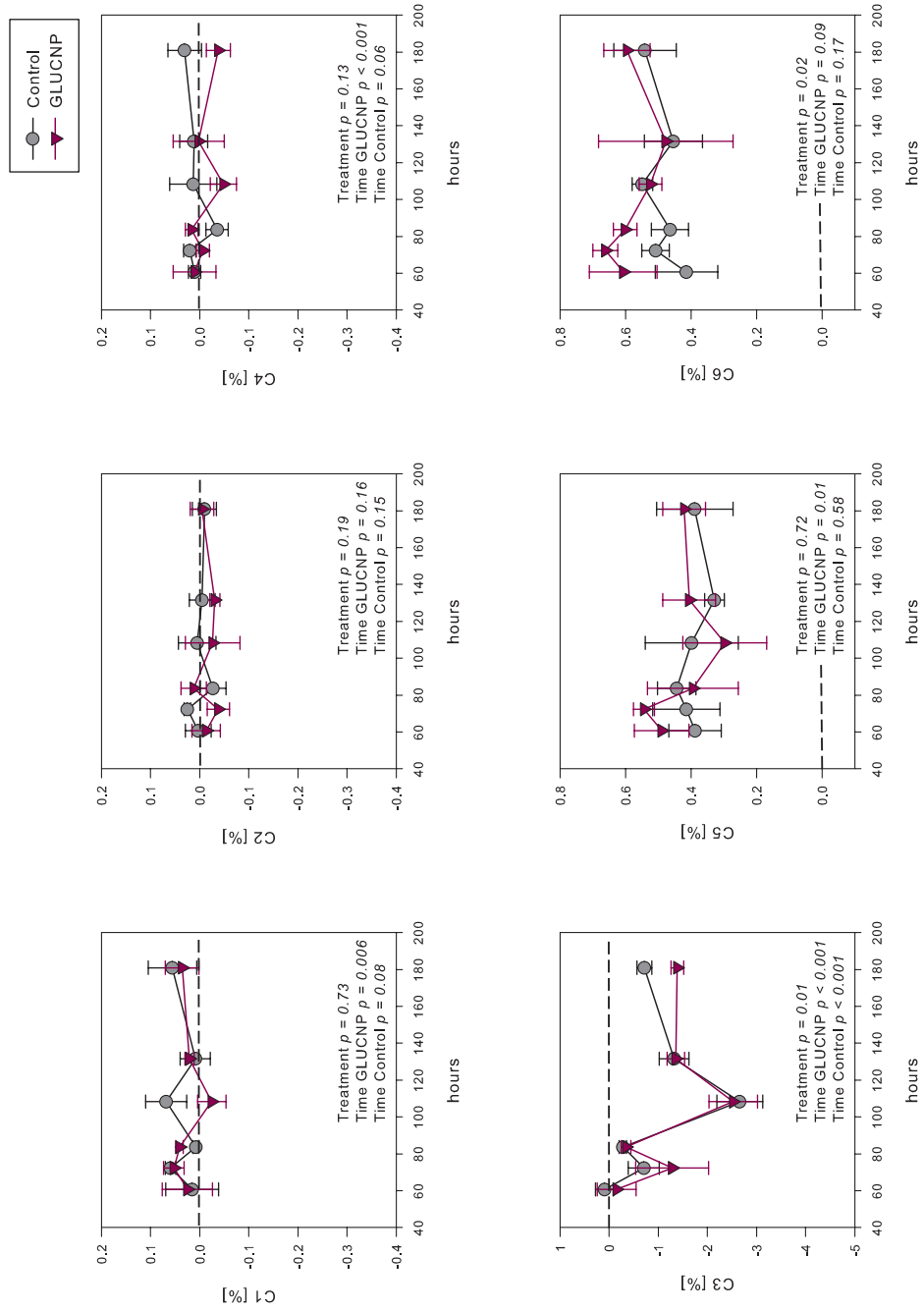
Figure 10 Temporal dynamics of fluorescence components in GLUCNP and control bioreactors
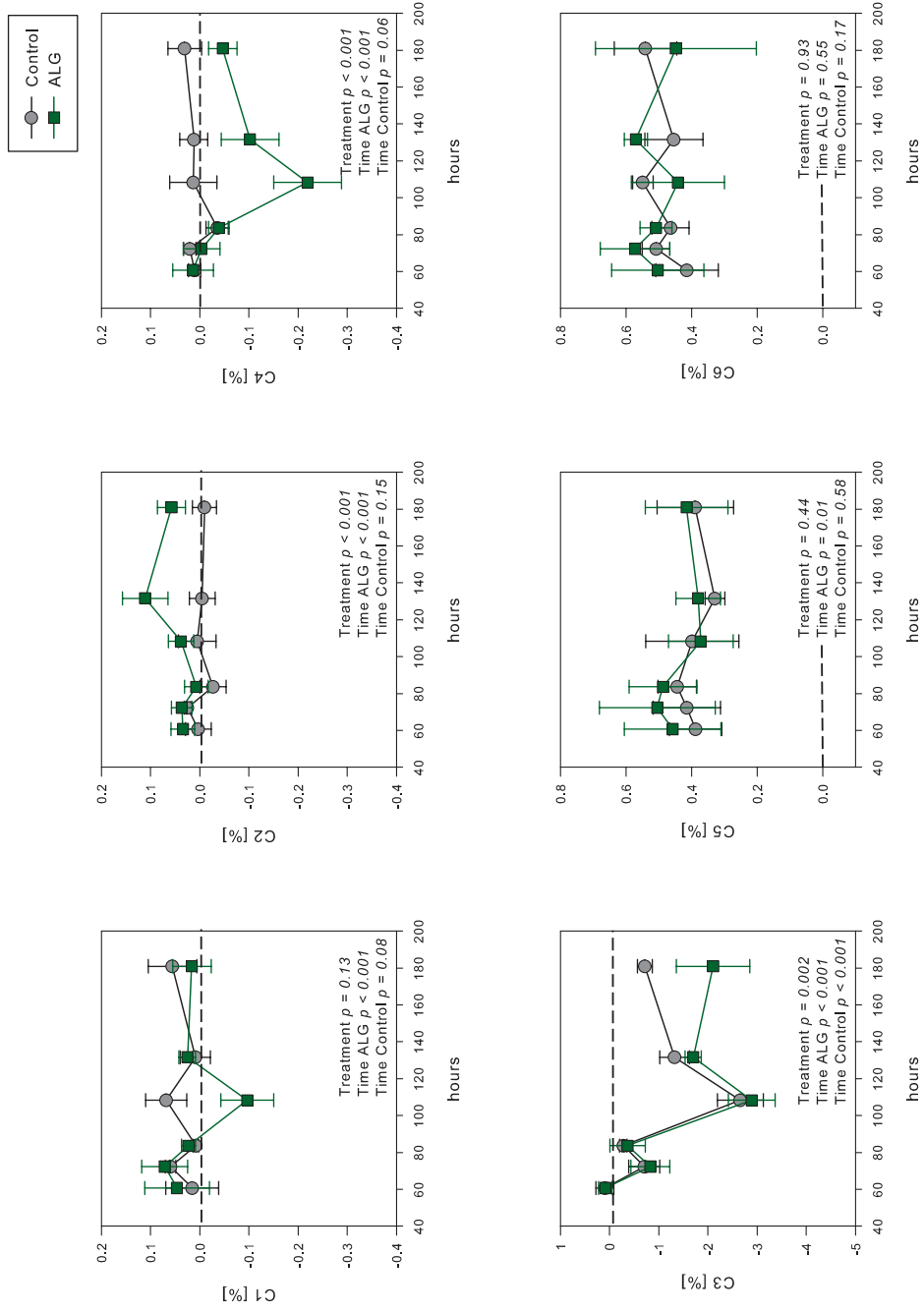
Figure 11 Temporal dynamics of fluorescence components in ALG and control bioreactors
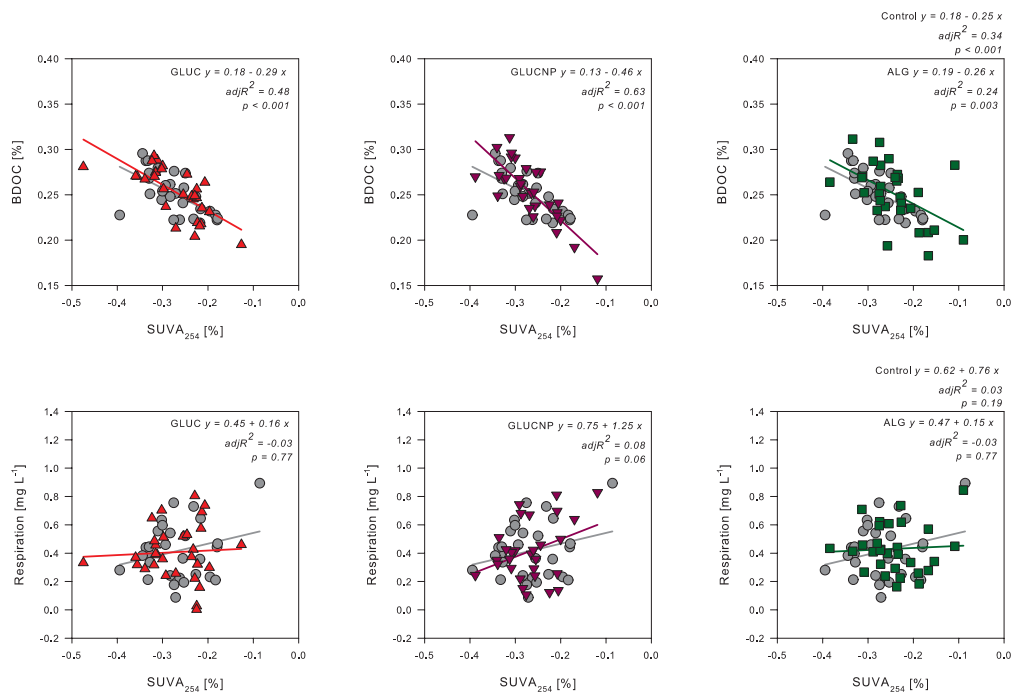
Figure 13 Linear Regression of SUVA$_{254}$ and BDOC or oxygen consumption
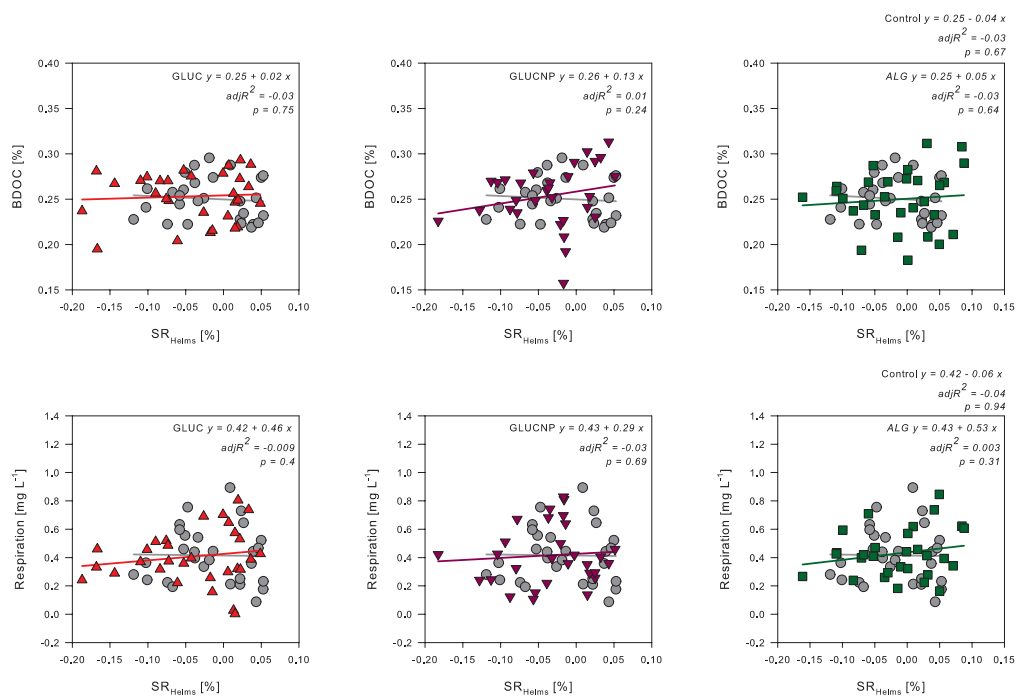

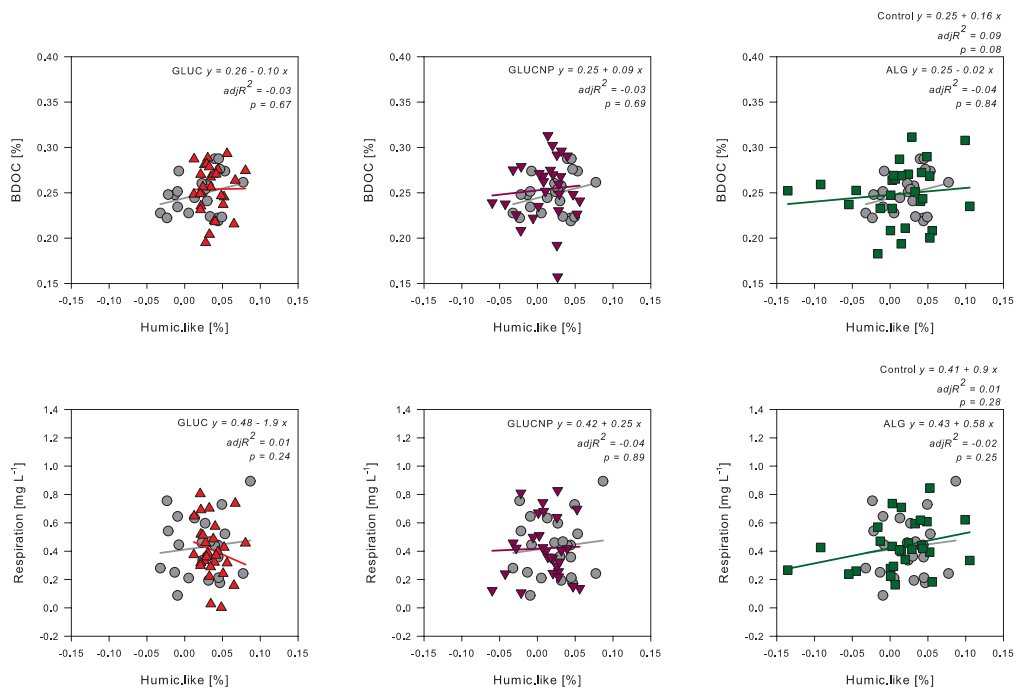Figure 12 Linear Regression of SR$_{Helms}$ and BDOC or oxygen consumption

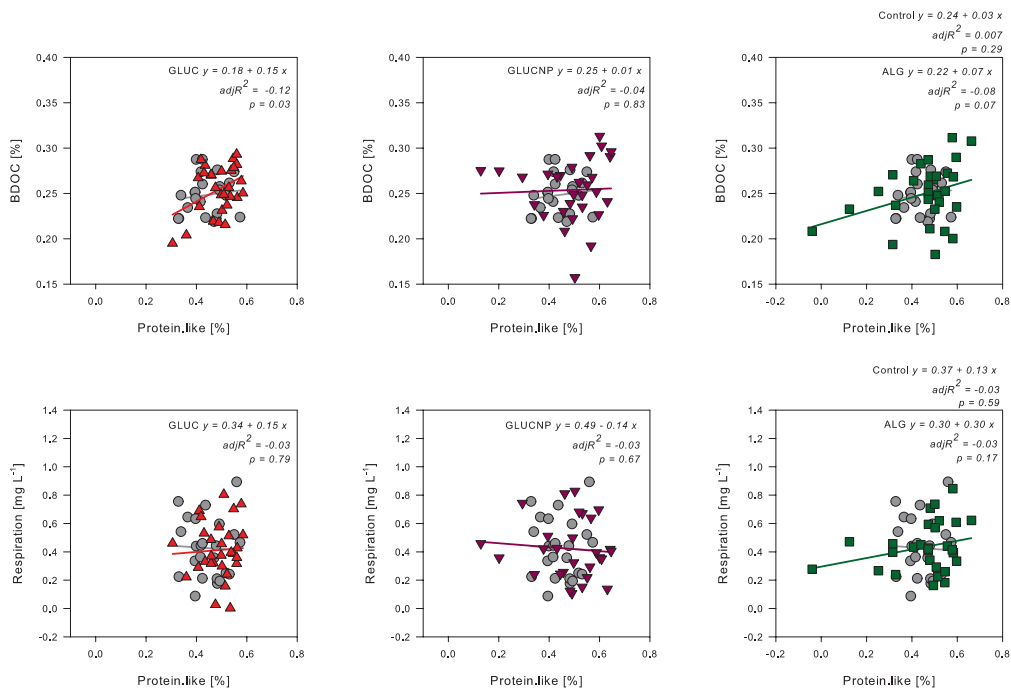Figure 15 Linear Regression of humic-like components and BDOC or oxygen consumption

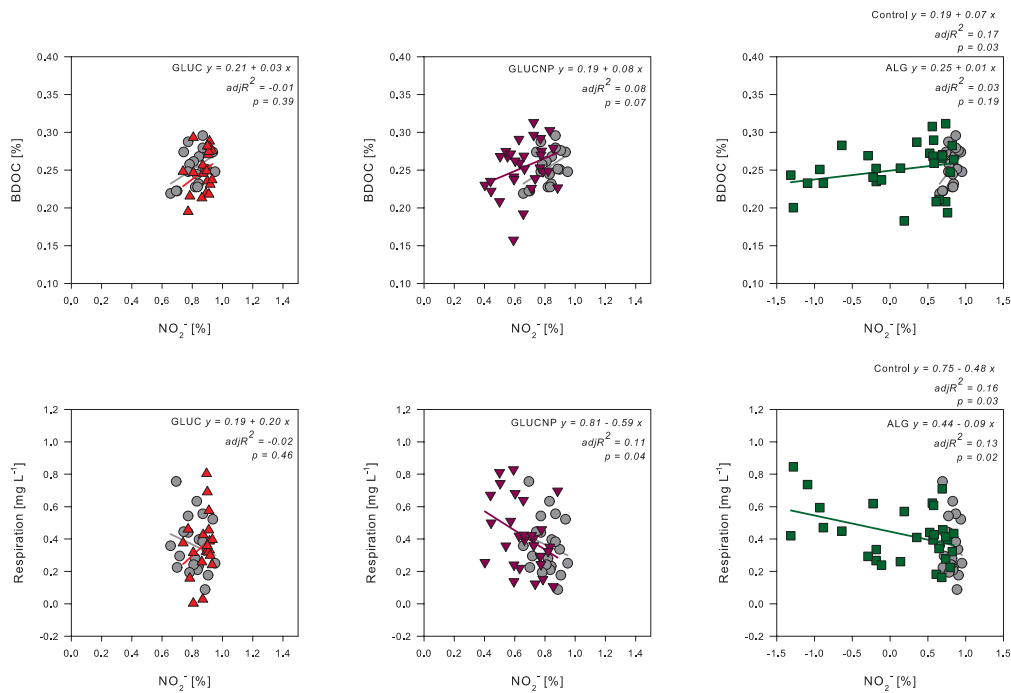Figure 14 Linear Regression of protein-like components and BDOC or oxygen consumption

34

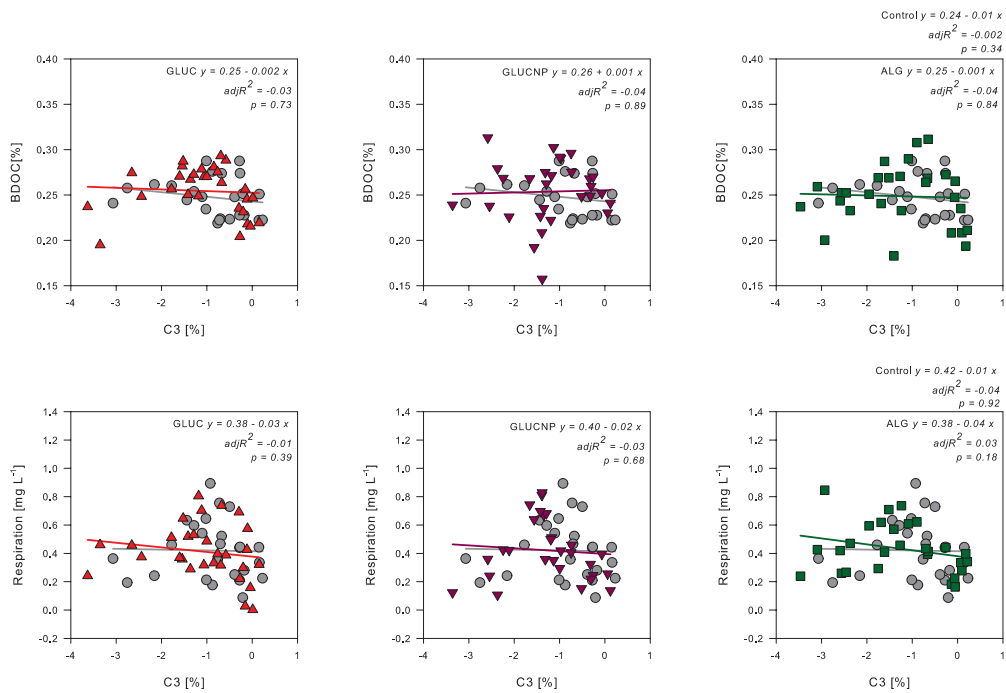Figure 16 Linear Regression of nitrite and BDOC or oxygen consumption

Figure 17 Linear Regression of C3 and BDOC or oxygen consumption

Table 5 Multiple Linear Regression

	BDOC [%]			Oxygen consumption [mg L^{-1}]		
	formula	adjR^2	p-value	formula	adjR^2	p-value
GLUC	$y = 0.13 + 0.29*SR_{Helms} – 0.37* SUVA_{254} – 0.01*C3$ SR$_{Helms}$ (p < 0.001) SUVA$_{254}$ (p < 0.001) C3 (p < 0.001)	0.81	< 0.001	$y = 0.36 + 1.62*SR_{Helms} – 0.11*C3$ SR$_{Helms}$ (p = 0.03) C3 (p = 0.03)	0.13	0.06
GLUCNP	$y = 0.1 + 0.33*SR_{Helms} – 0.55* SUVA_{254} – 0.01*C3$ SR$_{Helms}$ (p < 0.001) SUVA$_{254}$ (p < 0.001) C3 (p = 0.001)	0.87	< 0.001	n.s.	–	–
ALG	$y = 0.11 + 0.2*SR_{Helms} – 0.47* SUVA_{254} – 0.07*Protein.like$ SR$_{Helms}$ (p = 0.008) SUVA$_{254}$ (p < 0.001) Protein.like (p = 0.01)	0.67	< 0.001	$y = 0.3 + 2.0*Humic.like – 0.09*C3$ Humic.like (p = 0.008) C3 (p = 0.005)	0.27	0.008
Control	$y = 0.09 + 0.21*SR_{Helms} – 0.47* SUVA_{254} – 0.008*C3 + 0.07*Protein.like$ SR$_{Helms}$ (p = 0.007) SUVA$_{254}$ (p < 0.001) C3 (p = 0.04) Protein.like (p < 0.001)	0.75	< 0.001	n.s.	–	–

Discussion

In this microcosm experiment a mixture of RDOC and LDOC was used to simulate the supply of allochthonous and autochthonous organic matter during advective flow of groundwater and surface water to the hyporheic zone. The main target of this diploma thesis was to assess the alteration of the DOC pool by heterotrophic biofilm processes and link DOC transformations with microbial metabolic activity. Bioreactors with growing heterotrophic biofilms were fed with filtered stream water from a pre-alpine stream amended with RDOC (degraded willow extract from *Salix fragilis*) plus specific LDOC treatments. The LDOC treatments (GLUC, GLUCNP, ALG) served as resource subsidies similar to those that occur under environmental conditions (e.g. algal leachate). Characterisation of the added and remaining DOC pools should give further information of what is most likely to be assimilated by heterotrophic biofilms, and how it is transformed. Optical analyses were used to gain further insights into diverse chemical fractions of the dissolved organic carbon pool and spectroscopic parameters statistically combined with BDOC and oxygen consumption provide information about DOC dynamics in metabolic pathways of the heterotrophic microbial biofilms.

Humic-like Components

The DOC pool was characterized by six different fluorescence fractions - four humic-like and two protein-like components. Most of them are well known in the literature, but C3 was rarely found before. Stedmon & Markager (2005) describe a component similar to C3 as produced and removed very rapidly. Further they proposed microbial processes responsible for this DOC fraction but only after exposure to visible light. In our microcosm experiment C3 can also be described as a highly and rapidly produced humic-like component but in contrast to Stedmon & Markager (2005) light was not of relevance because heterotrophic biofilms were growing under dark conditions to exclude photoautotrophs. The effect of C3 was distinct from other humic-like components (C1, C2, C4) and also significantly different behaviour in bioreactors with added algal extract and glucose plus nutrients was observed.

37

The dynamic effect of the humic-like components (C1, C2, C4) hardly altered over time while C3 was produced to great extent during the microcosm experiment as mentioned above. Humic-like fractions of the bulk DOC are thought to be slowly cycling and more recalcitrant (Cory & Kaplan 2012) and also in this experiment we could show that humic-like compounds were not rapidly removed or processed. In contrast component C3 was significantly higher produced in all bioreactors with algal extract and glucose plus nutrients ($p < 0.001$). In the literature component C4 (peak C) is described as refractory (Osburn et al. 2011) since it is derived from microbial degradation (Stedmon & Markager 2005). Supplementary to these results C4 was taken up more by heterotrophic biofilms in the algal extract bioreactors, which further may indicate more degradation of C4 in presence of LDOC. Additionally, in the algal extract bioreactors more production of the humic-like component C2 was observed; however, so called priming effects of spiked LDOC on RDOC respiration could not be detected in this microcosm experiment.

Protein-like Components

In various studies amino acid carbon has been shown to be an excellent indicator for bioavailability of DOC (e.g. Amon et al. 2001, Graeber et al. 2012) and Balcarczyk et al. (2009) found protein-like fractions (tryptophan, tyrosine) positively retated to the proportion of DOC mineralised.

In this microcosm experiment proteinacous carbon was surprisingly not positively related to the bioavailability of DOC ($p > 0.5$). Further, the protein-like fraction in the bulk DOC pool was not significantly correlated with oxygen consumption ($p > 0.5$) but rather between the protein-like fraction and the glucose and algal extract amended bioreactors (GLUC $_{adj}R^2 = -0.12, p = 0.03$; ALG $_{adj}R^2 = -0.08, p = 0.07$). Since tyrosine-like (peak T) and tryptophan-like (peak B) components are considered to be autochthonous derived components (Osburn et al. 2011), both are assumed to be labile possibly incorporated into microbial biomass in this microcosm experiment.

Melillo et al. (1984) proposed that condensation reactions are responsible for proteins binding to carbohydrates or phenolics and therefore becoming

incorporated into heterocyclic rings of humic substances. Furthermore Volk et al. (1997) found high densities of amino acids, polypeptides and proteins bound to humic substances decomposed by biofilm. Hence proteinaceous material (C5, C6) removed during this experiment may be bound to humic substances and decomposed by heterotrophic biofilms, resulting in an increased aromaticity.

On the other hand the total fluorescence DOC of the chemical fractions is not proposed to be perfectly equal to the total DOC and furthermore may be insufficient to describe the biodegradability of DOC as a whole.

Bioavailability and fate of DOC

Many studies use spectroscopic parameters (e.g. SR_{Helms}, $SUVA_{254}$) to characterise the complex pool of DOC. In this experiment DOC bioavailability was not controlled by molecular weight, in agreement with Fischer at al. (2002) who proposed that DOC bioavailability is determined by abundance, presence and steric accessibility of chemical functional groups rather than molecular weight.

Surprisingly, a significant inverse relationship between BDOC and $SUVA_{254}$ was observed (see figure 12). BDOC is often shown to be positively related to $SUVA_{254}$ (e.g. Keil & Kirchman 1994; Maie et al. 2006) but there are nonetheless studies that were able to show inverse relationships between the loss of DOC and $SUVA_{254}$ (e.g. Fellman et al. 2008). Similarly, in this microcosm experiment the aromaticity of DOC was always higher after passing the bioreactors (increasing aromaticity, see table 2).

Increasing biomass (Bengtsson et al. in prep.) in this microcosm experiment may have involved build-up of EPS matrix during biofilm assembly, which could have further enhanced aromaticity by detaching single EPS fragments to the water column. Although polysaccharides are supposed to be the dominant EPS components, proteins, DNA and humic substances were also found in biofilm matrices and may act as electron acceptors or donors permitting redox activities in the matrix (Flemming & Wingender 2010). This would fit with proposed nitrification occurring in the bioreactors during the experiment.

Another possibility may be high enzymatic activity by heterotrophic biofilms and enzyme release on a small-scale in the biofilm environment. Enzymes consist of

39

proteins containing aromatic compounds such as tryptophan, tyrosine or phenylalanine, and also co-enzymes are often of heterocyclic compounds (Fresht 1999). However, enzymes degrade many aromatic compounds and also the protein-like fraction is removed during the experiment, hence results do not reinforce very well this hypothesis.

Surprisingly, the protein-like fraction does not seem to be a large part of the BDOC and the protein-like fraction was also responsible for only very poor prediction of BDOC in the multiple linear regression models (see table 6). Furthermore, BDOC was not related to oxygen consumption (figure 8). The protein-like components are, as mentioned above, assumed to be built into microbial biomass, most likely including into oxidizing nitrifiers. Nitrate and phosphate were not limiting factors for DOC uptake, but nitrite removal indicates that most likely nitrification occurred in the bioreactors during the experiment. The oxidizing reaction from NH_4^+ to NO_2^- to NO_3^- is rate-limited by the first step (NH_4^+ to NO_2^-). Although ammonium was under the detection limit during the experiment it is possible that NH_4^+ sources existed on micro-scales in the biofilm environment, and were immediately utilized. Two aerobic processes by microbes are thought to be responsible for the transformation from NH_4^+ to NO_2^-: a chemolithoautotrophic process and heterotrophic nitrification (Kirchman 2012). Heterotrophic nitrification is proposed to have slower rates but based on this process microbes do not gain energy from nitrogen oxidation, whereas chemolithoautotrophic mircobes have 10^3 to 10^4 times higher oxidizing rates but are mostly described in soils (Kirchman 2012). In oxic environments bacteria of the betaproteobacterial genera, such as *Nitrosospira* and *Nitrosomas* are considered to be the main microbes oxidizing ammonia but further, substantial abundances of ammonia-oxidation archaea has been shown in many environments, from soil to aquatic ecosystems (Kirchman 2012). However, more taxonomic information is necessary to clearly answer the question what type of nitrification occurred in this microcosm experiment.

In summary, BDOC slightly decreased and oxygen consumption increased over time. Since no significant relationship was found between these two parameters

in any of the bioreactors, bioavailable DOC was perhaps built into biomass, for example into oxidizing nitifiers, such as *Nitrosospira*.

Conclusion

LDOC content did not strongly affect RDOC degradation, thus priming could not be detected in this microcosm experiment. Although more production or removal of individual fluorescence components in bioreactors with higher nutrient concentrations (ALG, GLUCNP) could be observed, this experiment cannot clearly address priming effects by heterotrophic biofilms in interstitial waters. However, this research topic is relatively young in freshwater science and further studies are necessary to estimate priming effects mediated by microbial biofilms in stream water sediments more precisely.

Characterizing the DOC fractions gave insight into the alteration of different chemical fractions of the carbon pool in this experiment. More than 80 % of the bulk DOC was dominated by humic-like substances, but of rather low dynamics, whereas the protein-like fractions (~20 % of bulk DOC) were relatively strongly removed. Although the proteinacous carbon is assumed to be highly bioavailable (e.g. Balcarczyk et al. 2009), in this study a relationship between BDOC and protein-like components could not be found, suggesting the proteinacous material was subsequently bound to humic-like substances and transformed, incorporated or decomposed by oxidizing nitrifiers. This hypothesis is reinforced by the fact that increasing oxygen consumption was not responsible for the removal of the protein-like fraction, but nitrite was removed and additionally the aromaticity ($SUVA_{254}$) was increasing during the experimental phase.

To summarize, high nutrient availability combined with the rapidly produced humic-like component C3 and removed proteinacous material have had immediate effects on microbial activities in heterotrophic biofilms (most probably by oxidizing nitrifiers). Dynamics of humic-like substance were significantly distinct from protein-like components that showed higher variations over time when DOC supply was spiked with labile material. These results emphasize the functional complexity of the hyporheic activity and microbial metabolic dynamics in hyporheic sediments are proposed to influence

carbon fluxes through fluvial networks to a remarkable extent. Further studies are necessary to understand the link between microbial metabolism and DOC transformation via hyporheic flowpaths in more detail, including the influence of benthic biofilms. However, this study highlights the effects that hyporheic zones may also have on adjacent ecosystems, and their potential impact on global carbon budgets.

Zusammenfassung

In den letzten Jahren Wissenschaftsforschung ist zunehmend deutlicher geworden, dass Fließgewässer Ökosysteme nicht nur Transport-Funktionen haben, sondern den Kohlenstoff-Eintrag aus angrenzenden Gebieten in großen Mengen speichern und in Form von CO_2 in die Atmosphäre ausgasen können. Trotz ihrer relative geringen Oberfäche tragen Fließgewässer damit erheblich zu dem globalen Kohlenstoff-Budget bei und in Zeiten von Klimaveränderung und Treibhausgaseffekten ist es von besonderer Bedeutung, die ökologischen Funktion der Oberflächengewässer zu verstehen und ihre globalen Beiträge hervorzusehen. Je nach Struktur der Gewässersohle kann der Kies- und Sandlückenbereich (Hyporheisches Interstitial) unterschiedlich stark ausgeprägt sein. Das so genannte Hyporheal, in dem sich Grundwasser mit Oberflächenwasser mischt, ist ein Lebensraum für viele Mikroorganismen und oft auch für Larvenstadien größerer Organismen. Im hyporheischen Interstitial sind vor allem heterotrophe Biofilme verantwortlich für die metabolischen Prozesse und gekoppelt an hohe Abundanzen von Mikroorganismen können sie Kohlenstoffflüsse nachhaltig beeinflussen.

In diesem Mikrokosmos-Experiment sind Bioreaktoren mit heterotrophen Biofilmen besiedelt und mit gefilterten Wasser plus verschieden labilen gelösten organischen Kohlenstoff- Zusätzen (DOC) gespeist worden. Optisch spekroskopische Messungen wurden verwendet um den DOC-Pool genauer zu charakterisieren und zeitliche Dynamiken wurden statistisch mit der Verfügbarkeit von DOC und der Sauerstoff-Konsumation kombiniert. Sechs verschiedene Fluoreszenz-Komponenten konnten identifiziert werden. Der gesamte DOC-Pool war dominiert von humin-ähnlichen Stoffen (~80%), einen kleinerer Teil machten die protein-ähnlichen Stoffe aus (~20%). Während die protein-ähnlichen Stoffe abgebaut wurde, unterlagen die meisten der humin-ähnlichen Stoffe nur sehr geringere Dynamiken und veränderten sich, abgesehen von einer Komponente, kaum während des Experimentes. Auffällig war, dass die Bioverfügbarkeit des DOC mit keinem Parameter korrelierte, ausser einer inversen Korrelation mit der Aromatizität. Es wird vorgeschlagen, dass vor allem proteinogenes DOC in Biomasse eingebaut wurde und die Aromatizität gestiegen

ist durch, zum Beispiel, erhöhten Aufbau der Biofilm- Matrix oder enzymatischer Aktivität der Mikroorganismen. Zusammenfassend lässt sich sagen, dass die verschiedenen DOC-Fraktionen im Hyporheal wahrscheinlich sehr unterschiedlich Dynamiken unterliegen können und, wenn gleich auch mehr Untersuchungen notwendig sind, konnte durch dieses Mikrokosmos-Experiment gezeigt werden, dass die metabolische Aktivität hyporheischer Biofilme einen gewissen Einfluss auf den Stoffluss auch auf angrenzende Ökosysteme haben kann und hinsichtlich des globalen Kohlenstoffhaushaltes in Zukunft stärker berücksichtigt werden sollten.

Acknowledgments

References

Battin, T. J., Kaplan, L. A., Findlay, S., Hopkinson, C. S., Marti, E., Packman, A. I., ... & Sabater, F. (2008). Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geoscience*, *1*(2), 95-100.

Balcarczyk, K. L., Jones, J. B., Jaffé, R., & Maie, N. (2009). Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry*, *94*(3), 255–270. doi:10.1007/s10533-009-9324-x.

Bretschko, G. (1981). Vertical distribution of zoobenthos in an alpine brook of the Ritrodat-Lunz study area. *Verh. Internat. Verein. Limnol*, *21*, 873-876.

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, *51*(4), 325–346. doi:10.1016/0304-4203(95)00062-3.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., et al. (2007). Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. *Ecosystems*, *10*(1), 172–185. doi:10.1007/s10021-006-9013-8.

Confer, D., & Logan, B. (1998). A conceptual model describing macromolecule degradation by suspended cultures and biofilms. *Water Science and Technology*, *37*(4-5), 231–234. doi:10.1016/S0273-1223(98)00112-7.

Cory, R. M., & Kaplan, L. A. (2012). Biological lability of streamwater fluorescent dissolved organic matter. *Limnology and Oceanography*, *57*(5), 1347–1360. doi:10.4319/lo.2012.57.5.1347.

Cory, R. M., & McKnight, D. M. (2005). Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental science & technology*, *39*(21), 8142–9.

Dodds W.K. & Whiles M.R. (2010) *Freshwater Ecology: Concepts and Environmental Applications of Limnology*. Academic Press, San Diego, California.

Elwood, J.W., J.D. Newbold, R.V. O'Neill & W. van Winkle, l983. Resource spiralling: An operational paradigm for analyzing lotic ecosystems. p. 3-27. In: Fontaine, T.D., Bartell S. M. (Eds.). *Dynamics of lotic ecosystems*. Ann Arbor Science.

Fellman, J. B., D'Amore, D. V, & Hood, E. (2008). An evaluation of freezing as a preservation technique for analyzing dissolved organic C, N and P in surface water samples. *The Science of the total environment*, *392*(2-3), 305–12. doi:10.1016/j.scitotenv.2007.11.027.

Findlay, S. E., Sinsabaugh, R. L., Sobczak, W. V., & Hoostal, M. (2003). Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnology and Oceanography*, *48*(4), 1608-1617.

Fischer, H., Wanner, S. C., & Pusch, M. (2002). Bacterial abundance and production in river sediments as related to the biochemical composition of particulate organic matter (POM). *Biogeochemistry*, *61*(1), 37-55.

Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature reviews. Microbiology*, *8*(9), 623–33. doi:10.1038/nrmicro2415.

Fersht, A. (1998). *Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding*. WH Freeman.

Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research*, *30*(8), 1749-1758.

Graeber, D., Gelbrecht, J., Pusch, M. T., Anlanger, C., & Von Schiller, D. (2012). Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams. *The Science of the total environment*, *438*, 435–46. doi:10.1016/j.scitotenv.2012.08.087.

Guenet, B., Danger, M., Abbadie, L., & Lacroix, G. (2010). Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology*, *91*(10), 2850-2861.

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature reviews. Microbiology*, *2*(2), 95–108. doi:10.1038/nrmicro821.

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., & Mopper, K. (2008). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography*, *53*(3), 955–969. doi:10.4319/lo.2008.53.3.0955.

Kaplan, L.A. & Newbold, J.D. (2000). Surface and subsurface dis- solved organic carbon, p. 237–258. In: J. B. Jones and P. J. Mulholland. *Streams and ground waters*. Academic Press.

Kaplan, L.A. & Newbold, J.D. (2002). The role of monomers in stream ecosystem metabolism. In: Findlay, S. E., Sinsabaugh, R. L. (Eds.) *Aquatic Ecosystems, Interactivity of Dissolved Organic Matter*. Academic Press, San Diego, pp 97–119.

Kim, J. H., Zarzycka, B., Buscail, R., Peterse, F., Bonnin, J., Ludwig, W., ... & Sinninghe Damsté, J. S. (2010). Contribution of river-borne soil organic carbon to the Gulf of Lions (NW Mediterranean). *Limnology and Oceanography*, *55*(2), 507-518.

Kim, S., Kaplan, L. a., & Hatcher, P. G. (2006). Biodegradable dissolved organic matter in a temperate and a tropical stream determined from ultra-high resolution mass spectrometry. *Limnology and Oceanography*, *51*(2), 1054–1063. doi:10.4319/lo.2006.51.2.1054.

Kirchman D.L. (2012). *Processes in microbial ecology*. Oxford University Press, Oxford.

Keil, R. G., & Kirchman, D. L. (1994). Abiotic transformation of labile protein to refractory protein in sea water. *Marine Chemistry*, *45*(3), 187-196.

Lane, C. S., Lyon, D. R., & Ziegler, S. E. (2012). Cycling of two carbon substrates of contrasting lability by heterotrophic biofilms across a nutrient gradient of headwater streams. *Aquatic Sciences*, *75*(2), 235–250. doi:10.1007/s00027-012-0269-0.

Maie, N., Parish, K. J., Watanabe, A., Knicker, H., Benner, R., Abe, T., ... & Jaffé, R. (2006). Chemical characteristics of dissolved organic nitrogen in an oligotrophic subtropical coastal ecosystem. *Geochimica et cosmochimica acta*, *70*(17), 4491-4506. doi:10.1016/j.gca.2006.06.1554.

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T., & Andersen, D. T. (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, *46*(1), 38–48. doi:10.4319/lo.2001.46.1.0038.

Melillo, J. M., Naiman, R. J., Aber, J. D., & Linkins, A. E. (1984). Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. *Bulletin of Marine Science*, *35*(3), 341-356.

Meyer, J. L. (1994). The microbial loop in flowing waters. *Microbial Ecology*, *28*(2), 195-199.

Newbold, J. D., Elwood, J. W., O'Neill, R. V., & Winkle, W. V. (1981). Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*(7), 860-863.

Ogle, S. M., Breidt, F. J., & Paustian, K. (2005). Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. *Biogeochemistry*, *72*(1), 87–121. doi:10.1007/s10533-004-0360-2.

Osburn, C. L., Wigdahl, C. R., Fritz, S. C., & Saros, J. E. (2011). Dissolved organic matter composition and photoreactivity in prairie lakes of the U.S. Great Plains. *Limnology and Oceanography*, *56*(6), 2371–2390. doi:10.4319/lo.2011.56.6.2371.

Popp, M., Wanek W., Richter A., Kasper-Giebl A., Battin T. J., & Dorninger M. Endbericht 2006 – 2009. EcoCatch LUNZ „*Auswirkungen des Globalen Wandels auf ökosystemare Prozesse und Leistungen:* Immissionen, Retention und Austrägevon Schadstoffen und Nährstoffen".

Scully, N. M., Maie, N., Dailey, S. K., Boyer, J. N., Jones, R. D., & Jaffé, R. (2004). Early diagenesis of plant-derived dissolved organic matter along a wetland, mangrove, estuary ecotone. *Limnology and Oceanography*, *49*(5), 1667-1678.

Sobczak, W. V., & Findlay S. (2002). Variation in bioavailability of dissolved organic carbon among stream hyporheic flow-paths. *Ecology*, *83*(11), 3194–3209.

Stedmon, C. A., Markager, S., & Bro, R. (2003). Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry*, *82*(3-4), 239–254. doi:10.1016/S0304-4203(03)00072-0.

Stedmon, C. A., & Markager, S. (2005). Tracing the production and degradation of autochthonous fractions of dissolved organic matter using fluorescence analysis. *Limnology and Oceanography*, *50*(5), 1415–1426. doi:10.4319/lo.2005.50.5.1415.

Stewart, P. S., & Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nature reviews. Microbiology*, *6*(3), 199–210. doi:10.1038/nrmicro1838.

Thurman, E. M. (1985). *Organic geochemistry of natural waters* (Vol. 2). Kluwer Academic Pub.

Triska, F. J., Duff, J. H., & Avanzino, R. J. (1993). The role of water exchange between a stream channel and its hyporheic zone in nitrogen cycling at the terrestrial—aquatic interface. In *Nutrient Dynamics and Retention in Land/Water Ecotones of Lowland, Temperate Lakes and Rivers* (pp. 167-184). Springer Netherlands.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, *37*(1), 130-137.

Volk, C. J., Reserche, A., Gcncrale, C., Laffitte, M., Volk, C. B., & Kaplan, L. A. (1997). Chemical composition of biodegradable dissolved organic matter in streamwater, *Limnology and Oceanography, 42*(l), 39–44.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. a, Fram, M. S., Fujii, R., & Mopper, K. (2003). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental science & technology*, *37*(20), 4702–8.

Wetzel, R. G.R. (1992). Gradient-dominant ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. *Hydrobiologia*, 229 (1992), pp. 181–198.

Young, R. G., Matthaei, C. D., & Townsend, C. R. (2008). Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society*, *27*(3), 605–625. doi:10.1899/07-121.1.

Curriculum Vitae

Judith Andrea Rosentreter

PERSONAL INFORMATION

Born July 03, 1983 in Göttingen, Germany
Nationality: Germany, Austria

Address: Adambergergasse 4/14
        1020 Wien
        Austria

Email: judith.andrea.rosentreter@univie.ac.at

EDUCATION

University of Vienna, Austria   *April 2013*
- Diploma in Ecology/Biology (equiv. M.Sc)
- Diploma thesis, title: "Assessing dissolved organic carbon properties and dynamics in a microcosm experiment simulating the hyporheic zone"

University of Vienna, Austria   *March 2006 – October 2009*
- Study of Biology (equiv. BSc)

University Erlangen-Nurnberg, Germany   *October 2003 – March 2006*
- Teaching qualification at the primary school and the non-selective secondary school, with the main emphasis on secondary school
- Subjects: Arts, Biology, Mathematics, German
- First State Examination

Philipps-University Marburg, Germany   *October 2002 – October 2003*
- Economics

Grammar school, Goettingen, Germany   *1995 – 2002*
- University-entrance qualification

TEACHING EXPERIENCE

Teaching assistant at the Department of Organic Chemistry   *since March 2010*
Practical course on Organic Chemistry; e.g. peptide synthesis, isolation of natural material, derivatisation of monosaccharaides

FURTHER SKILLS & SELECTED COURSES

- **Languages:** fluent in German and English, basic knowledge of French and Spain

- **IT-Skills:** R, SigmaPlot, SPSS, Statgraphics

  Selected courses:
- Tutorial 'R-Statistics' (winter 2012)
- Seminar 'Ecology of Microbial Biofilms' (winter 2012)
- Field and lab course 'Biogeochemistry' (Lunzer Lake, Austria, summer 2012)
- Field course 'Primary Production' (Neusiedler Lake, Austria, summer 2011)
- Field course 'Aquatic Biocoenosis' (Lunzer Lake, Austria, summer 2011)
- Practical course 'Aquatic Microbial Ecology' (summer 2011)
- Practical course 'Experimental Design and Statistical Methods in Limnology' (winter 2010)
- Field course 'Hydrology and Stream Ecology' (winter 2010)
- Field course on Conservation of Mediterranean landscape and biodiversity (Sardinia, Italy, summer 2010)
- Practical course 'Organic Chemistry' (winter 2009)

- **SIL-Austria Meeting 2012,** Pörtschach, Austria

- **European Geosciences Union General Assembly 2012**, Vienna, Austria
  Poster: DOM transformations in stream biofilms shown by fluorescence spectroscop,
  NR Burns, JA Rosentreter, MM Bengtsson, K Wagner, E Herberg and TJ Battin

- **Soil Organic Matter 5, 2012**, Ascona, Switzerland
  Poster: Organic matter transformations in heterotrophic stream biofilms,
  NR Burns, JA Rosentreter, MM Bengtsson, K Wagner, E Herberg and TJ Battin