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Increasing addition of autochthonous to allochthonous carbon in nutrient-rich aquatic systems stimulates carbon consumption but does not alter bacterial community composition

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Abstract

Dissolved organic carbon (DOC) concentrations – mainly of terrestrial origin – are increasing worldwide in inland waters. The biodegradability of the DOC varies depending on quantity and chemical quality. Heterotrophic bacteria are the main consumers of DOC and thus determine DOC temporal dynamics and availability for higher trophic levels. It is therefore crucial to understand the processes controlling the bacterial turnover of additional allochthonous and autochthonous DOC in aquatic systems. Our aim was to study bacterial carbon (C) turnover with respect to DOC quantity and chemical quality using both allochthonous and autochthonous DOC sources. We incubated a natural bacterial community with allochthonous C (^{13}C-labeled beech leachate) and increased concentrations and pulses (intermittent occurrence of organic matter input) of autochthonous C (algae lysate). We then determined bacterial carbon consumption, activities, and community composition together with the carbon flow through bacteria using stable C isotopes.

The chemical analysis of single sources revealed differences in aromaticity and fractions of low and high molecular weight substances (LMWS and HMWS, respectively) between allochthonous and autochthonous C sources. In parallel to these differences in chemical composition, we observed a higher availability of allochthonous C as evidenced by increased DOC consumption and bacterial growth efficiencies (BGE) when solely allochthonous C was provided. In treatments with mixed sources, rising concentrations of added autochthonous DOC resulted in a further, significant increase in bacterial DOC consumption from 52 to 68 % when nutrients were not limiting. This rise was accompanied by a decrease in the humic substances (HS) fraction and an increase in bacterial biomass. Stable C isotope analyses of phospholipid fatty acids (PLFA) and respired dissolved inorganic carbon (DIC) supported a preferential assimilation of autochthonous C and respiration of the allochthonous C. Changes in DOC concentration and consumption in mixed treatments did not affect bacterial community composition (BCC), but BCC differed in single vs. mixed incubations.

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Our study highlights that DOC quantity affects bacterial C consumption but not BCC in nutrient-rich aquatic systems. BCC shifted when a mixture of allochthonous and autochthonous C was provided simultaneously to the bacterial community. Our results indicate that chemical quality rather than source of DOC per se (allochthonous vs. autochthonous) determines bacterial DOC turnover.

1 Introduction

The major organic carbon (C) pool in aquatic ecosystems is dissolved organic carbon (DOC; Cole et al., 2007). DOC consists of a heterogeneous mixture of different C compounds that vary in chemical quality and include both autochthonous DOC, e.g., C released by aquatic algae and macrophytes, and allochthonous C originating from terrestrial areas surrounding aquatic ecosystems. Both the quantity and quality of autochthonous and allochthonous C sources may alter the internal C-cycling of the recipient aquatic ecosystem. The terrestrial organic C input into freshwater systems accounts for ca. 2.9 $Pt\,C\,yr^{-1}$ worldwide (Tranvik et al., 2009). Contributions of terrestrial DOC to aquatic systems increased in the past decades (Hansson et al., 2013) and are related to rising atmospheric CO_2 concentrations, climate warming, continued N deposition, decreased sulfate deposition, land use and hydrological changes due to increased precipitation and/or droughts (reviewed in Porcal et al., 2009) thus highlighting the importance of studying the turnover of allochthonous C in relation to autochthonous C sources.

DOC serves as an important substrate and energy source for the majority of microorganisms in pelagic systems (Azam et al., 1983). Metabolic activities of the pelagic bacterial community thus control DOC turnover, which in turn, is determined by DOC concentrations and quality. Although it is well known that bacterial production positively correlates with DOC concentrations (Cole et al., 1988), little is known how the ratio between allochthonous and autochthonous C sources and the chemical quality of DOC influences bacterial activities in aquatic systems. Bacterial community composi-

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tion (BCC) may also be crucial for DOC turnover. It has been suggested that DOC as a C source can act as a strong control of BCC (Judd et al., 2006; Docherty et al., 2006). In marine systems it has been shown that different phylogenetic groups consume specific dissolved organic compounds (Cottrell and Kirchman, 2000). Van Hannen et al. (1999) and Grossart et al. (2007) related changes in BCC to organic matter quality, but Langenheder et al. (2005) recognized only weak shifts in BCC in response to a new DOC source. Despite the importance of DOC in pelagic systems, the role of DOC quantity and quality on bacterial communities and their activities remains equivocal.

It is important to combine chemical characterization of DOC with bacterial DOC consumption measurements to provide information on bioavailability of the total DOC pool. Bacterial consumption of DOC can be understood in terms of "DOC reactivity" in which labile DOC is processed within hours to days, whereas refractory DOC is degraded within weeks to years or even longer (reviewed in Søndergaard and Middelboe, 1995). In nature, fresh organic matter is not consistently introduced into aquatic systems, but rather through intermittent events such as the breakdown of an algal bloom or heavy rains. We hereafter refer to those events as pulses. The pulsed organic matter input can change bacterial activities and, hence, the C turnover in the system (Farjalla et al., 2006). The pulsed inputs are, to our knowledge, not considered in models although being a potentially important mechanism to consider when modeling DOC and C cycling in aquatic systems (Tranvik et al., 2009).

We aimed to evaluate the effects of fresh allochthonous and autochthonous C sources on C turnover and BCC, and to elucidate the degradation of distinct DOC fractions by natural bacterial communities. We incubated a fixed amount of leaf leachates (DOC_{leaf}) and added different concentrations of an algal lysate (DOC_{algae}) together with bacterial communities from the littoral zone of a temperate shallow lake. The setup is suitable to measuring the effect of increasing autochthonous C sources, e.g. DOC release during breakdown of algal blooms, on bacterial activities and BCC. We hypothesized that bacterial C turnover is stimulated by the addition of autochthonous DOC

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to a given allochthonous C source and that mixing both C sources results in a shift in BCC.

2 Methods

2.1 Experimental set-up

We sampled water from the littoral zone (0.5 m depth) of Schulzensee, a small, shallow, eutrophic lake in northeastern Germany. The lake water was pre-filtered through 0.8 µm to exclude grazers and the filtrate with the bacterial community was concentrated 25-fold by tangential flow filtration using a 0.2 µm filter. The bacterial concentrate was stored overnight at 4 °C to minimize activities and changes in the community prior to inoculation the next day. The incubation medium consisted of artificial lake water (Lehman, 1980) with nitrate (1.6 mg L^{-1}) and phosphate (3.6 µL m^{-1}) additions to avoid nutrient limitations.

We incubated allochthonous dissolved organic carbon (DOC_{leaf}; 7 mg C L^{-1}) for 12 days with increasing concentration levels of autochthonous DOC (DOC_{algae}; 1, 2, 3, and 4 mg C L^{-1}) in 500 mL Erlenmeyer flasks. There were 3 replicates for each treatment and 4 replicates for controls without DOC addition and leachate controls (Fig. 1). We increased the DOC_{algae} concentration by increasing the number and intensity of pulses, thus simulating natural conditions (Fig. 1).

DOC_{leaf} was produced by leaching ^{13}C-enriched beech leaves (*Fagus sylvatica*, L.) from trees grown under a $^{13}CO_2$ atmosphere in greenhouses in Nancy, France. We leached dried beech leaf disks (diameter 1.1 cm) for 48 h in double distilled water at 4 °C in the dark. The DOC_{algae} was extracted from a cyanobacterium (*Aphanizomenon flos-aquae*, L. SAG 31.87) and a green algal (*Desmodesmus* sp.) culture by centrifugation, dissolution in double distilled water, hydrolysis (20 min at 120 bar and 121 °C) and ultrasonication (1.5 min at 10 W). Both DOC_{leaf} and DOC_{algae} were filtered through a pre-rinsed 0.2 µm polycarbonate filter (Whatman, Dassel, Germany) before inoculation.

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We created the following controls: (a) no DOC addition, (b) addition of only DOC_{algae} and (c) addition of DOC_{leaf} ($7\,mg\,C\,L^{-1}$). In the 4 treatments, a base concentration of DOC_{leaf} ($7\,mg\,C\,L^{-1}$) was supplemented with different concentrations of DOC_{algae}: 1, 4, 9 and $16\,mg\,C\,L^{-1}$; these treatments are hereafter referred to as 1, 2, 3, and 4, respectively (Fig. 1). To avoid relatively high DOC concentrations at the beginning of the incubation and mimic pulsed events of organic C input, we supplied the DOC_{algae} in a stepwise manner over 9 days. Treatments 1, 2, 3, and 4 received pulses of 1, 2, 3 and $4\,mg\,C\,L^{-1}$ on day 0; treatments 2, 3 and 4 received a second pulse (2, 3, and $4\,mg\,C\,L^{-1}$, respectively) on day 3, treatments 3 and 4 received a third pulse (3 and $4\,mg\,C\,L^{-1}$) after 6 days, and treatment 4 received a fourth pulse ($4\,mg\,C\,L^{-1}$) after 9 days. Incubations were carried out for 12 days at 24 h aeration and 18 °C with a 16 : 8 h light/dark cycle and a light intensity of 72 µE. At the beginning and the end of the incubation (0 and 12 days, respectively), DOC concentration and chemical quality as well as bacterial biomass were determined for each control and treatment. At the end of the experiment, additional samples for DOC stable isotope analysis, bacterial production (BP), community respiration (CR), carbon isotope analysis of dissolved inorganic carbon (DIC), as well as concentrations and carbon isotope analysis of phospholipid-derived fatty acids (PLFA) were taken.

2.2 Chemical analysis

DOC concentrations ($mg\,C\,L^{-1}$) were determined with an organic carbon analyzer (Shimadzu, TOC-V CPH, Duisburg, Germany) as non-purgeable carbon after acidification. The quality of DOC was characterized by size exclusion chromatography with online carbon and nitrogen detection (Liquid Chromatography – Organic Carbon Detection-Organic Nitrogen Detection; LC-OCD-OND; Huber et al., 2011). A detailed description of both methods is given in Appendix A1.

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2.3 Bacterial activities

Bacterial production (BP) was determined by incorporation of ^{14}C-leucine following the protocol of Simon and Azam (1989). One incubation (5 mL) per replicate and one blank per treatment were run at a final concentration of 80 nmol L^{-1} leucine (specific activity 306 mCi $mmol^{-1}$) determined as saturation concentration. After processing the samples as described in Attermeyer et al. (2013) and filtering onto a 0.2 µm cellulose nitrate filter, the incorporated leucine was measured as disintegrations per minute (dpm) on a liquid scintillation analyzer (TriCarb 2810 TR, PerkinElmer Inc., Illinois, USA). Net dpm were converted to pmol L^{-1} d^{-1} according to Simon and Azam (1989). BP for water samples is given as µg C L^{-1} d^{-1} applying an isotope dilution factor of 2 (Kirchman, 1993).

Community respiration (CR) was determined as oxygen depletion over time and converted to C consumed. Oxygen measurements in the water were done with a non-invasive sensor dish reader (Optode, PreSens, Regensburg, Germany). The software directly converted the measured phase amplitude into the respective oxygen concentrations (see manufacturer's description). The oxygen concentrations in the water were recorded every 15 min to calculate the slope of a linear regression. The amount of consumed oxygen was converted to mg C L^{-1} d^{-1} by using 1 as a conversion factor (Berggren et al., 2012).

Additionally, we calculated bacterial growth efficiencies (BGE) from BP and CR as follows: BGE = BP/(BP + CR). We thereby assumed that the CR was primarily derived from bacterial community respiration because our prefiltration excluded all larger organisms.

2.4 PLFA analysis and determination of stable carbon isotope ratios of PLFA

Total lipids were extracted from 200 mL water samples and fractionated on silicic acid columns. The resulting phospholipids were converted to fatty acid methyl esters (FAME) followed by analysis on GC-MS, as described in Appendix A2.

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The carbon isotope composition was analysed using a gas chromatography-combustion-isotope-ratio-monitoring-mass-spectrometer system (GC-C-IRMS), as described in Bastida et al. (2011), with a Helium flow rate of 2 mL min^{-1}. The following temperature program was used: 1 min at 70 °C, heating with 20 °C min^{-1} to 120 °C, heating with 2 °C min^{-1} to 250 °C, followed by heating with 20 °C min^{-1} to 320 °C and hold for 10 min. Between 1 and 5 µL of the samples were injected with splitless mode. Each sample was measured at least in duplicate. The carbon isotope ratio of the FAMEs is reported in δ-notation (per mill) relative to the Vienna Pee Dee Belemnite standard (V-PDB) according to Slater et al. (2001) and Coplen (2011). The isotope ratios of the FAMEs reported were corrected for the carbon introduced during derivatisation according to Abraham et al. (1998).

A correction factor of +0.5 ‰ was used for respired DIC, and of +4 ‰ for $i+a15:0$ PLFAs in order to account for metabolic isotopic fractionation (Boschker et al., 1999; Karlsson et al., 2007). We calculated the fraction of metabolized C that contributed to respiration (R_{leaf}) and assimilation (A_{leaf}) in experiments by assuming that the δ^{13}C of metabolized C ($\delta^{13}C_{R/A}$, R stands for respired and A for assimilated) is a mixture derived from the two sources DOC_{algae} and DOC_{leaf}, such that

$$R_{\mathrm{leaf}} = \left(\delta^{13}C_R - 0.5 - \delta^{13}DOC_{algae}\right) / \left(\delta^{13}DOC_{leaf} - \delta^{13}DOC_{algae}\right) \quad \text{and} \tag{1}$$

$$A_{\mathrm{leaf}} = \left(\delta^{13}C_A + 4 - \delta^{13}DOC_{algae}\right) / \left(\delta^{13}DOC_{leaf} - \delta^{13}DOC_{algae}\right). \tag{2}$$

The δ^{13}C for DOC_{algae} was set to −16.77 ‰ and the δ^{13}C for DOC_{leaf} to −4.02 ‰. Those data were both taken from the single source incubations (algae and leachate control) at the end of the experiment (t_{12}).

2.5 Determination of stable carbon isotope ratios in DOC and respired DIC

The δ^{13}C-value of the respired C was determined at the end of the experiment by using the Keeling plot method (Keeling, 1958; Karlsson et al., 2007). At the beginning and after 48 h of incubation, we measured the concentration and carbon isotope composition

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of DIC. The samples were transferred to oxygen free, N_2-flushed glass vials (Labco, 12 mL, UK) containing 100 µL phosphoric acid (85 %, Merck, Darmstadt, Germany) for acidification. Analyses of the δ^{13}C-values of DIC and the concentration of the CO_2 released upon DIC acidification were carried out using a Thermo Delta V Advantage isotope ratio mass spectrometer interfaced to a GasBench-II automated headspace sampler. The internal precision of analyses based on internal standards was 0.22 ‰. Subsamples (50 mL) from each treatment for isotope analysis of DOC were filtered above a pre-rinsed cellulose acetate filter (0.2 µm, Sartorius AG, Göttingen, Germany), freeze-dried and transferred into tin capsules before measurement. These samples were then analyzed on an elemental analyzer (Flash HT, Thermo-Finnigan) connected to a Con-flow IV (Thermo-Scientific). Internal precision of the EA-IRMS system was 0.1 ‰.

2.6 Bacterial community analysis

In addition to the PLFA community analysis, we performed denaturing gradient gel electrophoresis (DGGE) with general bacterial primers to determine changes in the bacterial community structure between the treatments. DNA was extracted from 0.2 µm polycarbonate filters with phenol/chloroform after Nercessian et al. (2005) and amplified with eubacterial 16S primers (341f-GC; Muyzer et al., 1993, 907r; Teske et al., 1998). The DGGE was performed following the protocol of Muyzer et al. (1993) using the Ingeny PhorU DGGE-System (Ingeny International BV, GP Goes, Netherlands, polyacrylamide concentration: 7 %; gradient from 40–70 %) as described in Allgaier and Grossart (2006).

2.7 Statistics

We conducted non-parametric tests to detect differences between the two controls and the treatments. The statistical tests were regarded as significant when p was < 0.05. We performed linear regressions to assess the relationships between DOC concen-

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tration and DOC consumption, as well as DOC consumption and bacterial biomass. These statistical analyses were carried out with SPSS 19. The DGGE-fingerprint was analyzed as the Sørensen similarity coefficient of the presence/absence of distinct DNA bands in different samples, and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was constructed using the Vegan package in R (2.14.1; Oksanen et al., 2005). Additionally, we performed non-metric multidimensional scaling (nMDS) in order to get information on the degree of similarity between the samples.

3 Results

3.1 Quality of the inoculum

The LC-OCD analysis showed that the autochthonous DOC_{algae} was chemically more complex than the allochthonous DOC_{leaf} (Fig. 2). The low molecular weight (LMWS) fraction was significantly higher in the DOC_{leaf} (51 %) than in the DOC_{algae} (34 %). HS accounted for 49 % of DOC_{leaf} and 44 % of DOC_{algae}. In contrast, the DOC_{leaf} contained a negligible amount of high molecular weight substances (HMWS), whereas this fraction accounted for 22 % of the DOC_{algae} (Fig. 2). The specific UV absorption of the HS peak gave insight into the aromatic nature of both C sources. The DOC_{leaf} had a very low (0.8 $L\,mg^{-1}\,m^{-1}$) aromaticity while the DOC_{algae} had a high aromaticity (9.7 $L\,mg^{-1}\,m^{-1}$) reflecting a high proportion of aromatic and unsaturated structures in the HS fraction of the DOC_{algae}. In addition, the molecular weight of the DOC_{algae} was much higher than that of the DOC_{leaf} (Fig. 2).

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3.2 Bacterial DOC consumption

3.2.1 Total DOC consumption

We measured the decrease in total DOC from the initially added DOC (inoculated) until the end (t_{12}) of the incubation to determine the amount of DOC consumed by the bacterial community. For a better comparison, we calculated the percentage of DOC consumed. The mean DOC consumption in the algae control was 6 % lower (46 %) than the mean DOC consumption in the leachate control (52 %) although the difference was not significant (Mann Whitney U; $U = 12$; exact $p = 0.057$).

Total bacterial DOC consumption in the mixed treatments was highest when total DOC concentration was at a maximum (Fig. 1, treatment 4). After 12 days of incubation, 68 % (from 25.1 mg C L^{-1} to 8 ± 0.1 mg C L^{-1}) of the added total DOC was decomposed in treatment 4, but only 52 % (from 10.4 mg C L^{-1} to 5 ± 0.6 mg C L^{-1}) in treatment 1 (Fig. 3). We performed a linear regression using the total added DOC as an independent variable and the DOC consumed as a dependent variable. The proportion of DOC consumed increased linearly with added DOC ($y = -2.72 + 0.79x$; $R^2 = 0.996$).

3.2.2 Distinct consumption of LMWS, HS, and HMWS DOC fractions

The 3 organic matter fractions (LMWS, HS, and HMWS) remained relatively constant in the leachate controls after 12 days of incubation. The HMWS fraction, which accounts for ca. 10 % of the organic matter in this control, was introduced with the bacterial inoculum. The algae control reveals a relative increase in HMWS and a decrease in HS. The degradation of aromatic structures showed a contrasting pattern in the two controls. The aromaticity of the HS fraction increased in the leachate control relative to the DOC_{leaf} source, but decreased in the algae control compared to the DOC_{algae} source (stars in Fig. 2).

Analysis of DOC fractions in the treatments with mixed DOC sources revealed changes over time, particularly in the HS fraction (Fig. 3). Overall, all DOC fractions

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showed a decline in their concentrations after 12 days of incubation. However, this decline in concentration was highest for the HS fraction, as can be seen by their relative proportions (Fig. 3). The inoculated HS fractions were similar in all treatments, which were mainly due to the similar proportion of HS in the two C sources (Fig. 2). The percentage of decomposed HS increased from treatment 1 to 4. The HS fraction in treatment 4 decreased from 61 to 48 % amounting to a consumption of 7.8 mg C L^{-1} HS. In comparison, HS consumption in treatment 1 was 3.4 mg C L^{-1} (Fig. 3). Accordingly, at t_{12} the percentage of the HMWS fraction increased from treatment 1 to 4. The initial percentage of the LMWS fraction in both added C sources differed slightly (in treatment 1: 22 % corresponding to 2.3 mg C L^{-1} and in treatment 4: 17 % corresponding to 4.3 mg C L^{-1}) and its relative contribution to total DOC did not change significantly until the end of the experiment (in treatment 1: 18 % corresponding to 0.9 mg C L^{-1} and in treatment 4: 21 % corresponding to 1.7 mg C L^{-1}) indicating that the absolute LMWS consumption was highest in treatment 4 (2.6 mg C L^{-1}).

In addition, the LC-OCD analysis revealed a change in the proportion of aromatic and unsaturated structures. There was a higher degradation of aromatic structures and high molecular sizes in treatment 3 and 4 compared to that in treatment 1 and 2 (Fig. 2). At the end of the incubation, the aromaticity in treatment 3 and 4 was lower than for treatment 1 and 2 despite having added DOC_{algae} to these treatments, containing the highest amounts and proportions of aromatic structures.

3.3 Bacterial biomass and activities

The total amount of FAME and the amount of the FAMEs $i+a15:0$, the latter used as an indicator of heterotrophic bacterial biomass, were both positively correlated to the amount of DOC consumed (Fig. 4). The mean concentration of total FAME was ca. 1.9 times higher in treatment 4 than in treatment 1 (23.7 ± 4.3 pmol L^{-1} vs. 45.6 ± 21.4 pmol L^{-1}), whereas bacterial FAME were ca. 3.1 times elevated in treatment 4 than in treatment 1 (1.1 ± 0.4 pmol L^{-1} vs. 3.4 ± 2 pmol L^{-1}). The higher bacterial biomass in treatment 4 parallels the higher concentration of added DOC in treatment

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4 vs. treatment 1 (ca. 2.9 times). Both can be expressed in a linear regression with a steeper slope for the heterotrophic bacterial biomass (26.8) than for total FAME (10.7; Fig. 4).

Overall, bacterial growth efficiencies (BGEs) were relatively high and ca. 50 % of the C from DOC was incorporated into bacterial biomass; however, differences were not significant between different treatments (Kruskal–Wallis, df = 3, p = 0.516; Fig. 5) or controls (df = 5, p = 0.117).

3.4 Carbon flow

We aimed to identify the DOC sources respired or assimilated by heterotrophic bacteria via stable C isotope analysis. Unfortunately, we were not able to determine the stable C isotope composition of DIC for each treatment due to methodological problems. In some samples we did not measure a decrease in DIC concentration and could not calculate the Keeling plot. Based on the data obtained, we calculated the percentage of DOC_{leaf} respired or assimilated by the bacterial community according to the mixing model described in the methods (Fig. 6). Although the trends are not significant, we can still see a decrease in the percentage of DOC_{leaf} respired with the increase in DOC_{algae} concentrations, which is more pronounced in the assimilated C fraction (Fig. 6). A linear regression for the respired and assimilated fraction of leaf leachate revealed a roughly two-fold higher slope for the assimilated C (−3.18; red dotted line for assimilated C and −1.39; black striped line for respired C; Fig. 6).

3.5 Bacterial community structure

Bacterial community analyses based on PLFA and DGGE methods showed similar results to each other (Fig. 7). The treatments were distinguishable from the controls. Particularly, bacterial communities of the two controls clearly differed from those of the 4 treatments (Fig. 7b). The variability between the triplicates, however, was relatively high. Nevertheless, the distances between all triplicates of the controls with only one

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C source added and the treatments with a mixture of both C sources were evident, thus representing an obvious difference revealed by both, the PLFA concentrations and DGGE banding patterns.

4 Discussion

We set out to address the hypothesis that bacterial turnover and BCC of a mixture of both allochthonous and autochthonous DOC sources changes with quantity of autochthonous C and chemical quality. To answer this inquiry, we combined chemical analyses of differing DOC fractions with bacterial activity measurements, elucidation of the C flow by stable isotope analysis of incorporated and respired C, and changes in bacterial community composition (BCC). This multifaceted approach allowed us to gain new insights into bacterial DOC turnover, and in particular the effects of the quality and quantity of distinct C sources and fractions on bacterial dynamics and C turnover.

4.1 Quality and bioavailability of C sources: allochthonous vs. autochthonous

Allochthonous DOC was composed of compounds with relatively low molecular size and aromaticity in the HS fraction. It is controversial whether distinct DOC fractions such as LMWS should be considered labile or refractory to bacterial utilization. Amon and Benner (1996) proposed a size continuum model whereby bioavailability decreases with decreasing molecular weight. However, Kaiser and Sulzberger (2004) found bioreactivity, i.e. bacterial utilisation, was higher for LMWS than HMWS in the Tagliamento River. In line with this study, our results indicate a high microbial accessibility of allochthonous C with relatively low molecular sizes.

Despite several chemical differences in DOC_{leaf} vs. DOC_{algae}, both controls with a single type of DOC did not show significant differences in total DOC consumption or bacterial growth efficiency. Although differences between the controls were not significant, the DOC_{leaf} addition resulted in a higher bacterial DOC degradation and a 10 %

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higher mean BGE and thus incorporation into bacterial biomass. A heterogenous allochthonous C source can lead to significant differences in bioavailability (Berggren et al., 2007). A part of this heterogeneity, and subsequent bioavailability, may result from pre-processing of organic substrates by microbes before the C enters the DOC pool. Sun et al. (1997) found that leachate of dissolved organic matter (DOM) can be more biologically available than bulk riverine DOM and results by Pérez and Sommaruga (2006) indicated a more efficient utilization of algal lysate compared to soil-derived DOM. Therefore, besides chemical composition, the diagenetic alteration is also a major factor determining DOC bioavailability. This suggests that contributions of organic matter that is less processed by microbes to DOC is more bioavailable than older and processed DOC (Amon and Benner, 1996). The freshly leached C in our study did not undergo microbial decomposition before the inoculation and thus may explain its high bioavailability and high LMWS fraction. However, the generally high BGE in our experiment (compared to a mean BGE of ca. 30 % in a cross-system overview; del Giorgio and Cole, 1998) indicates that the DOC of both sources were readily available regardless of differences in chemical quality, pointing to an efficient incorporation of DOC into bacterial biomass and subsequent transfer to higher trophic levels.

Consequently, the bioavailability of distinct DOC sources is dependent on different factors and cannot be generalized. For example, the higher fraction of aromatic structures in DOC_{algae} of the control requires more energy for its microbial break down. Whereas the aromaticity of the HS fraction decreased in the algae control at the end of the incubation, it increased in the leachate control (Fig. 2). The decrease in aromaticity of the HS fraction in the algae control can be explained by a preferential utilization of aromatic compounds of the HS fraction. In contrast, increased aromaticity of the HS fraction in the leachate control can be explained by bacterial production of aromatic compounds of the HS fraction (Ogawa et al., 2001). In our study, we found the combination of chemical analyses and bacterial consumption measurements suitable for assessments of bacterial DOC availability, which depends on the age and chem-

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ical quality of the entire DOC pool rather than on its source (e.g. allochthonous vs. autochthonous).

4.2 Bacterial DOC availability of mixed sources

The addition of the two DOC sources and variable concentrations of autochthonous C in the treatments allowed us to follow DOC availability and consumption in relation to DOC quantity and quality. On the one hand, we changed the ratio of both DOC sources and, on the other hand, we were able to disentangle consumption of different fractions in the DOC pool. Although increasing DOC concentrations are generally not related to higher bacterial DOC consumptions (Basu and Pick, 1997), in our experiment the consumption of the microbially unprocessed and thus bioavailable DOC was related to DOC quantity (Fig. 3). Interestingly, DOC consumption was not related to the strength and frequency of the DOC pulse. An additional pulse experiment (Appendix B) did not reveal any significant differences in DOC consumption when the same total concentration of a single C source was added in pulses (total DOC = 24 mg C L^{-1}; Appendix B) differing in amount and frequency (Kruskal–Wallis, df = 3; p = 0.059). However, differences were significant when adding different concentrations of DOC without pulsing at the beginning of the experiment (Kruskal–Wallis; df = 3; p = 0.016). Thus, total DOC amount, and not pulsing, influenced bacterial DOC consumption in our experiment when nutrients were sufficiently supplied.

This increase in DOC consumption was paralleled by a significant increase in microbial biomass. This effect was more pronounced when taking FAME (relative to t_0), specific to heterotrophic bacteria, into account (Fig. 4). BGE, however, remained rather constant (Fig. 5) indicating that cell-specific activities were almost the same between the treatments. This suggests that, irrespective of a given DOC quality, higher DOC concentrations led to an increased bacterial biomass and thereby availability even when the ratio between DOC sources changes.

LC-OCD analysis unravelled preferences in the degradation of discrete C fractions. The relative degradation of HS and aromatic structures was higher with increasing

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DOC_{algae} concentrations (Fig. 3). HS, the most abundant fraction of DOC in natural aquatic ecosystems (Thurman, 1985) and in our experiment (Figs. 2 and 3), represents an important energy source for microorganisms (Moran and Hodson, 1990). Recently, Guillemette and del Giorgio (2011) related distinct fractions of the DOC pool to short- and long-term bacterial DOC consumption highlighting the importance of chemical quality of the DOC pool. Previous studies have shown that bacterial growth can be stimulated, e.g., in humic DOC-dominated high water periods of Amazonian aquatic ecosystems (Amado et al., 2006) or in boreal lakes (Karlsson et al., 2003). In our study, bacterial growth remained high on the HS fraction from both sources (Fig. 5) indicating the important role of allochthonous and autochthonous HS as bacterial substrates. Besides similar cell-specific bacterial DOC consumption rates, the higher bacterial biomass seems to particularly increase the decomposition of the HS fraction.

The generally high bioavailability of both DOC sources might be partially related to the non-limiting concentrations of N and P in our set-up, as found in nutrient rich aquatic systems. Nutrient addition, however, excludes effects of nutrient availability on bacterial DOC degradation and hence better accounts for effects related to the intrinsic features of DOC. Nevertheless, the scenario we are mimicking is the input of fresh organic matter after an algal breakdown or leaf fall, when high amounts of nutrients usually leak into the aquatic system at the same time (Hutalle-Schmelzer et al., 2010; Attermeyer et al., 2013). Despite nutrient limitation, other abiotic environmental factors such as temperature and irradiation may drive bacterial C turnover (Anesio et al., 2005). To better evaluate the impact of photooxidation on bacterial DOC consumption, we performed a separate light incubation. However, no differences in the three DOC fractions (LMWS, HS and HMWS) could be measured between dark and light treatments (Appendix C). Thus, changes in bacterial C utilization can be related solely to quality and quantity of DOC in our experimental set-ups.

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4.3 Effects of single and mixed DOC sources on BCC dynamics

In parallel to changes in bacterial activity, it remains questionable whether quantity or quality of DOC is crucial for bacterial community composition (BCC) dynamics. Eiler et al. (2003) showed that DOC quantity potentially influences BCC growing on aged humic DOC, although these changes were less pronounced in the systems with > 0.54 nM DOC. The amount of added DOC resulted in a higher total DOC consumption, bacterial biomass and an efficient usage of the HS fraction in our treatments. In contrast, BCC shifted when both sources were mixed in the treatments, but BCC did not shift with increasing additions of autochthonous DOC. Hence, BCC seems to be more affected by the higher chemical complexity of total DOC due to the presence of both allochthonous and autochthonous DOC sources.

4.4 Assimilation and respiration of DOC

BGE in all controls and treatments were high indicating an efficient incorporation of both sources into bacterial biomass. Although our data did not allow to statistically prove differences in bacterial C usage between treatments, we detected different trends in bacterial turnover of both C sources via respiration and assimilation (Fig. 6). The proportion of DOC_{algae} incorporated into bacterial biomass in comparison to the proportion respired was higher with increasing concentrations of DOC_{algae}, indicating preferential incorporation of DOC_{algae} into bacterial biomass when both DOC sources were mixed.

Linking the concept of bioavailability to metabolic processes, we conclude that DOC_{leaf} with generally lower molecular sizes was respired to a higher extent than DOC_{algae}, and that the respired fraction is less variable than that of C incorporated into bacterial biomass. In general, BP is much more variable than BR (del Giorgio et al., 1997) which could also explain the higher variance in incorporated C using the mixing model (Fig. 6). Carlson et al. (2002) revealed a stimulation of BR after the addition of labile C and suggested that bacteria used this source to maintain general cell processes such as cell motility, elimination of waste or repair of the cellular machinery (Carlson

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et al., 2007). With this additional energy the bacteria can also produce exoenzymes to degrade the more refractory carbon, rendering it available for incorporation into bacterial biomass. Another reason for a better incorporation of DOC_{algae} could be related to its chemical composition, which allows for better integration into bacterial biomass. Algal lysates mainly consists of biomass, which consists of compounds ideal for microbial biomass build up (Krog et al., 1986; Farjalla et al., 2006). Our results implicate that the bioavailable proportion of DOC_{leaf} was higher than that of DOC_{algae}. However, the bioavailable proportion of DOC_{leaf} was used to gain energy via respiration whereas that of DOC_{algae} was mainly assimilated.

5 Conclusions

Our study sheds light on bacterial DOC consumption and BCC dynamics in relation to DOC quantity and quality when fresh organic matter is entering aquatic systems. Our study highlights the importance of DOC quantity for bacterial DOC consumption and DOC quality for BCC. When nutrients are not limiting, increasing DOC concentrations generally lead to an increase in bacterial biomass which, in parallel to a relatively high BGE, results in an enhanced C turnover and utilisation of the more aromatic HS fraction, particularly of the DOC_{algae}. Interestingly, the pulsed additions of fresh DOC, mimicking heavy rain events or algal breakdowns, have a minor effect on bacterial C dynamics as long as the sum of DOC is at the same level. Additionally, stable C isotope analysis reveals the tendency for leaf leachate to be preferentially respired leaving the system as carbon dioxide. In contrast, the algal-derived material is preferentially incorporated into the bacterial biomass, thus remaining in the system and being channelled to higher trophic levels. BCC only shifted when both sources are supplied, pointing to a higher influence of chemical quality and structural complexity on BCC. In conclusion, distinction of DOC based on its sources seems to be of low relevance when addressing bacterial DOC consumption in nutrient replete aquatic ecosystems, whereas quantity

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and chemical quality of bulk DOC constitute important controlling factors for bacterial C consumption and BCC, respectively.

Appendix A

Methods

A1 Chemical analysis

DOC concentrations (mg C L^{-1}) were determined with an organic carbon analyzer (Shimadzu, TOC-V CPH, Duisburg, Germany). After filtration through a 0.2 µm pre-rinsed cellulose nitrate filter, DOC was analyzed as non-purgeable carbon after acidification with 1 % (v/v) 2 mol L^{-1} HCl. The carbon dioxide generated by catalytic high-temperature oxidation (850 °C) was measured online by infrared absorption. The quality of DOC was characterized by size exclusion chromatography with online carbon and nitrogen detection (Liquid Chromatography – Organic Carbon Detection – Organic Nitrogen Detection; LC-OCD-OND; Huber et al., 2011). Briefly, the chromatographable DOC portion of the filtered samples passes through a size-exclusion column packed with resin (Toyopearl HW 50S, volume of 250 mm × 20 mm). Phosphate buffer (0.029 mol L^{-1}, pH 6.5) was used as eluent at a flow rate of 1.1 mL min^{-1}. The first detector measured the absorbance at 254 nm. DOC was detected with infrared (IR) absorbance of CO_2 after ultraviolet (UV) oxidation of DOC at 185 nm in a cylindrical UV thin-film reactor (Graentzel-reactor). Fractions were identified by using fulvic and humic acid standards from the International Humic Substances Society and analyzed with a suitable software program (FIFFIKUS; DOC-Labor Huber, Karlsruhe, Germany). DOC was summarized in three fractions: high molecular weight substances (HMWS, e.g. polysaccharides), humic or humic-like substances (HS) including buildings blocks and low molecular weight substances (LMWS), which include both low molecular weight acids and low molecular weight neutral substances. HMWS and HS are known to be

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recalcitrant to microbial degradation (Hessen and Tranvik, 1998) and LMWS are more labile due to their low molecular weight (Saunders, 1976). The ratio between the spectral absorption coefficient (SAC in m^{-1}, at 254 nm) and the organic carbon of the humic fraction (in $mg\,C\,L^{-1}$) was calculated as aromaticity (so called SAC/OC: specific UV-absorption of the HS peak, $L\,mg^{-1}\,m^{-1}$).

A2 PLFA analysis and determination of stable carbon isotope ratios of PLFA

Total lipids were extracted from 200 mL water samples filtered over a 0.2 µm polyvinyl fluoride filter using a modified Bligh and Dyer method as described by Frostegård et al. (1991) and changed after Steger et al. (2011). After lipid fractionation on silicic acid columns (BondElut® LRC-Si, Agilent Technologies, Santa Clara, USA), the phospholipids in the polar fraction were dried under a gentle stream of nitrogen and converted to fatty acid methyl esters (FAME) by a mild alkaline methanolysis (Guckert et al., 1985). Before analysis, the completely dried FAME fraction was dissolved in n-hexane containing 20.06 $ng\,\mu L^{-1}$ of the fatty acid 21 : 0 as an internal standard. For identification and quantification of the FAMEs, a gas chromatograph coupled to a mass spectrometer (Agilent, Palo Alto, USA) was used as described in Bastida et al. (2011). One µL aliquots were injected with either splitless mode or at different split ratios of up to 1 : 50. FAMEs were identified by comparison of fatty acid mass spectra of standards (bacterial acid methyl ester mix, Supelco, Munich, Germany) and to the NIST MS database. The concentration of each FAME was quantified relative to the internal standard. The fatty acid nomenclature was used according to Bastida et al. (2011).

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Appendix B

Additional pulse experiment

B1 Experimental set-up

We performed an additional pulse experiment to test whether pulsing in DOC addition has an influence on bacterial DOC consumption. We used lake water and the natural bacterial community plus nutrients (oligotrophic Lake Stechlin in northern Germany; for more details see Allgaier and Grossart, 2006) and a single DOC source (beech DOC_{leaf}). The DOC_{leaf} was added at the beginning of the experiment without any pulses, but at increasing DOC concentrations. DOC concentrations increased by $6\,mg\,C\,L^{-1}$ from 6 to $24\,mg\,C\,L^{-1}$ in 250 mL Erlenmeyer flasks (see Fig. 1). Additionally, we performed three incubations with the same total DOC concentrations but with an increasing number of pulses (see Fig. 1). All incubations were done in triplicates for 12 days under the same conditions as in the main experiment. We measured DOC concentrations (procedure described in the methods section of the manuscript) and calculated DOC consumption after 12 days.

B2 Results

The DOC consumption increased with increasing DOC concentrations (from 45 % in treatment 1 × 6 to 58 % in 1 × 12, 64 % in 1 × 18, and 67 % in treatment 1 × 24, respectively) but were the same (67 % in treatment 1 × 24 and 69 % in treatment 4 × 6) for the $24\,mg\,C\,L^{-1}$ treatment without pulses and in all other pulsed incubations with the same total DOC concentrations (Fig. A1). The treatments without pulses were significantly different (Kruskal–Wallis; $df = 3$ and $p = 0.016$). In contrast, treatments with varying DOC pulses were not significantly different (Kruskal–Wallis; $df = 3$ and $p = 0.059$). This confirms that the pulses had no influence on bacterial DOC consumption, whereas differences in DOC concentrations significantly affected it.

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Appendix C

Liquid Chromatography with Organic Carbon Detection (LC-OCD) of illuminated and non-illuminated DOC_{leaf} without microorganisms

C1 Experimental set-up

We incubated beech leachate DOC (DOC_{leaf}) in sterile conditions to test whether the presence of light in our incubation has an influence on DOC quality. Therefore, we set up 250 mL Erlenmeyer flasks with beech DOC_{leaf} at 7 mg C L^{-1} diluted with double distilled water. We incubated three Erlenmeyer flasks in light comparable to the incubation conditions of the main experiment and three Erlenmeyer flasks in complete darkness. After one week we froze the samples until further chemical analysis. The samples were analysed with the Liquid Chromatography – Organic Carbon Detection – Organic Nitrogen Detection (LC-OCD-OND) as described in the method section of the manuscript.

C2 Results

We detected no significant differences between the light and dark treatments (Fig. B1). Upon illumination, there was a slight decrease in HS and an accompanied increase in LMWS. In general, however, the quality revealed by LC-OCD did not change by illumination when comparing the start (t_0) with the end of the incubation (Mann–Whitney U; $p > 0.05$ for t_0 vs. t_7 non-light and for t_0 vs. t_7 light) and in the light and dark treatment at the end of the experiment (Kruskal–Wallis; $p > 0.05$ for dark vs. light).

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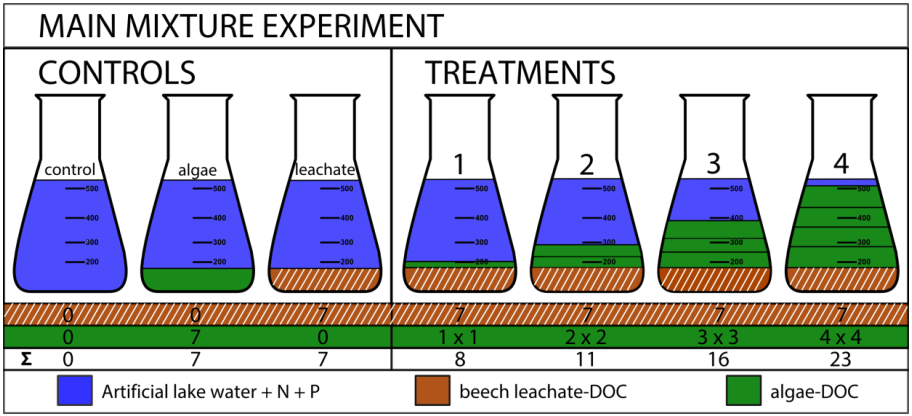

Fig. 1. Experimental set-up for the main mixture experiment with controls (left) and treatments (right) with pulses of DOC_{algae} 1 to 4. At the bottom, the brown striped line gives the amount of added DOC_{leaf} ($7\,mg\,C\,L^{-1}$), the green line the amount of DOC_{algae} and numbers of pulses, and the white line at the bottom the sum of DOC added. All units are given in $mg\,C\,L^{-1}$.

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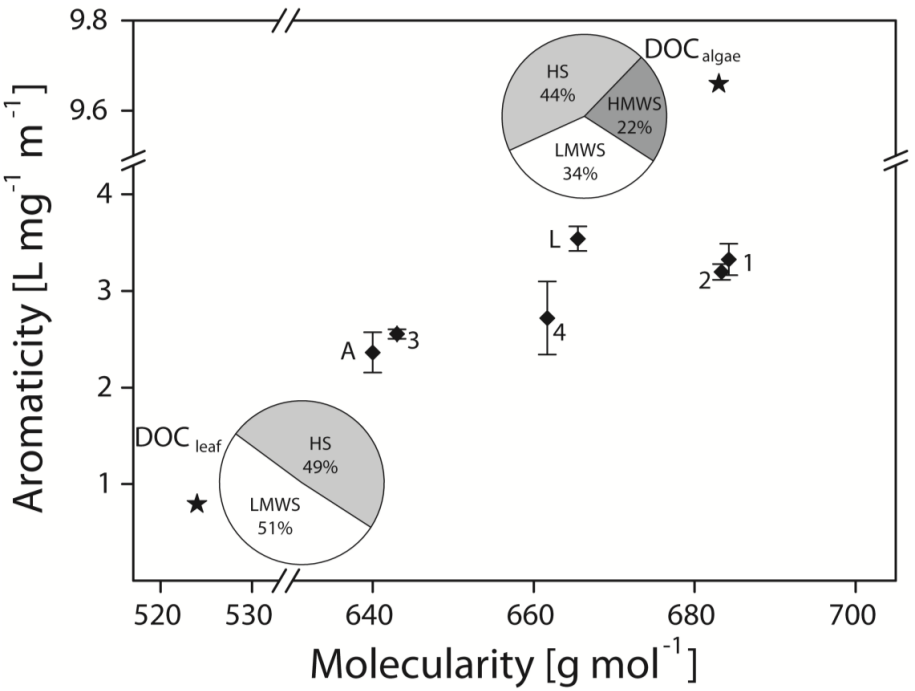

Fig. 2. Humic substances diagram as shown after Huber et al. (2011) with aromaticity (SAC/OC ratio in L mg^{-1} m^{-1}) plotted against molecularity (nominal molecular weight in g mol^{-1}) of the algae (A) and leachate (L) control and the treatments (1, 2, 3, 4) at the end of the experiment (t_{12}; rhombi) and of the initial DOC_{algae} and DOC_{leaf} added as DOC sources to the treatments (stars). The pie charts illustrate the chemical composition of both DOC_{algae} and DOC_{leaf} sources as revealed by Liquid Chromatography – Organic Carbon Detection (LC-OCD), a method which distinguishes between low molecular weight substances (LWMS), humic or humic-like substances (HS) including building blocks and high molecular weight substances (HMWS).

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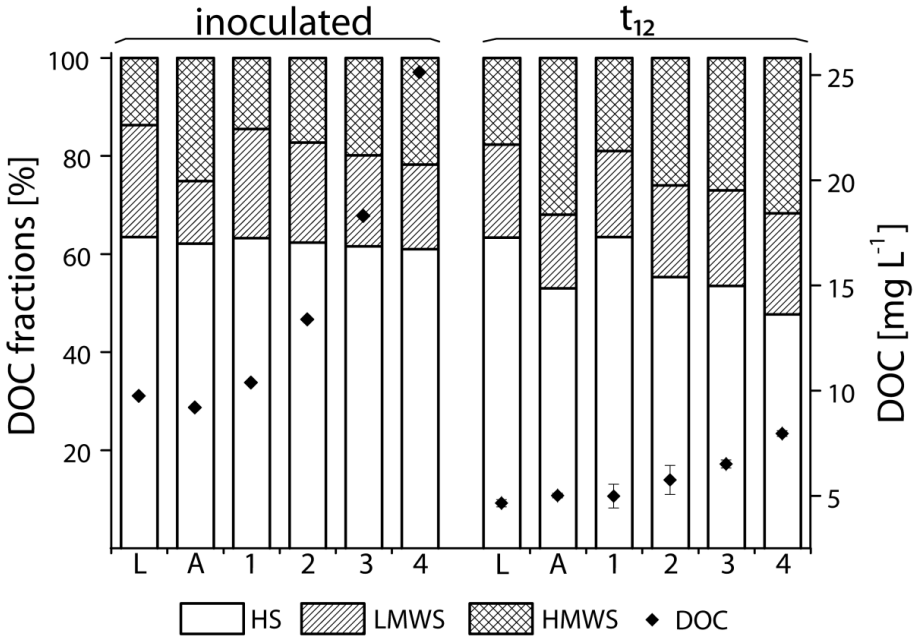

Fig. 3. Percentage of inoculated and final (t_{12}) dissolved organic carbon (DOC) fractions in leachate (L) and algae (A) controls and treatments (1–4). According to Huber et al. (2011) the fractions are humic-like substances and building blocks (HS), low molecular weight substances (LMWS), and high molecular weight substances (HMWS). The rhombi give DOC concentrations at the beginning (inoculated) and the end (t_{12}) of the experiment. At t_{12} we plotted the mean ± standard deviation for the DOC concentration; where no standard deviation bars are visible they are small and hidden by the respective rhombus.

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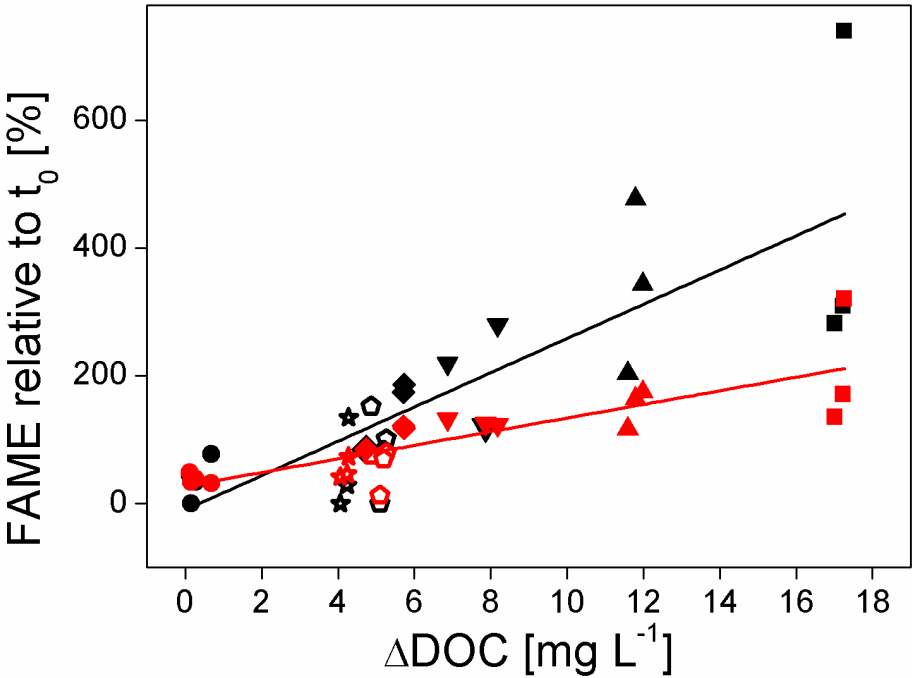

Fig. 4. Relative changes in the total phospholipid fatty acid methyl ester (FAME; in red) and $i + a15:0$ FAME (in black) plotted against consumed DOC (ΔDOC in mg C L^{-1}) from all controls and treatments. Different symbols represent the different controls and treatments: circle = control without DOC addition; open star = algae control; open pentagon = leachate control; rhombus = 1; triangle down = 2; triangle up = 3 and square = 4. The percentages refer to the relative changes in concentrations from the beginning (t_0) to the end of the experiment (t_{12}). The red line is the linear fit for total FAME ($y = 27.5 + 10.7x$ and $R^2 = 0.69$) and the black line for $i + a15:0$ FAME ($y = -9.5 + 26.8x$ and $R^2 = 0.65$).

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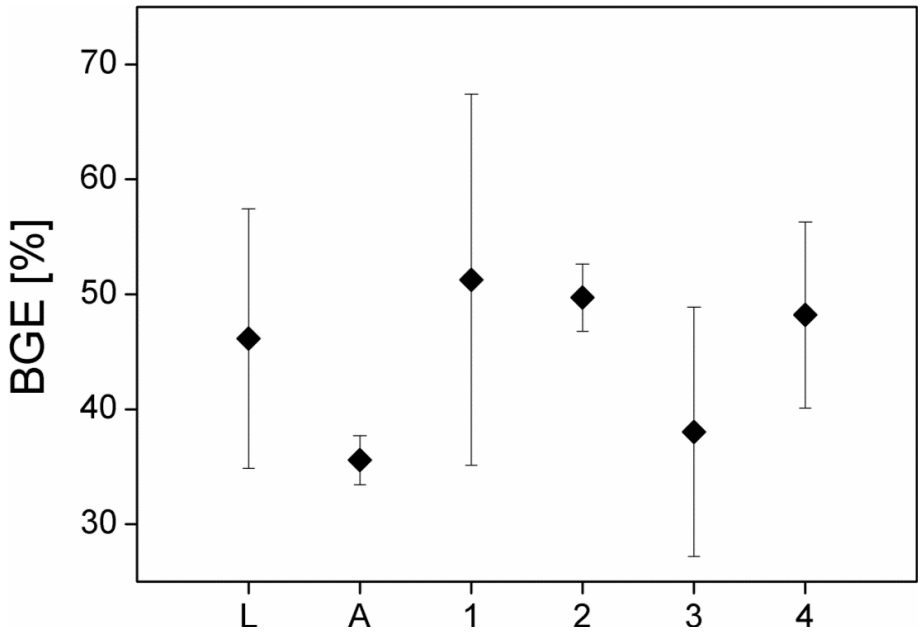

Fig. 5. Bacterial growth efficiencies (BGE) in controls with either DOC_{leaf} (L) or DOC_{algae} (A) addition and in each treatment containing both DOC sources at the end (t_{12}) of the experiment calculated from respiration and bacterial production.

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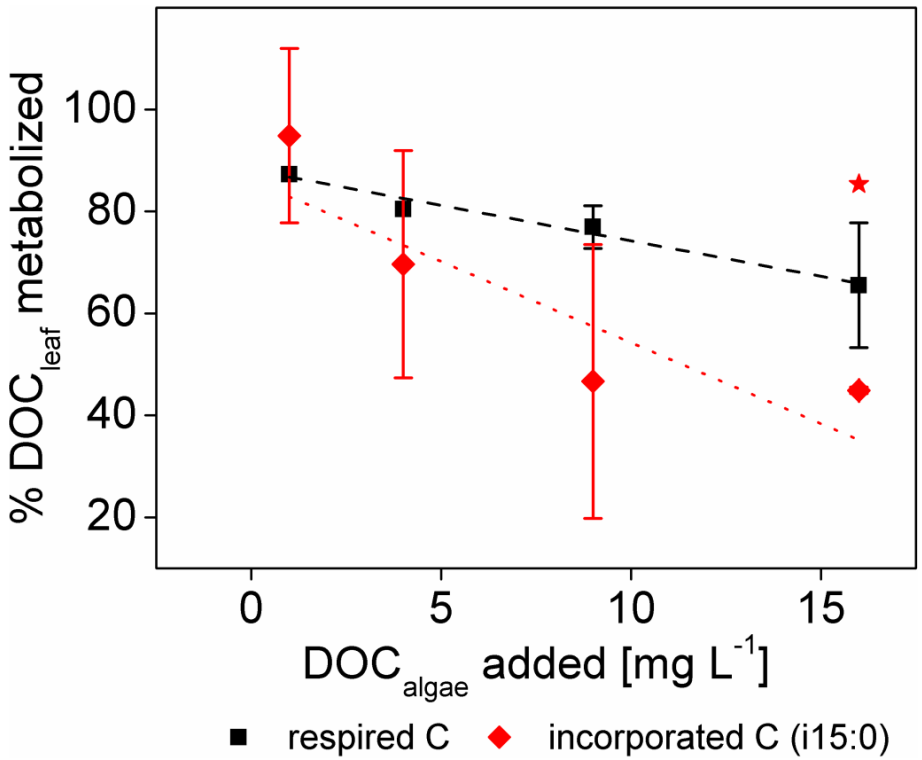

Fig. 6. Percentage of DOC_{leaf} metabolized via respiration (black square) or assimilation into *i* 15 : 0 fatty acid methyl ester (FAME; red rhombi; indicative for heterotrophic bacteria) of each treatment calculated from a two-source mixing model at the end of the experiment. The star marks an outlier which was not included in the regression and the black striped line is for the respired C and the red dotted line for the incorporated C.

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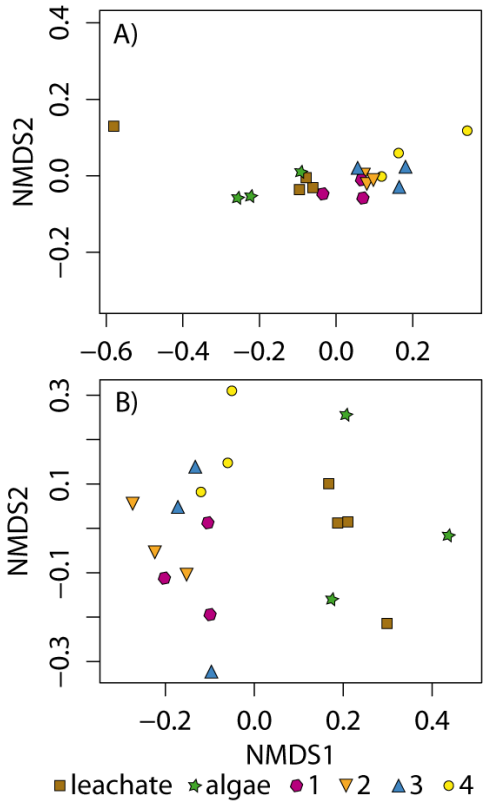

Fig. 7. Nonmetric multidimensional scaling (nMDS) plots representing differences between the algae and leachate control and the four treatments of **(A)** phospholipid-derived fatty acids (PLFA) concentration patterns and **(B)** denaturing gradient gel electrophoresis (DGGE) banding patterns. Bray–Curtis dissimilarities were calculated for the concentrations of 13 different PLFA and for banding patterns (Sørensen similarity coefficient) from DNA amplified with general eubacterial primers of each treatment and triplicate at the end of the experiment. The stress value is: for **A** = 0.013 and for **B** = 0.166.

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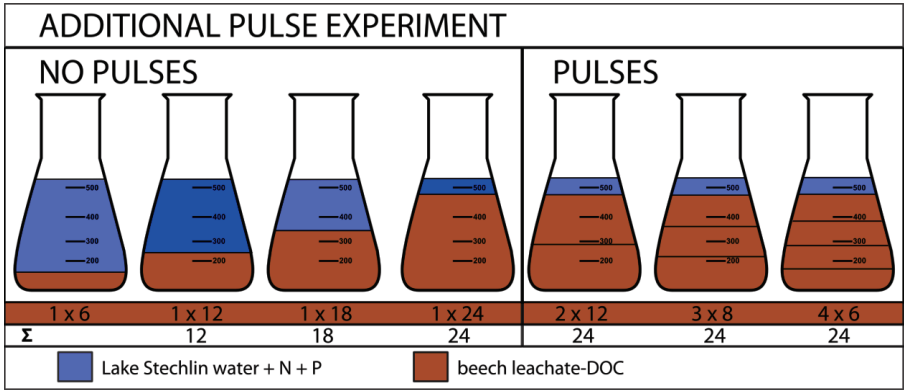

Fig. A1. Experimental set-up of the additional pulse experiment showing the two parts with and without pulses.

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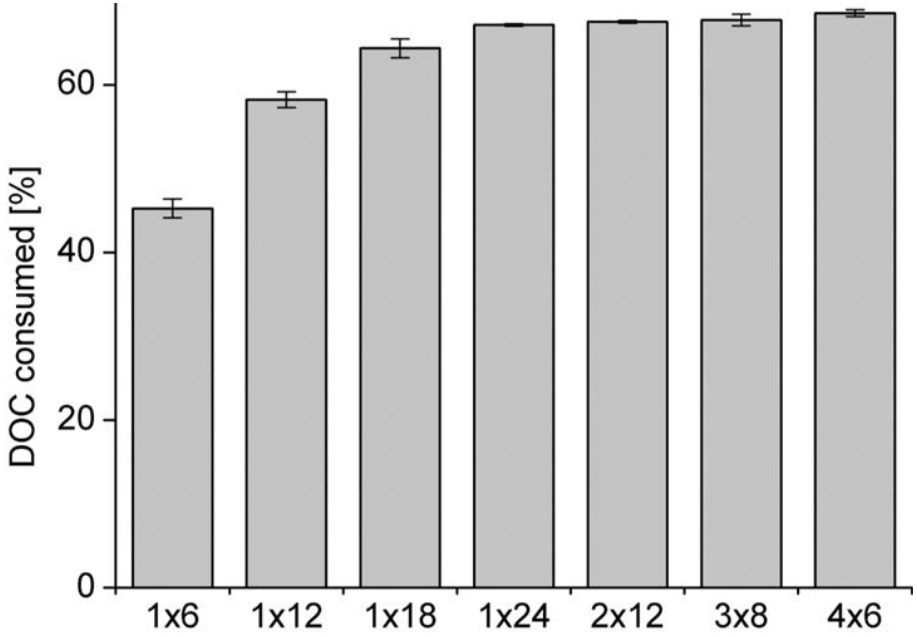

Fig. A2. DOC consumed (mean ± standard deviation; in %) after 12 days in the four treatments with increasing DOC (1 × 6, 1 × 12, 1 × 18, 1 × 24) and in the three treatments with pulsed DOC input (2 × 12, 3 × 8, 4 × 6).

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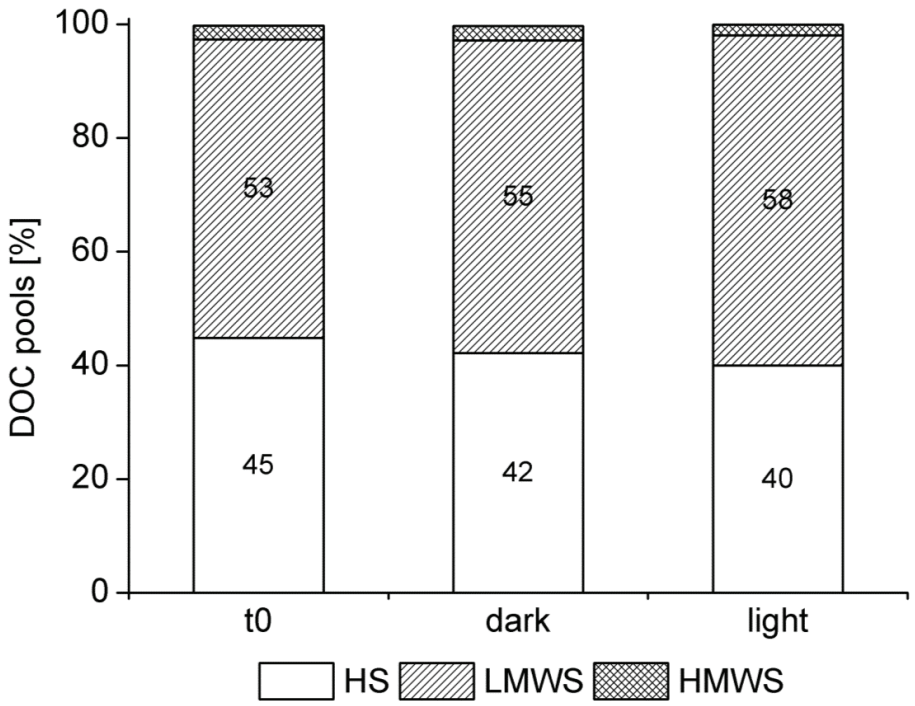

Fig. B1. Percentage of measured DOC fractions at the start (t_0) and end (t_7) of the incubation in both dark and light treatment. According to Huber et al. (2011) we claimed the fractions HS which represent humic like substances and building blocks, LMWS consist of low molecular weight substances and HMWS depict high molecular weight substances.

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