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Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on bacterial community composition

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Abstract. Dissolved organic carbon (DOC) concentrations – mainly of terrestrial origin – are increasing worldwide in inland waters. Heterotrophic bacteria are the main consumers of DOC and thus determine DOC temporal dynamics and availability for higher trophic levels. 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 (phytoplankton lysate). We then determined bacterial C consumption, activities, and community composition together with the C flow through bacteria using stable C isotopes. The chemical analysis of single sources revealed differences in aromaticity and low- and high-molecular-weight substance fractions (LMWS and HMWS, respectively) between allochthonous and autochthonous C sources. Both DOC sources (allochthonous and autochthonous DOC) were metabolized at a high bacterial growth efficiency (BGE) around 50 %. In treatments with mixed sources, rising concentrations of added autochthonous DOC resulted in a further, significant increase in bacterial DOC consumption of up to 68 % when nutrients were not limiting. This rise was accompanied by a decrease in the humic substance (HS) fraction and an increase in bacterial biomass. Changes in DOC concentration and consumption in mixed treatments did not affect bacterial community composition (BCC), but BCC differed in single vs. mixed incubations. 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 phytoplankton and macrophytes) and allochthonous C originating from terrestrial areas surrounding aquatic ecosystems. Allochthonous C inputs to aquatic ecosystems can vary in time and quality, e.g., particulate or dissolved forms (Carpenter et al., 2005). In forested watersheds, substantial amounts of leaves can fall into lakes and dissolve into the water column (Gasith

and Hasler, 1976; France, 1995; Vander Zanden and Gratton, 2011).

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 has increased in the past decades (Hansson et al., 2013), which is related to rising atmospheric CO_2 concentrations, climate warming, continued N deposition, decreased sulfate deposition, and 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 heterotrophic bacteria in pelagic systems (Azam et al., 1983). Metabolic activities of the pelagic bacterial community thus can control DOC turnover, which in turn can be, among other ways, 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. In addition, the bacterial community composition (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). Van Hannen et al. (1999) and Grossart et al. (2007) related changes in BCC to organic matter quality. In marine systems it has been shown that different phylogenetic groups consume specific dissolved organic compounds (Cottrell and Kirchman, 2000). Therefore, different DOC sources can support different bacterial communities, but the knowledge about the effect of mixing different DOC sources on BCC remains equivocal. To our knowledge there is just one study that evaluated the response of a natural bacterial community to the mixing of DOC sources from different origins (Fonte et al., 2013).

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).

We aimed to evaluate the effects of fresh allochthonous and autochthonous DOC on bacterial decomposition and BCC, and to elucidate the degradation of distinct DOC fractions by natural bacterial communities. We incubated the DOC sources in (1) single-source incubations and (2) mixed treatments with a fixed amount of leaf leachates (DOC_{leaf}) and added different concentrations of an algal lysate (DOC_{phyto}) together with bacterial communities from the littoral zone of a temperate shallow lake. We hypothesized that (1) both sources, irrespective of origin, are efficiently decomposed by the bacterial community. Further, we expect (2) increased DOC degradation with increased addition of DOC_{phyto} and that (3) mixing both C sources results in a shift in BCC.

2 Methods

2.1 Experimental setup

We sampled water from the littoral zone (0.5 m depth) of Schulzensee, a small, shallow, eutrophic lake. The lake is located in northeastern Germany, in a forest dominated by pine trees. The lake water was prefiltered 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 µg L^{-1}) additions to avoid nutrient limitations.

The leaf leachate (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. The leaves were harvested after they had fallen from the tree, then dried and stored at room temperature. We leached brown beech leaf disks (diameter 1.1 cm) for 48 h in double-distilled water at 4 °C in the dark. The phytoplankton lysate (DOC_{phyto}) was extracted from a cyanobacterium (*Aphanizomenon flos-aquae*, L. SAG 31.87) and a green algal (*Desmodesmus* sp.) culture by centrifugation, followed by dissolution in double-distilled water, hydrolysis (20 min at 120 bar and 121 °C), and ultra-sonication (1.5 min at 10 W). The phytoplankton cultures were not axenic and are representative of those lakes (Yamamoto, 2009; Wu et al., 2011). Both DOC_{leaf} and DOC_{phyto} were filtered through a prerinsed 0.2 µm polycarbonate filter (Whatman, Dassel, Germany) before inoculation; both procedures are similar to the ones described in Kritzberg et al. (2006).

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

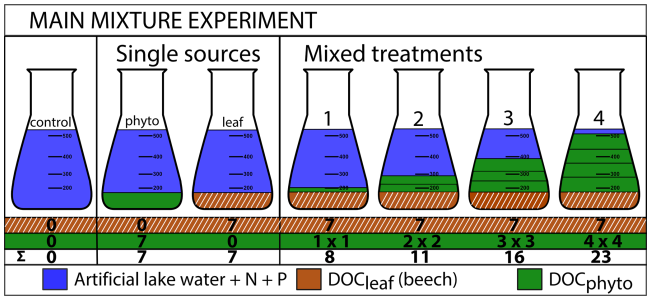

Fig. 1. Experimental setup for the main mixture experiment with controls (left), single-source incubations (middle), and mixed treatments (right) with pulses of DOC_{phyto} 1 to 4. At the bottom, the brown striped line gives the amount of added DOC_{leaf} (7 mg $C\,L^{-1}$), the green line the amount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

We created a control with no DOC addition, single-source incubations with addition of only DOC_{phyto} and addition of DOC_{leaf} (7 mg $C\,L^{-1}$). In the four mixed treatments, a base concentration of DOC_{leaf} (7 mg $C\,L^{-1}$) was supplemented with different concentrations of DOC_{phyto}: 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, we supplied the DOC_{phyto} 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 the control, the single-source incubations, and mixed treatments. 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), and 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). This method allows to distinguishing between high- and low-molecular-weight substances (HMWS and LMWS, respectively) and humic substances (HS). A detailed description of both methods is given in the supplemental material (Supplement A1).

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 Stable carbon isotope analysis

We determined the two pathways of C flow, assimilation and respiration, through bacteria via two separate stable C isotope approaches. On the one hand, the assimilated C into bacterial biomass is analyzed with compound-specific stable carbon isotope analysis of PLFA (Boschker and Middelburg, 2002). On the other hand, the respired C was measured after an incubation of water samples for a determined period, and concentration and isotopic composition ($\delta^{13}C$) of dissolved inorganic carbon (DIC) were measured at the start and end of the incubations. Keeling plot analyses subsequently revealed the $\delta^{13}C$ of the respired C (Karlsson et al., 2007).

2.4.1 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 the supplemental material (Supplement A2).

The carbon isotope composition was analyzed 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 by 20 °C min^{-1} to 120 °C, heating by 2 °C min^{-1} to 250 °C, followed by heating by 20 °C min^{-1} to 320 °C and maintaining the temperature for 10 minutes. Between 1 and 5 µl of the samples were injected with splitless mode. Each sample was measured at least in duplicates. The carbon isotope ratio of the FAMEs is reported in δ-notation (per mil) relative to the Vienna Pee Dee Belemnite standard (VPDB) according to Slater et al. (2001) and Coplen (2011). The isotope ratios of the FAMEs reported were corrected for the carbon introduced during derivatization according to Abraham et al. (1998).

2.4.2 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 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 prerinsed 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 ConFlo IV (Thermo Scientific). Internal precision of the EA-IRMS system was 0.1 ‰.

2.4.3 Calculations

A correction factor of −0.5 ‰ was used for respired DIC, and of +4 ‰ for $i/a15:0$ PLFA 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_{phyto} and DOC_{leaf}, such that

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

and

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

The δ^{13}C for DOC_{phyto} 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 at the end of the experiment (t_{12}).

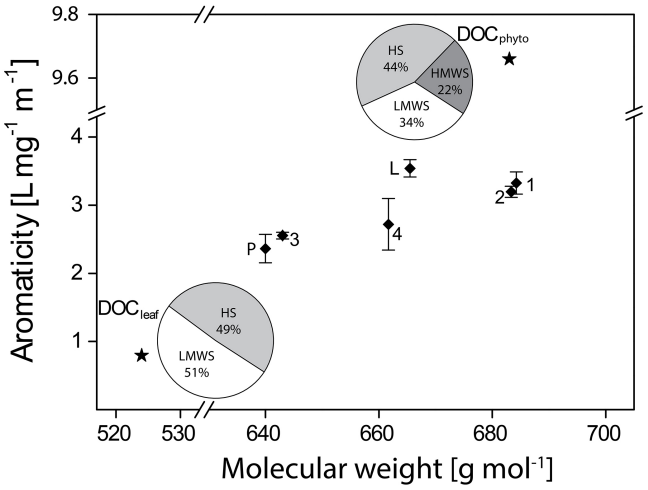

Fig. 2. Humic substances diagram as shown after Huber et al. (2011) with aromaticity (SAC / OC ratio in L mg^{-1} m^{-1}) plotted against molecular weight (in g mol^{-1}) of the single phytoplankton (P) and leachate (L) incubation and the mixed treatments (1, 2, 3, 4) at the end of the experiment (t_{12}, rhombi) and of the initial DOC_{phyto} and DOC_{leaf} added as DOC sources to the treatments (stars). The pie charts illustrate the chemical composition of both DOC_{phyto} and DOC_{leaf} sources as revealed by liquid chromatography–organic carbon detection (LC-OCD), a method that distinguishes between low-molecular-weight substances (LMWS), humic or humic-like substances (HS) including building blocks, and high-molecular-weight substances (HMWS).

2.5 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 and 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 to 70 %) as described in Allgaier and Grossart (2006).

2.6 Statistics

We conducted non-parametric tests to detect differences between the two single-source incubations 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 concentration 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 amendments

The chromatographic analysis showed that the phytoplankton lysate (DOC_{phyto}) was chemically more complex than the leaf leachate (DOC_{leaf}) (Fig. 2). The LMWS fraction was significantly higher in the DOC_{leaf} (51 %) than in the DOC_{phyto} (34 %). HS accounted for 49 % of DOC_{leaf} and 44 % of DOC_{phyto}. In contrast, the DOC_{leaf} contained a negligible amount of HMWS, whereas this fraction accounted for 22 % of the DOC_{phyto} (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_{phyto} 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_{phyto}. The aromaticity of DOC_{phyto} could be artificially increased as a result of the pretreatment (i.e., autoclaving) due to condensation processes. In addition, the molecular weight of the DOC_{phyto} was much higher than that of the DOC_{leaf} (Fig. 2).

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 between treatments, we calculated the percentage of DOC consumed. The mean DOC consumption in the DOC_{phyto} incubation was 6 % lower (46 %) than the mean DOC consumption in the DOC_{leaf} incubation (52 %) although the difference was not significant (Mann–Whitney U; $U = 12$; exact $p = 0.057$).

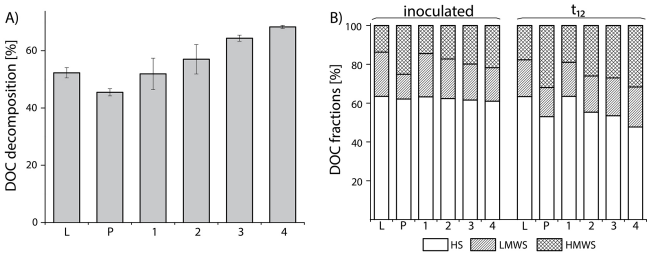

Fig. 3. Percentage of DOC decomposed **(A)** and inoculated and final (t_{12}) dissolved organic carbon (DOC) fractions **(B)** in leachate (L) and phytoplankton (P) single incubation and mixed treatments (1–4). According to Huber et al. (2011) the fractions are humic-like substances and building blocks (HS), low-molecular-weight substances (LMWS), and high-molecular-weight substances (HMWS). In A we plotted the mean ± standard deviation for the DOC decomposition.

Total bacterial DOC consumption in the mixed treatments was highest when total DOC concentration was at a maximum (Fig. 1, treatment 4). 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 ($y = -2.72 + 0.79$x; $R^2 = 0.996$).

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

The three organic matter fractions (LMWS, HS, and HMWS) remained relatively constant in the DOC_{leaf} incubation after 12 days. The HMWS fraction, which accounts for ca. 10 % of the organic matter, was introduced with the bacterial inoculum. The DOC_{phyto} incubation reveals a relative increase in HMWS and a decrease in HS. The degradation of aromatic structures showed a contrasting pattern in the two single-source incubations. The aromaticity of the HS fraction increased in the DOC_{leaf} incubation relative to the DOC_{leaf} source, but decreased in the DOC_{phyto} incubation compared to the DOC_{phyto} 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 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), but the percentage of decomposed HS increased from treatment 1 to 4. In addition, the

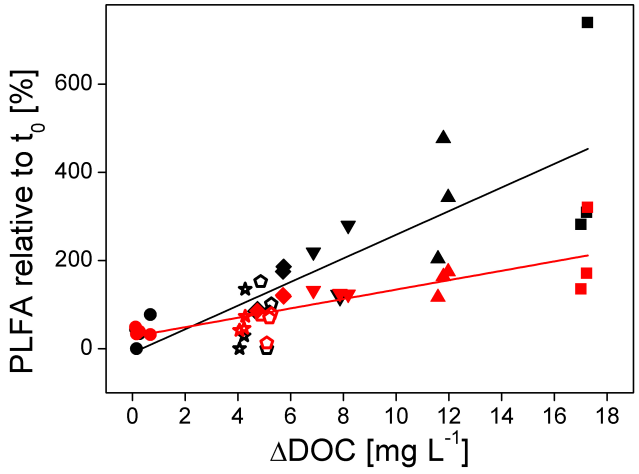

Fig. 4. Relative changes in the total phospholipid fatty acids (PLFA; in red) and $i+a15:0$ PLFA (in black) plotted against consumed DOC (ΔDOC in mg C L^{-1}) from all controls, single incubations and treatments. Different symbols represent the different controls and treatments: circle, control without DOC addition; open star, single DOC_{phyto}; open pentagon, single DOC_{leaf}; 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 PLFA ($y = 27.5 + 10.7$ times and $R^2 = 0.69$) and the black line for $i+a15:0$ PLFA ($y = -9.5 + 26.8$ times and $R^2 = 0.65$).

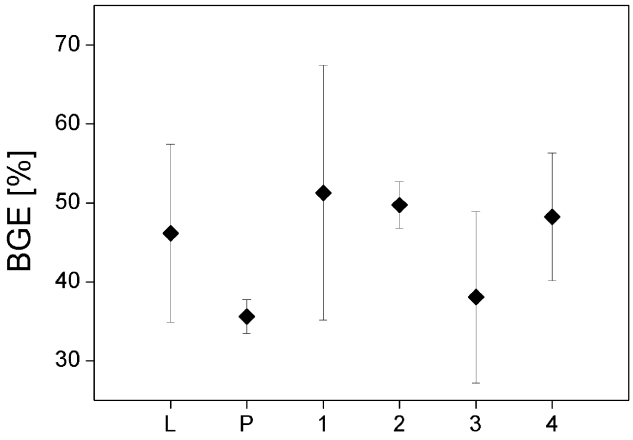

Fig. 5. Bacterial growth efficiencies (BGE) in single-source incubations with either DOC_{leaf} (L) or DOC_{phyto} (P) addition and in each treatment containing both DOC sources at the end (t_{12}) of the experiment calculated from respiration and bacterial production.

chromatographic 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_{phyto} to these treatments, containing the highest amounts and proportions of aromatic structures.

3.3 Bacterial biomass and activities

The total amount of PLFA and the amount of the PLFA $i+a15:0$, 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 PLFA 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 PLFA 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 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 PLFA (10.7, Fig. 4).

Overall, bacterial growth efficiency (BGE) was 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 single-source incubations ($df = 5$, $p = 0.117$).

3.4 Carbon flow

We aimed to identify the DOC sources respired (measurement of DIC respired and Keeling plot analysis) or assimilated (via PLFA) by heterotrophic bacteria via stable C isotope analysis. Unfortunately, we were not able to determine the stable C isotope composition of dissolved inorganic carbon (DIC) for each treatment due to methodological problems (two are missing in treatments 1 and 2, and one is missing in treatments 3 and 4). In these samples we did not measure an increase 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 models 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_{phyto} 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 denaturing gradient gel electrophoresis (DGGE) methods showed similar results (Fig. 7). The mixed treatments were clearly separated from the single-source incubations. In particular, bacterial communities incubated with the single sources clearly differed from those of the four treatments (Fig. 7b).

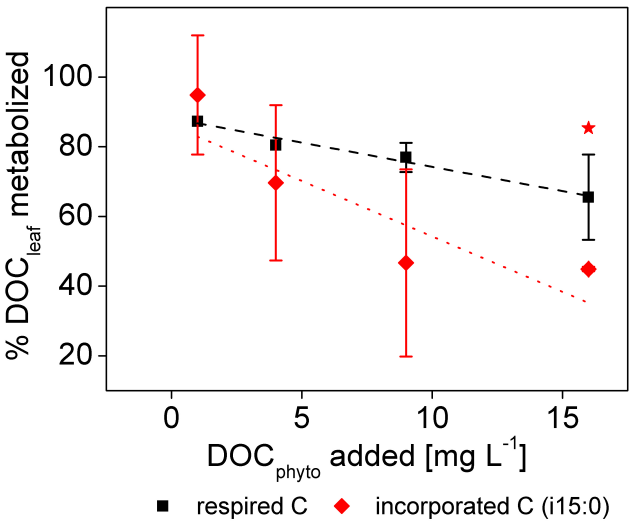

Fig. 6. Percentage of DOC_{leaf} metabolized via respiration (black square) or assimilation into *i*15 : 0 phospholipid fatty acids (PLFA; red rhombi, indicative of heterotrophic bacteria) of each treatment calculated from a two-source mixing model at the end of the experiment. The star marks an outlier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

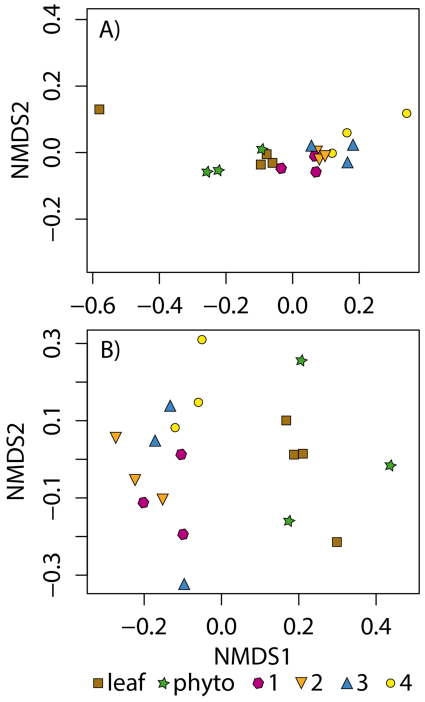

Fig. 7. Nonmetric multidimensional scaling (nMDS) plots representing differences between the phytoplankton (phyto) and leaf leachate single incubations and the four treatments of **(A)** phospholipid-derived fatty acids (PLFA) concentration patterns and **(B)** denaturing gradient gel electrophoresis (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 $A = 0.013$ and $B = 0.166$.

The variability between the triplicates, however, was relatively high. Nevertheless, the distances between all triplicates of the single-source incubations 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 measured an efficient bacterial decomposition of both sources in separate incubations and an increasing DOC decomposition with increasing additions of autochthonous DOC_{phyto} to allochthonous DOC_{leaf}. In addition, we revealed that bacterial decomposition of a mixture of both allochthonous and autochthonous DOC sources changes with quantity of autochthonous C and bacterial community composition (BCC) changes with chemical quality (mixing of both sources). 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 BCC. This 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 a low aromaticity in the HS fraction. It is controversial as to whether distinct DOC fractions such as LMWS should be considered labile or refractory to bacterial utilization. Kaiser and Sulzberger (2004) found bioreactivity, i.e., bacterial utilization, was higher for LMWS than HMWS in the Tagliamento River. In line with this study, our results indicate a high microbial accessibility of both DOC sources with relatively low molecular sizes.

The total DOC consumption and bacterial growth efficiency (BGE) was very high, suggesting a high availability of the phytoplankton lysate as well as the leaf leachate. A heterogeneous 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 preprocessing of organic substrates by microbes before the C enters the DOC pool. Sun et al. (1997) found that leachate of particulate organic matter (POM) can be more

biologically available than bulk riverine DOM and results by Pérez and Sommaruga (2006) indicated a more efficient utilization of algal lysates compared to soil-derived DOM. Therefore, besides chemical composition, the diagenetic alteration is also a major factor determining DOC bioavailability. This suggests that a contribution 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. 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 was 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_{phyto} requires more energy for its microbial break down. Whereas the aromaticity of the HS fraction decreased in the DOC_{phyto} incubation at the end of the incubation, it increased in the DOC_{leaf} incubation (Fig. 2). The decrease in aromaticity of the HS fraction in the DOC_{phyto} incubation can be explained by a preferential utilization of aromatic compounds of the HS fraction. In contrast, increased aromaticity of the HS fraction in the DOC_{leaf} incubation 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 chemical quality of the entire DOC pool rather than on its source (e.g., allochthonous vs. autochthonous).

4.2 Bacterial DOC availability of mixed sources

We changed the ratio of both DOC sources and 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 DOC sources increased with increasing DOC_{phyto} additions (Fig. 3). In the main experiment, this increase in DOC consumption was paralleled by a significant increase in microbial biomass. This effect was more pronounced when taking PLFA (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 change.

Interestingly, DOC consumption was not related to the strength and frequency of the DOC pulse. An additional pulse experiment (Supplement 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 $= 24\,\mathrm{mg\,C\,L^{-1}}$, Supplement B) differing in amount and frequency (Kruskal–Wallis: $\mathrm{d}f = 3$, $p = 0.059$). However, differences were significant when adding different concentrations of DOC without pulsing at the beginning of the experiment (Kruskal–Wallis: $\mathrm{d}f = 3$, $p = 0.016$). Thus, total DOC amount (i.e., quantity), and not pulsing, influenced bacterial DOC consumption in our experiment when nutrients were sufficiently supplied.

The chemical analysis unraveled preferences in the degradation of discrete C fractions. The relative degradation of HS and aromatic structures was higher with increasing DOC_{phyto} concentrations (Fig. 3). HS, the most abundant fraction of DOC in natural aquatic ecosystems (Thurman, 1985) and in our experiment (Figs. 2, 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, for example, 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.

Although our data did not detect statistical 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_{phyto} incorporated into bacterial biomass in comparison to the proportion respired was higher with increasing concentrations of DOC_{phyto}, indicating preferential incorporation of DOC_{phyto} into bacterial biomass when both DOC sources were mixed. To our knowledge, this is one of the first studies that has simultaneously determined the C flow via assimilation and respiration. These results, although not statistically significant, give a first glimpse on different pathways of autochthonous vs. allochthonous DOC within the bacterial community and should be followed up on in more detail in future studies.

The generally high bioavailability of both DOC sources might be partially related to the non-limiting concentrations of N and P in our setup, 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. Besides 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 (Supplement C). Thus, changes in bacterial C utilization can be related solely to quality and quantity of DOC in our experimental setups.

4.3 Effects of single and mixed DOC sources on BCC dynamics

In parallel to changes in bacterial activity, the question remains whether this is accompanied by a change in bacterial community composition (BCC). 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 but BCC did not shift with increasing additions of autochthonous DOC. Thus, we found no relation between BCC and metabolic function

However, BCC shifted when both sources were mixed in the treatments. A greater diversity of compounds due to the combination of two or more DOC sources can change BCC (Findlay et al., 2003; Carlson et al., 2002; Fonte et al., 2013). Such shifts in BCC were always accompanied by metabolic changes in these studies. However, most of these studies used a labile and a more refractory C source (Fonte et al., 2013). It was suggested that bacterial taxa partition along a C source gradient with different ratios of allochthonous vs. autochthonous C (Kritzberg et al., 2006; Jones et al., 2009). In our incubations, BCC seems to be more affected by the presence of both labile allochthonous and labile autochthonous DOC sources but not by the different ratios of the two DOC sources.

Surprisingly, we found no differences between the two single-source incubations. This could be due to the fact that the DGGE technique might only reveal the abundant bacterial taxa. Thus, this result needs to be further tested, with higher resolution techniques, for example, next generation sequencing.

5 Conclusions

Our study sheds light on bacterial DOC consumption and BCC dynamics in relation to DOC quantity and quality. When nutrients are not limiting, increasing DOC concentrations generally led to an increase in bacterial biomass, which, in parallel to a relatively high BGE, results in an enhanced C turnover and utilization of the more aromatic HS fraction, particularly of the DOC_{phyto}. Interestingly, the pulsed additions of fresh DOC have a minor effect on bacterial C dynamics as long as the sum of DOC is at the same level. BCC only shifted when both sources are supplied, pointing to a large 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 under nutrient-replete conditions, whereas quantity and chemical quality of bulk DOC constitute important controlling factors for bacterial C consumption and BCC, respectively.

Supplementary material related to this article is available online at http://www.biogeosciences.net/11/1479/2014/bg-11-1479-2014-supplement.pdf.

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