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Impact of photooxidation on microbial degradation of dissolved organic carbon in streamwater and shallow groundwater

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1. Abstract

Dissolved organic matter (DOM) source, composition, photochemical alteration and availability affect freshwater ecosystems, their carbon fluxes and thus the global carbon cycle. The aim of this study was to gain an understanding of how these components influence the optical properties and microbial processing of chromophoric DOM (CDOM). The experiments included monitoring of photochemical effects of irradiation on allochthonous and autochthonous dissolved organic carbon (DOC), as well as subsequent microbial degradation processes.

Excitation-emission spectroscopy and absorption spectra were used to describe and quantify the change in optical and fluorescent properties of CDOM from a calcareous headwater stream and from various adjacent water bodies. Microbial respiration was monitored to identify changes in the bioavailability of irradiated DOC samples. Exposure to a spectrum similar to natural sunlight significantly affected the optical properties of CDOM and consequently augmented the bioavailability of DOC to the microbial metabolism; autochthonous DOC from periphytic algae which served as a potential endmember of a DOC continuum in the streamwater did not follow this pattern.

The results suggest a large proportion of soil-derived carbon in the stream, which is traditionally thought to be refractory. However, my experimental results suggest that if this DOC is photooxidized, it becomes partially photo-bleached and converted into low-molecular-weight compounds, which enhance microbial metabolism. The photochemical alteration of CDOM provides the opportunity for microbial processing of the photooxidized substrates, eventually increasing CO_2 emissions and reducing CDOM concentrations in stream ecosystems.

2. Introduction

The chemical composition and structure of dissolved organic carbon (DOC) in freshwater ecosystems affect microbial metabolism and can therefore impact the global carbon cycle (Kaplan and Bott, 1989; Battin et al., 2008). DOC is composed of complex molecules like humic and fulvic acids (Hedges et al., 1994), largely derived from terrestrial vegetation (Allan and Castillo, 2007); Some of this allochthonous DOC was initially stored in soils for years to centuries (Trumbore, 1997) before it enters stream. This DOC is thought to be refractory because of its structural complexity, physicochemical protection from microbial degradation and because it has been extensively reworked by soil microorganisms already. In contrast, the autochthonous fraction of DOC, derived from aquatic primary production, typically contains constituents, which are lower in aromaticity. Algal derived substrates are considered labile and are rapidly metabolized (Azam and Cho, 1987).

Freshwater ecosystems depend on this putatively recalcitrant DOC as energy source since a large portion of the metabolized organic carbon is derived from terrestrial vegetation (Battin et al., 2008). Approximately 1.9 Pg carbon per year, derived from terrestrial ecosystems, are transported into freshwater ecosystems and then into the oceans (Cole et al., 2007). During this process part of the carbon evades as CO_2 and part is stored in sediments (Cole et al., 2007).

The enhanced UVB radiation caused by stratospheric ozone destruction was the reason for various studies focusing on the photolabile fraction of DOC, which shows a strong absorbance the visible light, in the UV-B and in the UV-A region (Osburn et al, 2001). This fraction is referred to as chromophoric or colored dissolved organic matter (CDOM) and is mainly composed of humic substances derived from terrestrial vascular plants (Boyle et al., 2009). CDOM has multiple consequences for aquatic ecosystems as it influences the aquatic light field and shields biota of potentially harmful UV-radiation (Walsh et al., 2003), for example.

Photochemical transformation of terrestrial DOC was shown to result in low-molecular-weight compounds (LMW) (Helms et al., 2008) and enhanced bacterial growth, though in the case of DOC from algae it was demonstrated to inhibit bacterial growth (Tranvik and Bertilsson, 2001). Photooxidation was also shown to enhance the cleavage of macromolecules (Kieber et al., 1989) and the release of CO_2 and CO (Mopper et al., 1991). Most of these studies were performed on DOC from the ocean (Osburn et al., 2009), whereas only few focused on freshwater DOC (Langenheder, 2006; Helms et al., 2008; Kragh et al., 2008).

Based on these findings, the following study focuses on testing the photochemical effects on DOC in a headwater stream and its adjacent landscape. In experiments, I used a spectrum similar to natural sunlight, monitored photochemically induced changes in optical and fluorescent properties and the implications for microbial respiration and growth. DOC was sampled from various locations along its pathway into the stream, thus taking into consideration that streams are complex ecosystems integrated in the surrounding landscape and in constant interaction with the groundwater and the fringing floodplains. Bacterial metabolism was monitored in these different waters to identify photo-induced changes in DOC bioavailability. Thus, the target of this study was to gain an understanding of how DOC source and composition in combination with photochemical alteration influence microbial degradation processes. This basic understanding of DOC dynamics can lead to further insights into carbon fluxes in stream ecosystems.

3. Material and methods

3.1 Experimental design and sampling

The experimental set up for this study encompassed the comparison of DOC from five different origins. The effect of UV radiation on the optical properties of both allochthonous and autochthonous CDOM was studied and the

influence on bacterial growth efficiency investigated. For that purpose, DOC from ground water, soil water, soil extract, algae extract and stream water was examined and compared. The sampling period ranged from October to November 2008, extracts were prepared in July.

Streamwater (SB) was sampled from the Oberer Seebach (Austria). The Seebach is a calcareous, 3rd order mountain stream of 11.5 km length. Its catchment is largely karstic. The stream originates from the Obersee and discharges into the Ybbs. DOC concentration is typically low in these waters (1 to 2 mg C L^{-1}).

Groundwater samples (GW) were pumped from shallow wells (0.8 m) installed in the floodplain 4 months before sampling. They were located approximately 20 m away from the Oberer Seebach.

Soil water (SW) was obtained from a depth of 0.1 m using a Tensiometer (VS-single or-twin; UMS), pre-installed in the floodplain, which employed a negative pressure of 250 hP.

These three samples were stored in plastic containers (pre-washed with 0.1 N HCL and subsequently rinsed thoroughly with Milli-Q water (Millipore). All glassware used was either combusted at 450°C for 4 hours or washed with 0.1 N HCL and flushed with MilliQ (Millipore). The samples were filtered (47 mm, Whatman GF/F glass-fiber filters, precombusted at 450°C for 4 hours) within 20 min after sampling in order to remove particulate material. The filtered water was then stored in the dark (4°C) until further processing.

Soil Extract (SE) was produced as follows: In June, floodplain soils (A horizon) were compounded with Milli-Q water (Millipore). Soil was removed via sieving and centrifugating (30 min, 5000 rpm) and the supernatant was finally filtered (GF/F, Whatmann) and frozen (-20°C).

Algae Extract (AE) was produced using periphytic algae from the streambed. The algae was scraped off the stones, collected in plastic containers together with stream water and frozen (-20°C). Prior to the experiments extracts were thawed at room temperature and subsequently GF/F (Whatman) filtered.

During the freezing period and the thawing process the samples were kept in the dark.

3.2 Irradiation

Prior to the experiments, all samples were filtered (0.2 µm, Millipore) to remove microbial cells and then diluted to a final DOC concentration of 1-2 mg C L^{-1} by adding groundwater from a deeper well. Groundwater was chosen because its ionic composition is similar to the stream water and it is low in DOC (< 1 mg C L^{-1}).

For a period of 12 hours (Tranvik and Bertilsson, 2001) samples were either irradiated in 1-liter beakers or incubated in the dark, serving as dark control. Samples were always kept at room temperature. The light source (Osram, Ultra-Vitalux, 230V, E27/ES; 300W) emitted in the UVA (320-400 nm), UVB (300-320nm), photosynthetically active radiation (PAR, 400-700 nm) and in the infrared region (from 780 nm) (Fig.1).

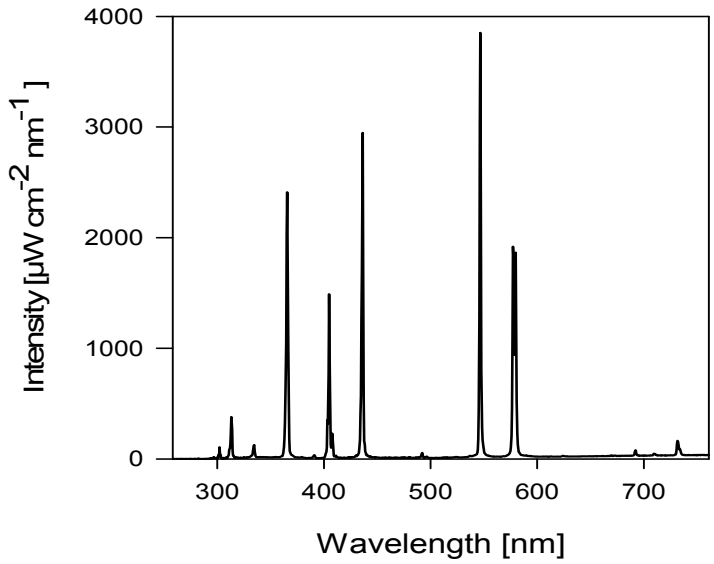

Figure 1 Measured spectrum of the light source used for irradiation (Osram, Ultra-Vitalux, 230V, E27/ES; 300W).

3.3 Incubation period

Five replicate samples (620 ml) of each irradiated and control treatments were transferred into combusted Schott bottles. In order to prevent limitation

of bacterial growth, inorganic nutrients to a final concentration of 2000 µg NO_3-N L^{-1}, 8 µg NH_4-N L^{-1} and 6 µg PO_4-P L^{-1} were added. These concentrations correspond to twice the respective concentrations in the stream water.

Each replicate was inoculated with the microbial community naturally occurring in the stream water. For that purpose, I concentrated microbial cells by first filtering 3 to 5 L stream water trough a GF/F filter (Whatman) to remove particles and major grazers, and then through a membrane filter (0.2 µm, Millipore). Cells were carefully removed from the membrane filter with a few millilitres of GF/F-filtered stream water. This cell suspension was always produced fresh on the day of inoculation and stored in the dark (4°C) until usage. The final cell abundance in the inoculated Schott bottles was always adjusted to 7 ± 3% of the initial cell abundance in the stream water (GF/F filtered; Whatman). The bottles were then incubated in the dark for 9 days at room temperature (20°C) on horizontal shakers. Duplicate samples of the irradiated and the dark treatments were kept in closed Schott bottles coated with aluminium foil for oxygen measurement.

3.4 DOC and nutrient analysis

DOC concentration was measured before and after irradiation as well as at the end of each experiment using a TOC analyzer (Sievers 900). Bioavailable DOC (BDOC) was calculated as the decrease in DOC over the incubation period of 9 days for each treatment. Nutrient concentrations were measured in the same intervals using a continuous flow analyzer (FLOW SYS RA 104, Systea).

3.5 Optical analyses

CDOM can be characterized by absorption spectra and excitation-emission matrices (EEM). These properties can be used as indicators of DOC source and composition (Hudson et al., 2007; Stedmon at al., 2003). Samples for

optical analyses were collected before and after the irradiation, and also after 9 days of incubation in the dark. Additional samples were obtained for absorbance analysis in 4 hour intervals during irradiation. Samples were immediately filtered (GF/F Whatman) and stored in acid-rinsed (0.1 N HCL) and combusted (450°C, 4 h) glass vials in the dark (4 °C).

3.6 Absorbance analysis

Absorbance measurements were performed within 5 days of sampling using a UV VIS spectrophotometer (Shimadzu, UV 17000, Pharma Spec) and 10 cm quartz cuvettes using Milli-Q water (Millipore) as the blank. Samples were acidified to a pH of approximately 2.

CDOM generally shows a strong absorbance in the short wave region, which decreases exponentially (Twardowsi et al, 2004). Commonly absorption spectra are characterized by the spectral slope parameter, which is calculated using least squares linear regression fitted to the log transformed spectra (Blough et al, 1993) or by nonlinear regression fitting (Stedmon et al, 2000) The slope parameter is usually calculated for a relatively large region, e.g. 300-700nm (Helms et al., 2009), 250-500 (Battin, 1998). However, there is no clear consensus on which method and wavelength regions are to be used for characterization (Twardowsi et al, 2004; Loiselle et al, 2009).

The absorption spectra can be described using the following equation:

$$a_\lambda = a_{\lambda 0} e^{l - S(\lambda - \lambda 0)}$$

where a_λ is the absorption coefficient at wavelength λ, $a_{\lambda o}$ is the reference wavelength and S is the spectral slope parameter. The absorption coefficients were calculated as follows:

$$a_\lambda = 2.303 \times \frac{A\lambda}{r}$$

where A is the absorbance at wavelength λ and r is the cuvette path length (m). (Green and Blough, 1994).

The low DOC concentration of the samples resulted in minor differences in absorption after irradiation (see below). Hence the slopes for two shorter intervals of the log-transformed spectra were calculated according to Helms et al (2008); the 275-295 nm ($S_{275-295}$) and the 350-400 nm ($S_{350-400}$) intervals. This was performed using the least squares linear regression function (MS Excel). The slope ratio (S_R) was computed as the ratio of $S_{275-295}$ to $S_{350-400}$ and subsequently used for comparison of the data. (Helms et al., 2008) The specific UV absorbance (SUVA 254 in mg C L^{-1} m^{-1}) was calculated by normalizing the absorption coefficient at wavelength λ (m^{-1}) by the DOC concentration (mg C L^{-1}). Weishaar et al. (2003) showed a strong correlation between the SUVA 254 and DOM aromaticity ($r^2 > 0.97$) for an array of humic substance isolates.

Furthermore, the apparent quantum yields (AQY) were calculated for each treatment and experiment following Osburn et al. (2009). They served as a measure of photorectivity and allowed the detection of photochemical induced changes. The AQY are defined as the average decrease in the absorption coefficient over the 280-550 nm region divided by the total mol photons absorbed m^{-2}. The average decrease in absorption was calculated using the following equation:

$$\Delta a_{avg.} = \frac{\sum(280-550)a(\lambda)_{initial} - a(\lambda)_{final}}{270}$$

where $a(\lambda)_{initial}$ and $a(\lambda)_{final}$ are the initial and final absorption coefficients (after exposure. $a(\lambda)_{initial}$ was corrected for the dark controls. The total quanta (I_a in mol photons m^{-2}) absorbed by the samples during the 12 hours of irradiation was computed as:

$$I_a = I_0(\lambda) \times (1 - e^{-a(\lambda)geo \times L})$$

where I_0 (λ) is the incident radiant flux (mol photons m^{-2} s^{-1}) measured at the surface of the water samples and $a(\lambda)_{geo}$ is the geometric average of the initial and final absorption coefficients. L is the pathlength of the sample the irradiation passes. The AQY were then calculated according to:

$$\boldsymbol{avg} = \frac{\Delta \boldsymbol{a}_{\boldsymbol{avg}}}{\boldsymbol{I}_{\boldsymbol{a}}}$$

3.7 Excitation-emission matrices

CDOM is composed of a complex chemical mixture including humic substances, amino acids, peptides, proteins and carbohydrates, for instance. Since excitation and emission wavelengths are specific to the molecule (Lakowicz, 1999), EEMs are indicative of fluorescent compounds present in the complex CDOM mixture (Stedmon et al., 2005).

When a molecule absorbs light, an electron is excited from the ground state to a higher energy level (an unoccupied orbital), which is referred to as singlet state (S_1,S_2,..). The electron then returns to the lowest level of the singlet state (non-radiative) and fluorescence occurs when the electron returns to the ground state. The difference in energy level between the lowest S_1 state and the ground state is emitted as fluorescence. Thus the emission wavelength is longer and poorer in energy than the excitation wavelength (the stokes`shift). With increasing complexity of a molecule, fluorescence shifts towards longer wavelengths (Stedmon et al., 2003; Hudson, 2007)

Coble (1996) found that not the use of a single excitation-emission pair is an adequate tool for characterizing CDOM, due to a shift in wavelengths depending on CDOM origin (e.g., marine versus freshwater), but the wavelength independent fluorescence maxima, coded as A, B, C, M and T (Table 1).

Table 1 Fluorophores and corresponding excitation and emission wavelengths (Coble, 1996; Parlanti et al., 2000).

Fluorophore Name used by Coble(1996)	Fluorophore Name used by Parlanti et al. (2000)	Exciation wavelength [nm]	Emission wavelength [nm]	Type
A	α´	237-260	400-500	Humic-like
C	α	300-370	400-500	Humic-like
B	β	225-237	309-321	Protein-like (Tyrosine)
		275	310	
T	δ	225-237	340-381	Protein-like (Tryptophan)
		275	340	
Chlorophyll a		431	670	
Chlorophyll b		435	659	

Excitation-emission matrices were obtained using a Fluorescence Spectrophotometer (Hitachi F-7000). Measurements were performed in 1 cm quartz cuvettes and using the water Raman peak of Milli-Q water (Millipore) as reference. Excitation spectra were collected at excitation wavelengths ranging from 200 to 450 nm at 5 nm increments. Emission scans were performed at 2 nm increments of emission wavelengths between 250 and 700 nm. The Fluorescence Spectrophotometer was set at a scan speed of 12,000 nm min^{-1} and a response time of 0.01 s. Fluorescence intensities were normalized to the average water Raman peak (λ_{ex}=350 nm; λ_{em}=397 nm) of Milli-Q water (Nieke et al., 1997). An average intensity of 170.42 ± 4.75 (average ± SD, n=8, 10 measurements per sample) of the water Raman peak was reported. Fluorescence intensity was further normalized to the DOC concentration (Parlanti et al., 2000).

3.8 Respiration measurement

During the 9 days of incubation, the concentration of dissolved oxygen was measured at least once a day at one-minute intervals for a period of 30

minutes. Non-invasive determination of oxygen contents could be achieved by using a 4-Channel Fiber-Optic Oxygen Meter (PreSens Precision Sensing GmbH, Regensburg, Germany), utilizing luminescence quenching by molecular oxygen. Prior to the incubations a sensor spot (SP-Pst3, 5mm) acting as a luminophore was glued inside each Schott bottle. A non-invasive fiber-optic probe communicated with the optode and transmitted the signal.

3.9 Microbial cell abundance and biovolume measurements

Samples for bacterial abundance analysis were collected daily from each replicate during the incubation period of 9 days. Samples were preserved with formaldehyde (2.5 % final concentration) and stored in the dark (4°C). Cell abundance was determined using epifluorescence microscopy (Zeiss AxioImager) and Sytox Green (0.1 N in DMSO) for staining. To determine the cell number, 30 fields were counted per sample.

Cell sizes were determined for at least 50 cells at the beginning of the inoculation period for each treatment. Due to low cell abundances at that point of time (T_{min}) this number was chosen to be representative, whereas at the point of maximal biomass (T_{max}) 200 cells were determined for each treatment. Digital images were taken using a digital camera (AxioCam MRc5) in combination with the AxioVision (4.6.1.0) software. Images were then analyzed using the software ImageJ (1.41o). The threshold was set by approximating it to manually measured cell sizes. Overlapping cells and particles with an area smaller than 0.1 µm were excluded.
Cell volumes were calculated using the following equation: $V= (w^2*\pi/4)/ (l-w) + (\pi*w^3/6)$. V is defined as the cell volume in μm^3 and w and l are cell width and length in µm. Cell carbon content was calculated employing the allometric relationship $C = 120*V^{0.72}$ (Norland, 1993). Microbial biomass for each treatment was calculated as the product of cell numbers and average cell carbon content.

Microbial growth rates (MGR) were determined as the increase in cell number over time from T_{min} until just before stationary phase employing the exponential model: $N_t = N_0 * e^{(kt)}$, where N_0 is the cell number at T_{min} and N_t is the cell number just before stationary phase, t is the time (h) and k is the growth rate (h^{-1}). Microbial growth efficiencies (MGE) were calculated as the increase in carbon biomass versus carbon biomass plus respiration (Kragh et al., 2008). The increase in carbon biomass was determined for each experiment and each treatment individually by subtracting the minimal biomass from the maximal biomass. Due to cell lyses, probably caused by the production process of the cell concentrate, day one instead of day zero was chosen to represent the minimum biomass at the beginning of the incubation period.

Respiration was determined as the change in oxygen content over time. Oxygen loss was converted into CO_2 production using a respiratory quotient of 1 (Smith and Prairie, 2004).

3.10 Statistical analyses

Statistics were performed using SPSS (14.0). A t-test was used to test for significant differences between treatments of DOC, SUVA 254, S_r and EEM values. It was tested for significant differences between initial samples (ini), dark controls (con) and irradiated samples (uv). The significance level was corrected for multiple comparisons (Dunn-Sidak correction, Sokal and Rohlf, 1994). Adapted significance levels for 2 comparisons (ini-con and ini-uv) are *0.025, **0.005, ***0.0005, whereas significant differences in values due to the 9-day incubation period were tested using the significance levels *0.05, **0.01, ***0.001. Differences in AQY, I_a, Δa_{avg} between samples were tested using a one-way ANOVA. Changes in BDOC content caused by irradiation were tested using a t-test. A possible increase of MGR in irradiated samples versus dark controls was tested using a Mann-Whitney U-test (1-tailed).

4. Results

4.1 DOC contents and SUVA 254

DOC concentration ranged from 0.95 to 1.71 mg C L^{-1} in the initial samples without UV treatment (Fig. 2. A). Irradiation resulted in a significant (t-test, $P < 0.0005$, n=5) increase in DOC concentration of 31.5±7.66 %. Dark controls, except algae extract (t-test, $P < 0.005$, n=5) did not significantly change in DOC concentration (t-test, $P > 0.025$, n=5). SUVA 254 ranged from 3.81 to 6.7 mg C L^{-1} m^{-1} initially (Fig. 2.B), but irradiation decreased these values on average by 26.2±4.84 % (t-test, $P < 0.0005$, n=5). In dark controls SUVA 254 decreased by 4.8±5.28 % (t-test, $P < 0.025$, n=5). After the incubation period of 9 days, SUVA 254 increased significantly in both treatments (t-test, $P < 0.005$, n=5) (Table 2). Nutrient concentrations (initial and final) are listed in table 3.

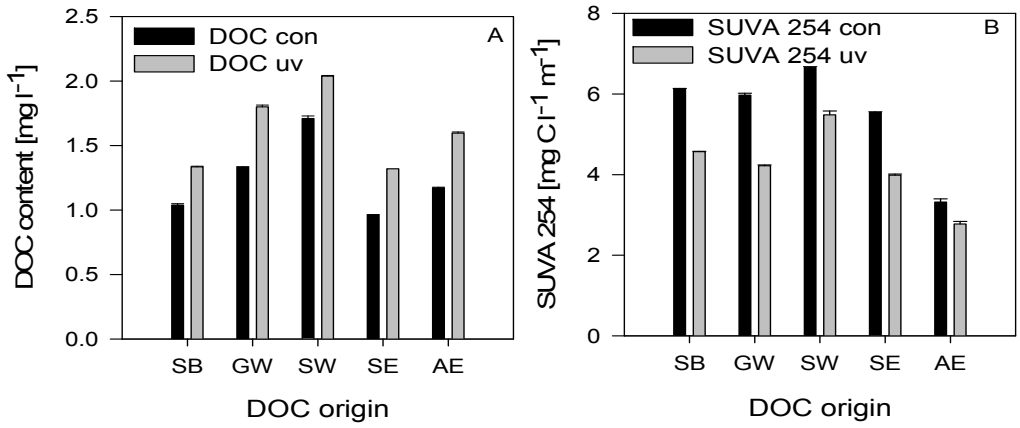

Figure 2 (A) Changes in DOC concentration (B) and SUVA 254 after 12 hours irradiation (uv) and dark control (con). Given is the mean±SD (n=5).

4.2 Absorbance

Helms et al. (2008) showed an inverse correlation between the S_R and molecular weight of CDOM. Therefore, I used S_R to obtain information about possible changes in molecular weight of DOC caused by irradiation. S_R

ranged from 0.75 to 0.85 initially, and irradiation increased on average S_R values by 30.3 ± 4.71% (Fig. 3). After 12 hours of irradiation, S_R differed significantly (t-test, $P < 0.0005$, n=5) from the initial S_R (Table 4). Most changes in optical properties occurred during the first 4 hours of irradiation. Significant (t-test, $P > 0.025$, n=5) changes in optical properties could not be shown for the dark controls. A shift in absorbance from the $S_{350\text{-}400}$ nm to the $S_{275\text{-}295}$ nm region occurred in all experiments after irradiation (Table 4).

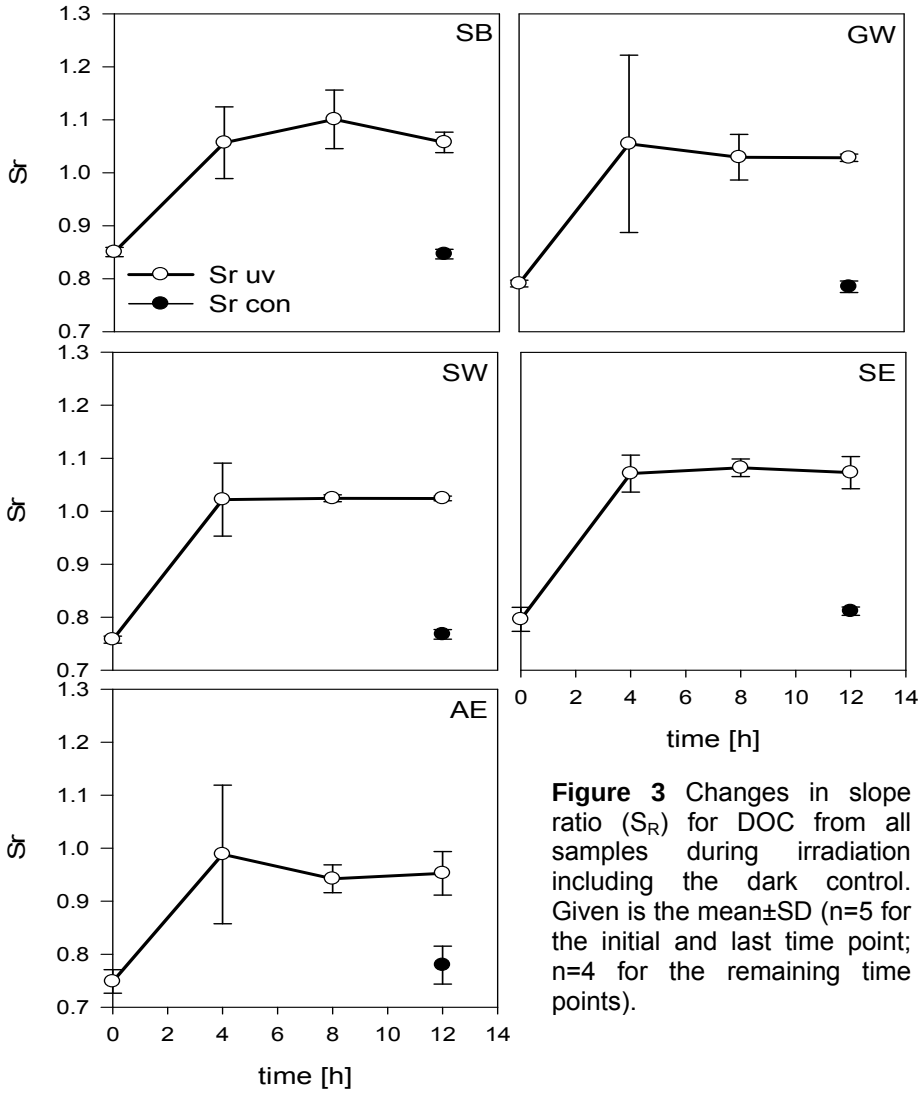

Figure 3 Changes in slope ratio (S_R) for DOC from all samples during irradiation including the dark control. Given is the mean±SD (n=5 for the initial and last time point; n=4 for the remaining time points).

We calculated the AQY to describe and compare the photoreactivity of CDOM derived from different sources. The average decrease in absorption

(Δa_{avg}) did not differ significantly (one-way ANOVA, $F_{4;\ 20df}$ =6.990, P>0.05) between streamwater, groundwater, soil water and algae extract; Δa_{avg} was significantly (one-way ANOVA, $F_{4;\ 20df}$ =6.990, P<0.05) lower for soil extract (Fig. 4 A). The total quanta (I_a) in absorbed by the samples during the 12 hours of irradiation ranged from 2.55 – 4.93 mol photons m^{-2} (Fig. 4A). Samples from streamwater revealed a significantly (one-way ANOVA, $F_{4;\ 20df}$=816.098, P<0.001) lower I_a than groundwater and soil water samples. However, it did not significantly (one-way ANOVA, $F_{4;\ 20df}$ =816.098, P>0.05) differ from soil extract and algae extract. A higher absorption was observed for soil extract compared to algae extract (one-way ANOVA, $F_{4;\ 20df}$ =816.098, P>0.05).Groundwater, soil water and soil extract had significantly (one-way ANOVA, $F_{4;\ 20df}$ =18.819, P<0.05) lower AQY compared to samples from streamwater and algae extract. The AQY of streamwater and algae extract did not differ significantly (one-way ANOVA, $F_{4;\ 20df}$ =18.819, P>0.05) (Fig. 4.B).

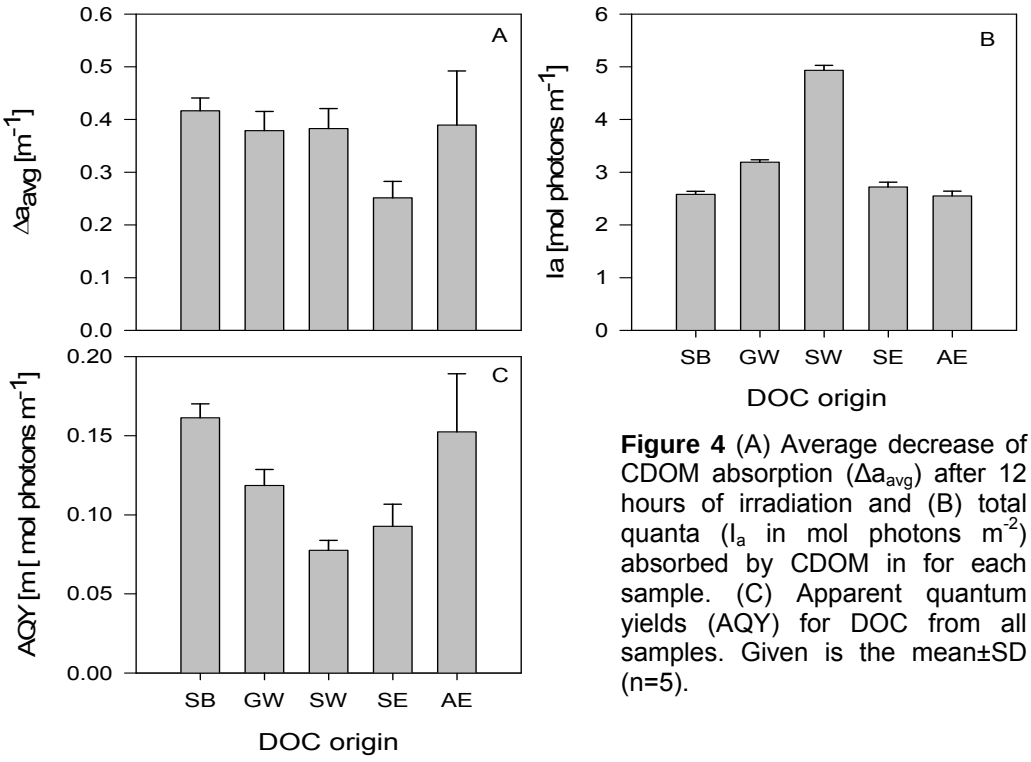

Figure 4 (A) Average decrease of CDOM absorption (Δa_{avg}) after 12 hours of irradiation and (B) total quanta (I_a in mol photons m^{-2}) absorbed by CDOM in for each sample. (C) Apparent quantum yields (AQY) for DOC from all samples. Given is the mean±SD (n=5).

4.3 Excitation Emission Matrixes

Excitation-emission spectroscopy was employed to qualitatively describe and monitor alterations of fluorescent fractions due to irradiation and microbial degradation processes. In experiments based on allochthonous material (soil water, soil extract, and groundwater) the humic like fractions (as indicated by the peaks encoded as A and C; Coble, 1996) showed higher fluorescence in comparison to DOC from algae extract samples (i.e., autochthonous carbon) (Fig. 5).

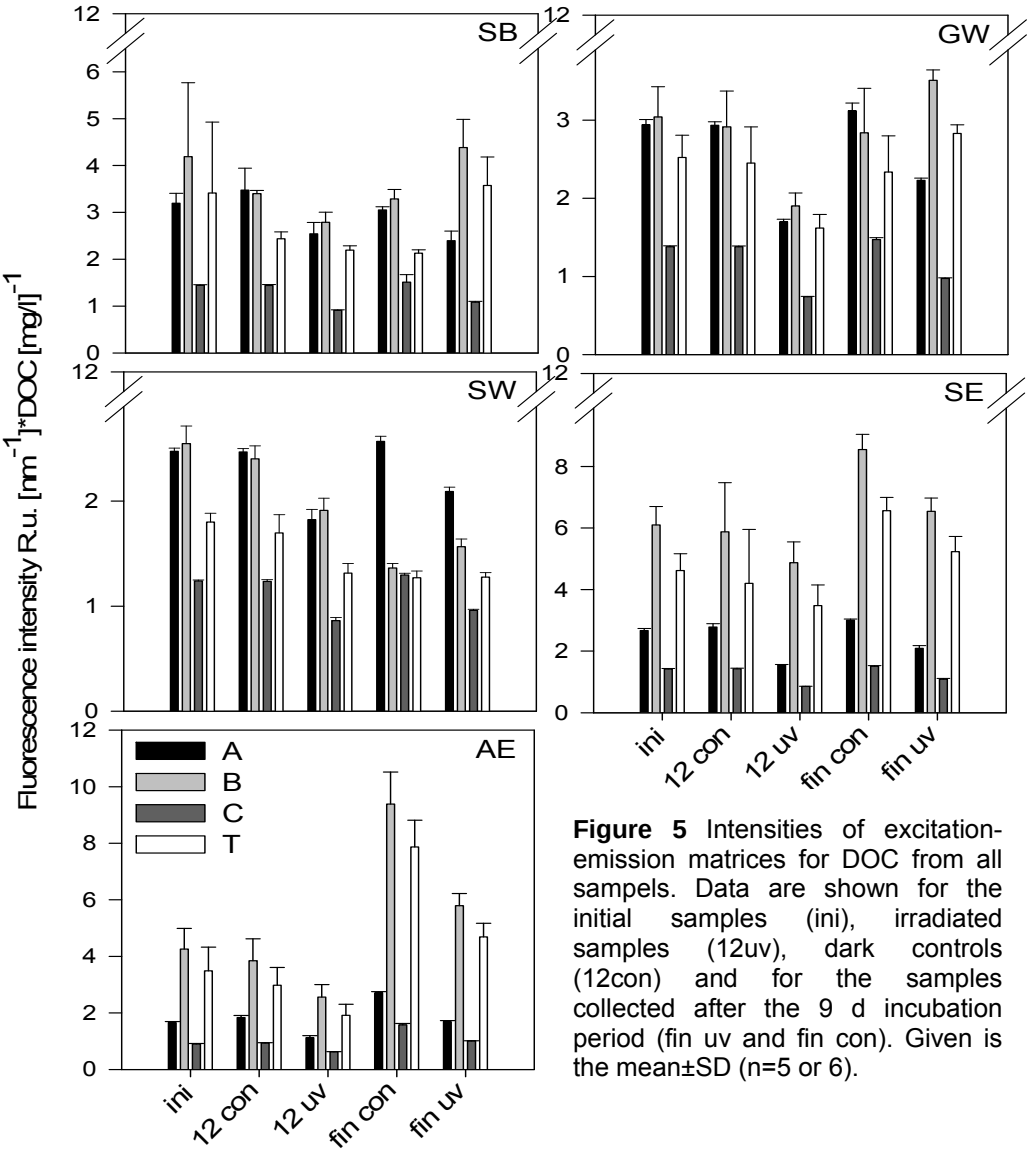

Figure 5 Intensities of excitation-emission matrices for DOC from all sampels. Data are shown for the initial samples (ini), irradiated samples (12uv), dark controls (12con) and for the samples collected after the 9 d incubation period (fin uv and fin con). Given is the mean±SD (n=5 or 6).

Samples from streamwater also showed a high humic-like peak comparable to allochthonous carbon. Algae extract samples showed a higher fluorescence in protein-like peaks. A significant (t-test, $P < 0.005$, n=5) decrease in fluorescence of humic-like substances after irradiation was observed. Fluorescence of the protein-like peaks B and T significantly (t-test, $P < 0.025$, n=5) decreased in all experiments, except for streamwater (t-test, $P > 0.025$, n=5) after irradiation. Fluorescence intensities in dark controls did not change (t-test, $P > 0.025$, n=5), with the exception of peak A and C in algae extract (t-test, $P < 0.005$, n=5). Peak B in both treatments (irradiated and dark control) increased significantly (t-test, $P < 0.05$, n=5) after the 9 day incubation period, apart from the dark controls of streamwater samples. An increase in fluorescence of humic-like fractions after the incubation period could be shown for all experiments (t-test, $P < 0.01$, n=6) except for streamwater samples. Here peak A in both treatments and peak C in dark control remained unchanged (t-test, $P > 0.05$, n=6).

4.4 Microbial growth and oxygen consumption

We consistently observed an increase in cell abundance and a decrease in oxygen concentration over the incubation period of 9 days (Fig. 6). Bacterial growth in the algae extract sample was approximately 5 times higher than in the other samples. Irradiation enhanced bacterial growth and respiration in the majority of the samples. Only for algae extract no major effect of irradiation on bacterial growth and respiration could be observed. Maximal microbial abundance occurred earlier in irradiated samples that contained a large portion of allochthonous material, than in dark controls. For algae extract maximal biomass was observed earlier in the dark controls.

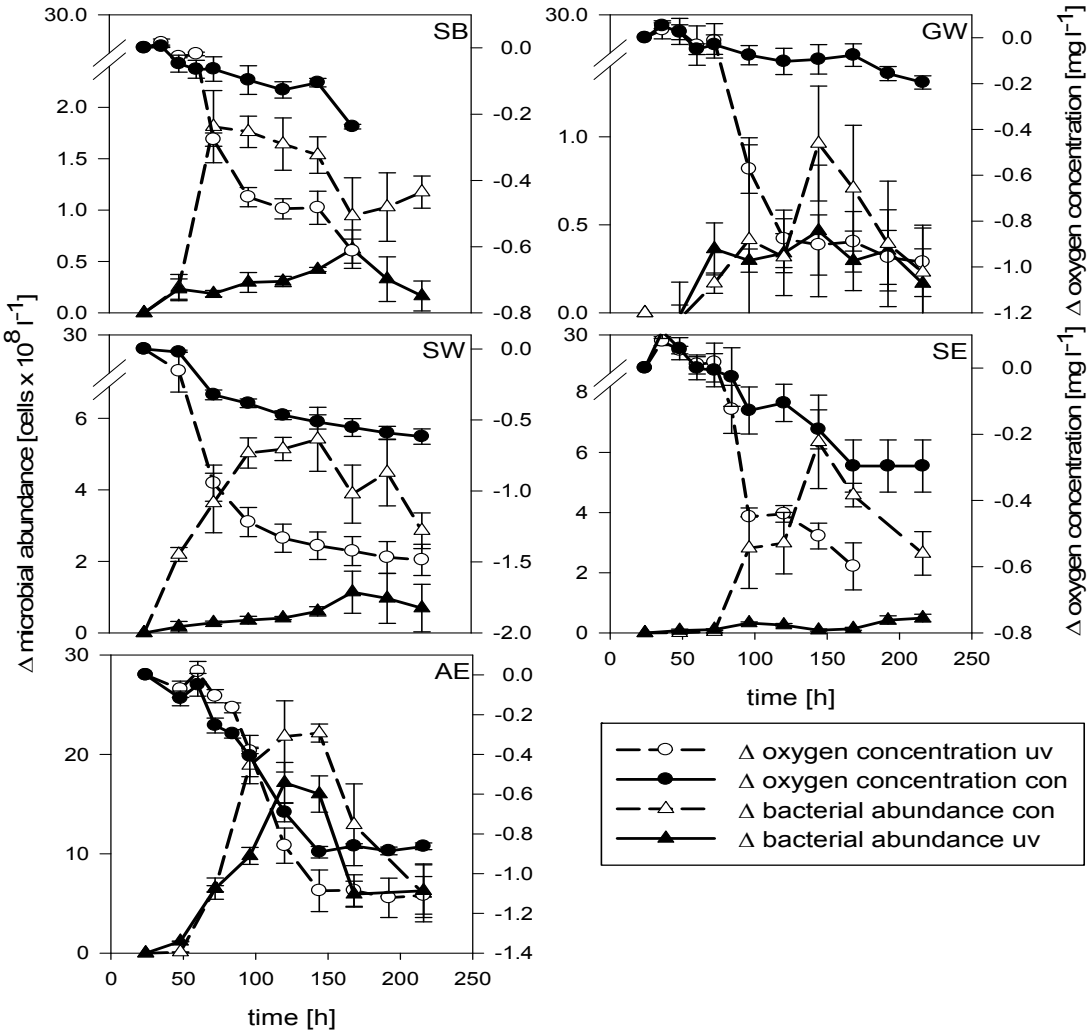

Figure 6 Increase in cell number as Δ microbial abundance (T_0-T_X) and decrease in oxygen content as Δ oxygen concentration (T_0-T_X) for all DOC origins during the 9 d incubation. Data are shown for irradiated and dark control treatments. Given is the mean±SD (n=3 for cell numbers, n=2 for oxygen concentration).

4.5 Bacterial growth rate and efficiency

BDOC from streamwater, groundwater, soil water, and soil extract samples increased significantly by 13.7± 4.09% after irradiation (t-test, P< 0.001, n=6). Algae extract — with highest BDOC initially — was not affected by irradiation (t-test, $t_{5.94df}$=1.925, P>0.05) (Fig. 7. A). Growth rates increased significantly (U-test, 1-tailed, P < 0.05, n=3) after irradiation, but showed no significant (U-test, 1-tailed, P > 0.05, n=3) response to irradiation of groundwater (Fig. 7.B).

Doubling times correspond to MGR (Fig. 7D). MGE was generally enhanced by irradiation, except for groundwater (Fig. 7C).

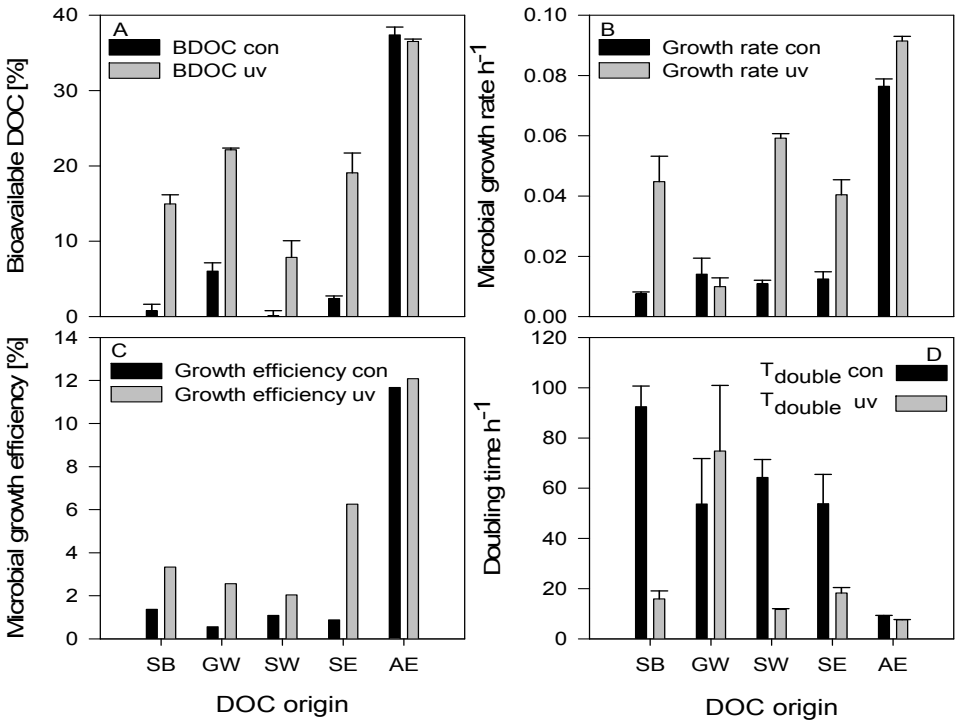

Figure 7 (A) Percentage (%) of BDOC, (B) bacterial growth rates, (C) growth efficiencies and (D) doubling times. Data are shown for irradiated and dark treatments. Given is the mean±SD (n=6 for A; n=6 for B and C)

A significant correlation of BDOC content of both treatments and MGR could be shown ($r= 0.79$, $P < 0.01$), as well as a correlation of BDOC and MGE ($r= 0.85$, $P < 0.01$) (Fig. 8 C, D). S_R values were not related to MGR ($r= 0.26$, $P > 0.05$) and MGE ($r= -0.011$, $P > 0.05$). A significant relationship between S_R and MGR was found when algae extract was excluded ($r= 0.71$, $P < 0.05$).

A weak "grouping effect" of both treatments for MGR and MGE by S_R was observed. Irradiated samples always exhibited higher S_R values, whereas a lower S_R was observed in dark controls (Fig. 8 A, B).

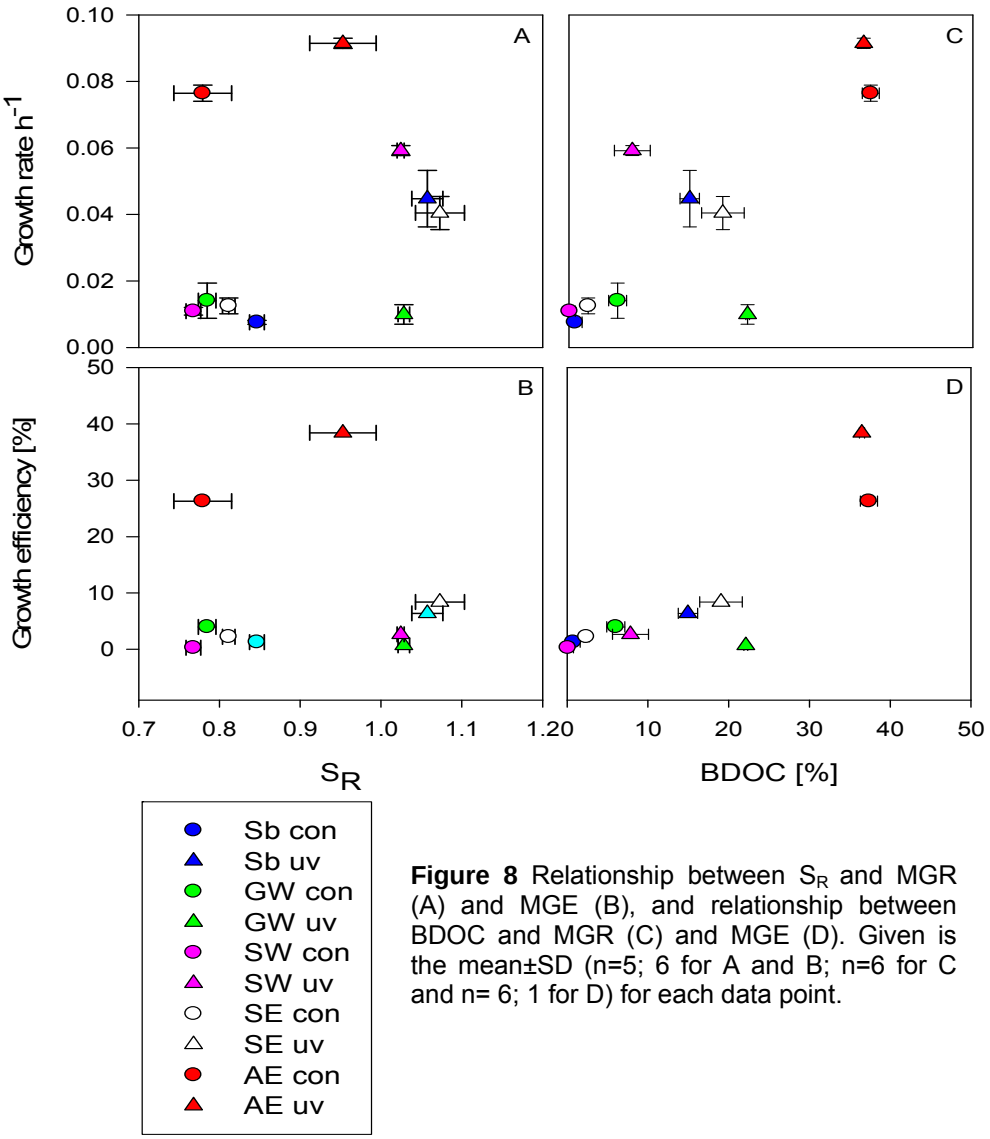

Figure 8 Relationship between S_R and MGR (A) and MGE (B), and relationship between BDOC and MGR (C) and MGE (D). Given is the mean±SD (n=5; 6 for A and B; n=6 for C and n= 6; 1 for D) for each data point.

5. Discussion

Most studies on photochemical CDOM transformation focus on marine (Osburn et al, 2009) and lake water rich in humics (Kragh, 2008; Helms, 2008).

Few have studied possible photochemical effects on streamwater (Belmont et al., 2009). In this study, I tested possible effects of photooxidation on the optical properties of CDOM in different waters along the flow path adjacent to a clear-water stream. I further studied the effects of photochemically altered CDOM on microbial respiration and growth. Exposure to a spectrum similar to natural sunlight significantly affected the optical properties of CDOM and consequently augmented the bioavailability of DOC to the microbial metabolism.

5.1 Optical analyses

DOC concentration slightly but consistently increased after irradiation, which contradicts the generally reported decreasing effect of UV irradiation (Judd et al., 2006). This may be attributable to the photochemical cleavage of initially large into smaller compounds, which may then facilitate the analytical (UV via Sievers) oxidation of DOC ultimately leading to higher concentrations. Irradiation resulted in a change of optical properties of DOC in all experiments. SUVA 254 was reduced by irradiation indicating a decrease in aromaticity (Weishaar et al., 2003); however the SUVA 254 in dark controls decreased significantly as well. The decrease of SUVA 254 of irradiated samples was on average 20 fold higher than in the dark controls.

Irradiation caused a shift in absorbance from the $S_{275\text{-}295}$ nm to the $S_{350\text{-}400}$ nm region, thus increasing S_R, which is indicative of a production of LMW compounds. Helms et al. (2008) suggested that chromophores associated with HMW CDOM are destroyed by photobleaching, causing CDOM to partially shift from the HMW to the LMW fraction. These authors showed an inverse relationship between S_R and molecular weight of CDOM. This is

consistent with the findings of Kieber et al. (1989) that photooxidative degradation of organic matter enhances the cleavage of macromolecules and the release of CO_2 and O_2.

The 9-day incubation period resulted in a decrease of S_R for DOC from all waters but not for all treatments. This may be due to microbial production or preservation of fractions that show absorbance in the long wavelength region. Helms et al. (2008) and Moran et al. (2000) found similar results after longer periods of incubation. Excitation Emission matrixes showed that in samples with allochthonous DOC (soil water, soil extract, and groundwater), humic-like fractions had higher fluorescence maxima initially when compared to samples with autochthonous DOC, such as the algal extracts. Samples from streamwater also showed a relatively high humic-like peak emphasizing the relevance of allochthonous carbon in the study stream. Generally a decrease in humic substances and protein-like fractions after irradiation was observed. These results indicate a photooxidative destruction of humic substances and proteins. The increase in fluorescence of protein-like fractions is most probably caused by cell lyses due to DOC limitation after the incubation period of 9 days. An increase in fluorescence of humic-like fractions after the incubation period could be observed in most experiments and treatments.

The total quanta (I_a) in absorbed by streamwater was significantly lower compared to groundwater and soil water; however, it did not significantly differ from soil extract and algae extract. A higher absorption was observed for soil extract compared to algae extract. Allochthonous carbon (groundwater, soil water and soil extract) had lower AQY compared to streamwater and algae extract. Though I_a values of these samples were high, the decrease in absorption coefficients was comparatively low, thus decreasing AQY. This indicates that a large quantity of photons is required to change the optical properties of allochthonous CDOM, whereas autochthonous CDOM is easily changed and therefore more photoreactive.

5.2 Microbial growth rate and efficiency

Tranvik and Bertilsson (2001) found that irradiation of low-aromaticity DOC from lakes resulted in more refractory material. Algae extract initially contained a high concentration of BDOC which was not changed by irradiation, whereas in allochthonous and streamwater samples BDOC content increased significantly. This suggests that mainly refractory DOC is affected by irradiation. Putatively refractory material, such as humics and lignin mainly derived from terrestrial vegetation, is photochemically transformed into less aromatic compounds. Subsequently microbial growth and respiration were generally stimulated by DOC previously exposed to irradiation. Microbial abundance peaked at different points of time. For instance, in samples with clearly allochthonous DOC the lag phase became particularly clear comparing dark controls to UV treatments. This implies that irradiation caused the production of a portion of labile substrates that fuelled microbial metabolism. In the dark controls growth was slower, indicating a higher portion of more refractory DOC. The experiment based on algae extract exhibited the highest bacterial growth. This was to be expected when considering the dependence of bacterial secondary production on autochthonous primary production as described by Peduzzi et al (2008), for instance.

Although allochthonous CDOM is less photoreactive, as indicated by the AQY, photochemical changes show a stronger effect of this CDOM on the bioavailability and bacterial growth compared to autochthonous CDOM. Photochemically pre-treated DOC was assimilated more rapidly in comparison to not irradiated DOC. This is consistent with the notion that photooxidative degradation of organic matter enhances the cleavage of macromolecules (Kieber et al., 1989) and results in the production of bioavailable LMW compounds.

Irradiation of allochthonous and autochthonous DOC enhanced MGR and MGE for streamwater, soil water and soil extract, which corresponds to indicated decreases in molecular weight and humic like fractions. As

expected, MGE and MGR correlated significantly to BDOC concentrations. An increased portion of BDOC supported bacterial metabolism and thus enhanced MGR and MGE. Though the BDOC concentration of groundwater was increased by irradiation no significant effect could be observed for MGR and MGE, which might be due to phosphate limitation. A “clustering effect” of MGR and MGE in both treatments by S_R was observed. Irradiated samples always exhibited higher S_R values, whereas a lower S_R was observed in dark controls. Irradiation resulted in a comparatively low increase of S_R for algae extract. A higher S_R initially would be expected when considering algal derived DOM to be bioavailable. Since DOC consists of a highly complex mixture of compounds (Thurman, 1985; Stedmon et al., 2005), S_R is indicative of changes in molecular weight (Helms et al., 2008), but does not detect all compounds that influence bioavailability. More research is needed to identify the individual DOC compounds and their behaviour to photooxidation and subsequent influence on bioavailability.

SB and soil water showed similar growth patterns, which is indicative of a large proportion of soil derived carbon in the stream and reflects the pool of humic substances indicated by EEM. This putatively refractory organic carbon can be photooxidized by natural solar irradiation during downstream transport and partially converted into LMW compounds low in humics and with potentially higher bioavailability. Most of the change in optical properties took place during the first 4 hours of irradiation. Since the residence time of the Lunzer Seebach is approximately 7.26 h it is reasonable to assume that the effects observed in the laboratory reflect natural occurrences.

The photochemical alteration of CDOM combined with storage and retention zones along the downstream transport (Battin et al., 2008) provides the opportunity for microbial processing of the photooxidized substrates, thus reducing CDOM concentrations in stream ecosystems and increasing CO_2 emissions. Hydrological connectivity between a stream and its adjacent water landscape is therefore an important factor controlling DOM dynamics and

microbial processing. It is imperative to understand the effects of photochemical alteration on CDOM from various sources in freshwater ecosystems and the subsequent microbial degradation processes to evaluate the role of stream ecosystems in the global carbon budget.

This study shows the significance of photochemical impacts on clear water streams with low DOC concentration. Taking into consideration that these streams are representative for alpine regions, predicted susceptible to climate change, these photochemical impacts deserve more research including optical analyses such as EEM and UV-VIS spectroscopy.

6. Appendix

6.1 Tables

Table 2 Decrease of the SUVA 254 after irradiation. Data is shown for the initial samples (ini), irradiated samples (12uv), dark controls (12con) and for the samples taken after the incubation period of 9 days (fin uv and fin con). Values are the mean of 5 (ini, 12uv, 12con), and 6 (fin con, fin uv) data points. SD is the standard deviation. Significant results for tests 12con- fin con and 12uv – fin uv are identified by asterisks (*0.05, **0.01, ***0.001). Dunn-Sidak corrected (Sokal and Rohlf, 1994) tests (ini – 12uv, ini –12con) were tested using corrected significance levels (*0.025, **0.005, ***0.0005).

DOC origin/treatment	SUVA 254 [mg C L^{-1} m^{-1}]	SD
SB initial	6.16	0.01
SB 12uv	4.57***	0.01
SB 12con	6.12*	0.02
SB fin uv	5.61***	0.11
SB fin con	6.33***	0.02
GW initial	6.08	0.02
GW 12uv	4.22***	0.02
GW 12con	5.97**	0.05
GW fin uv	5.62***	0.04
GW fin con	6.44***	0.04
SW initial	6.70	0.02
SW 12uv	5.48***	0.10
SW 12con	6.65	0.02
SW fin uv	6.22***	0.07
SW fin con	6.75**	0.04
SE initial	5.62	0.05
SE 12uv	3.98***	0.03
SE 12con	5.54*	0.02
SE fin uv	5.14***	0.06
SE fin con	6.08**	0.24
AE initial	3.81	0.05
AE 12uv	2.78***	0.06
AE 12con	3.32***	0.08
AE fin uv	3.57***	0.08
AE fin con	5.13***	0.21

Table 3 Nutrient concentration before (after nutrient addition, ini) and after the 9-day incubation period for both treatments (fin con, fin uv). (* Nutrients were not measured, but calculated) Given is the mean±SD (n= 3-5)

DOC origin/ treatment	N-NH4 [µg/l]	SD	N-NO3 [µg/l]	SD	P-PO4 [µg/l]	SD
SB ini	9.10	0.56	1827.77	7.39	5.87	0.21
SB fin uv	2.97	0.62	2031.57	17.12	1.83	0.73
SB fin con	3.02	1.31	1951.07	6.37	4.57	0.32
GW ini	10.33	0.25	1878.83	14.96	2.20	0.40
GW fin uv	3.60	0.64	1853.35	30.80	0.00	0.15
GW fin con	1.48	0.41	1775.03	13.52	0.37	0.34
SW ini*	8.00		2000.00		6.00	
SW fin uv	4.87	0.81	1906.43	45.92	7.58	0.69
SW fin con	4.22	0.48	1873.43	27.07	14.92	0.61
SE ini	10.48	0.26	1797.98	36.81	6.18	0.31
SE fin uv	5.15	0.96	1980.91	115.68	1.05	0.30
SE fin con	3.97	0.66	1911.48	144.10	5.00	1.22
AE ini	20.65	0.07	1114.35	2.47	1.90	0.14
AE fin uv	33.58	5.04	2099.70	6.21	0.37	0.56
AE fin con	15.87	1.54	1967.63	11.76	0.25	0.21

Table 4 Slopes of the $S_{275-295}$ and $S_{350-400}$ region and the slope ratio (S_R). S_R generally increased after irradiation (t-test, $P < 0.001$, n=5; Dunn-Sidak corrected, Sokal and Rohlf, 1994). $S_{275-295}$ values decrease after irradiation, whereas $S_{350-400}$ increase. Data is shown for the initial samples (ini), irradiated samples (12uv), dark controls (12con) and for the samples taken after the incubation period of 9 days (fin uv and fin con). Values are the mean of 5 (ini, 12uv, 12con), 4 (4uv, 8uv) and 6 (fin con, fin uv) data points. SD is the standard deviation. Significant results for tests 12con- fin con and 12uv – fin uv are identified by asterisks (*0.05, **0.01, ***0.001). Dunn-Sidak corrected (Sokal and Rohlf, 1994) tests (ini – 12uv, ini –12con) were tested using corrected significance levels (*0.025, **0.005, ***0.0005).

DOC origin/ treatment	$S_{275-295}$	SD	$S_{350-400}$	SD	avg. S_R	SD
SB initial	0.017	0.000	0.020	0.000	0.85	0.01
SB 4uv	0.019	0.000	0.018	0.001	1.06	0.07
SB 8uv	0.020	0.000	0.018	0.001	1.10	0.06
SB 12uv	0.022	0.000	0.021	0.000	1.06***	0.02
SB 12con	0.017	0.000	0.020	0.000	0.85	0.01
SB fin uv	0.019	0.000	0.019	0.001	1.01*	0.04
SB fin con	0.016	0.000	0.020	0.000	0.79***	0.01
GW initial	0.017	0.000	0.022	0.000	0.79	0.01
GW 4uv	0.019	0.001	0.018	0.003	1.05	0.17
GW 8uv	0.021	0.000	0.020	0.001	1.03	0.04
GW 12uv	0.021	0.000	0.021	0.000	1.03***	0.01
GW 12con	0.017	0.000	0.022	0.000	0.78	0.01
GW fin uv	0.020	0.000	0.021	0.001	0.95**	0.03
GW fin con	0.016	0.000	0.022	0.000	0.72***	0.01
SW initial	0.017	0.000	0.022	0.000	0.76	0.01
SW 4uv	0.019	0.000	0.018	0.002	1.02	0.07
SW 8uv	0.020	0.000	0.019	0.000	1.02	0.01
SW 12uv	0.020	0.000	0.020	0.000	1.02***	0.00
SW 12con	0.016	0.000	0.021	0.000	0.77	0.01
SW fin uv	0.018	0.000	0.018	0.002	1.03	0.08
SW fin con	0.015	0.000	0.021	0.001	0.72**	0.02
SE initial	0.016	0.000	0.020	0.001	0.80	0.02
SE 4uv	0.019	0.000	0.017	0.001	1.07	0.03
SE 8uv	0.020	0.000	0.018	0.000	1.08	0.02
SE 12uv	0.020	0.000	0.019	0.001	1.07***	0.03
SE 12con	0.016	0.000	0.020	0.000	0.81	0.01
SE fin uv	0.017	0.000	0.017	0.001	0.99**	0.02
SE fin con	0.014	0.000	0.017	0.001	0.79	0.04
AE initial	0.014	0.000	0.019	0.001	0.75	0.02
AE 4uv	0.016	0.001	0.016	0.002	0.99	0.13
AE 8uv	0.017	0.000	0.018	0.001	0.94	0.03
AE 12uv	0.017	0.000	0.018	0.000	0.95***	0.04
AE 12con	0.014	0.000	0.018	0.001	0.78	0.04
AE fin uv	0.014	0.000	0.016	0.001	0.86	0.03
AE fin con	0.011	0.000	0.015	0.001	0.73**	0.04

6.2 Zusammenfassung

Die Herkunft, Struktur, Zusammensetzung, die photochemische Veränderung und Bioverfügbarkeit von gelösten Organischen Material (DOM) beeinflussen Fließgewässerökosysteme und ihre Kohlenstoff Flüsse. Das Ziel dieser Studie ist das Verständnis, wie diese Komponenten chromophores DOM (DDOM) in seinen optischen Eigenschaften und mikrobieller Prozessierung beeinflussen. Die Experimente inkludierten das Monitoring von photochemischen Effekten von Strahlung auf autochthon und allochthon produzierten Kohlenstoff und anschließenden mikrobiellen Abbauprozessen.

Excitation-Emission Spektroskopie und Absorptions- Spektroskopie ermöglichten eine Beschreibung und Qualifikation der Änderung von optischen und fluoreszierenden Eigenschaften. Die mikrobielle Respiration wurde beobachtet um eine Änderung in der Bioverfügbarkeit von bestrahlten Proben des Lunzer Unterseebaches und von verschiedenen anschließenden Wasserkörpern zu identifizieren. Die Proben wurden einem Spektrum, ähnlich dem des natürlichen Sonnenlichts, ausgesetzt. Dadurch veränderten sich die optischen Eigenschaften signifikant und die Bioverfügbarkeit des DOC für den mikrobiellen Metabolismus wurde erhöht. Autochthones DOC von periphytischen Algen stammend folgte diesem Muster nicht.
Die Resultate indizieren, dass ein großer Anteil an organischem Kohlenstoff, welcher aus dem Auboden stammt, in den Bach eingeschwemmt wurde. Meine Resultate schlagen vor, dass dieser als refraktär betrachtete organische Kohlenstoff einer Photooxidation durch natürliches Sonnenlicht während eines Bach abwärts gerichteten Transportes unterliegt, und so teilweise in Komponenten eines geringeren Molekulargewichtes transformiert wird. Diese veränderten Substrate erhöhen den mikrobiellen Metabolismus. Die photochemische Veränderung von CDOM bietet die Möglichkeit einer mikrobiellen Prozessierung der photooxidierten Substrate, wodurch CO_2 Emissionen erhöht und die CDOM Konzentrationen reduziert werden.

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Education:

September 1991 until June 1995:	Primary school Strasshof
September 1995 until April 1998:	Secondary school Gänserndorf
April 1998 until December 2001:	German School London
December 2001 until June 2004:	Naturwissenschaftliches Oberstufenrealgymnasium Brigittenau (Grammar school with emphasis on natural science)
June 2004:	Matura (final exam)
Since October 2004:	studies of Biology at the University of Vienna
June 2006:	diploma examination(first part); Studies of Ecology
Since October 2008:	work on my diploma thesis; advisor: Dr.Tom J. Battin ("Impact of photooxidation on microbial degradation of dissolved organic carbon in streamwater and shallow groundwater")

Languages:

German, English, French

Computer literacy:

MS Word, MS Excel, MS PowerPoint, ArcGis, Geomedia, SPSS, Sigma Plot

Work experience:

1st July until 31. July 2003 at Raccolta, Molnar & Greiner GmbH, 1090 Wien (trading company) in reception and accounting