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Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter

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Bacteria play a central role in the cycling of carbon, yet our understanding of the relationship between the taxonomic composition and the degradation of dissolved organic matter (DOM) is still poor. In this experimental study, we were able to demonstrate a direct link between community composition and ecosystem functioning in that differently structured aquatic bacterial communities differed in their degradation of terrestrially derived DOM. Although the same amount of carbon was processed, both the temporal pattern of degradation and the compounds degraded differed among communities. We, moreover, uncovered that low-molecular-weight carbon was available to all communities for utilisation, whereas the ability to degrade carbon of greater molecular weight was a trait less widely distributed. Finally, whereas the degradation of either low- or high-molecular-weight carbon was not restricted to a single phylogenetic clade, our results illustrate that bacterial taxa of similar phylogenetic classification differed substantially in their association with the degradation of DOM compounds. Applying techniques that capture the diversity and complexity of both bacterial communities and DOM, our study provides new insight into how the structure of bacterial communities may affect processes of biogeochemical significance.

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Introduction

Carbon (C) cycling has received considerable attention in recent years, spurred by the increase of carbon dioxide concentrations in the atmosphere and the therewith-associated changes in climate (Solomon et al., 2007). In the wake thereof, attempts have been made to balance the global C budget and to develop a mechanistic understanding of its underlying dynamics. This has led to a revision of the traditional view in which inland waters were considered a passive ‘pipe’ that merely transported C from land to sea. It is now, however, recognised that inland waters make up an active compartment: one that mineralises, transforms and stores C of terrestrial origin besides transporting it to the oceans (Cole et al., 2007; Battin et al., 2009; Tranvik et al., 2009). Therefore and in view of future climatic changes, it is of great importance to comprehend which factors influence the mineralisation and transformation of terrestrially derived C in freshwater ecosystems.

It is the bacteria that essentially decompose this allochthonous dissolved organic matter (DOM) and introduce it into the aquatic food web (Pomeroy 1974; Azam et al., 1983; Jansson et al., 2007). Bacterial degradation of DOM is carried out by phylogenetically diverse communities, whose composition has been shown to be affected by the quality and quantity of DOM (for example, Logue and Lindström, 2008). Furthermore, differences in bulk bacterial processes (for example, bacterial respiration or production) related to changes in DOM quality and quantity point towards the existence of functionally distinct bacterial groups (for example, Kirchman et al., 2004). Yet, studies
investigating how the composition of bacterial communities affects the cycling of C in fresh waters have to date yielded inconclusive results; while some argue to having observed a close relationship between bacterial community composition (BCC) and C processing (Crump et al., 2003; Kirchman et al., 2004; Judd et al., 2006; Kritzberg et al., 2006; Langenheder et al., 2006; Bertilsson et al., 2007), others found inconsistent (Comte and del Giorgio, 2009, 2010; Lindström et al., 2010) or weak links (Langenheder et al., 2005). It has to be noted, though, that rather than actually demonstrating a direct relationship between BCC and C processing (see Langenheder et al., 2005, 2006), most studies illustrate that environmental parameters, such as DOM quality and quantity, affect community composition and functioning alike. Given the intertwined nature of BCC, the environment and bacterial functioning, studies directly addressing the relationship between aquatic BCC and C processing are clearly lacking.

This lack may be partly due to former methodological limitations. Despite their importance in aquatic systems, DOM and microbial diversity yet remain to be characterised for the most part (Curtis and Sloan, 2003; Hertkorn et al., 2008). As DOM is one of the most complex molecular mixtures on Earth (Hedges et al., 2000) and microbial communities are extremely diverse (Curtis and Sloan, 2004), studies going beyond bulk assessments of DOM as well as the most abundant members of microbial communities have been rather challenging. Recent technological advances in the field of molecular biology (for example, high-throughput sequencing) and adopting advanced instrumental approaches into analytical chemistry (for example, electrospray ionisation mass spectrometry (ESI-MS)) have, however, made it possible to obtain information of greater resolution and depth in this respect (see Kujiawinski, 2011 for an overview and Herlemann et al., 2014; Landa et al., 2014 and Shabarova et al., 2014 for studies that combine the two approaches). Such an in-depth and integrative characterisation of both complex DOM compounds and microbial communities is a prerequisite for exploring the relationship between microbial community composition and the processing of DOM.

Here we studied the link between the composition of aquatic bacterial communities and the degradation of DOM of terrestrial origin. The aim was to examine how bacterial communities different in composition differ in their processing of DOM. We hypothesised that bacterial assemblages of different origin differ in their ability and potential to degrade DOM, because they vary in composition. We tested this hypothesis by adopting a common garden experiment in which a uniform, terrestrially derived yet artificially prepared DOM medium was inoculated with aquatic bacterial communities collected from four sites of varying environmental character.

Materials and methods

Study sites and sampling

Study sites. The four environmental sites that were selected for this experiment are all situated within the Umeå River basin in the boreal zone of northern Sweden and differed in dissolved organic carbon (DOC) characteristics (Supplementary Table S1). Two aquatic samples were taken within the Krycklan catchment at the Svarterget long-term ecological research site (Laudon et al., 2013): that is, a humic headwater lake (EnvHL) and a groundwater (EnvGW) sample. The two remaining aquatic samples were collected downstream of the Krycklan catchment in the Vindelälven River (EnvVA), one of two major tributaries to the Umeå River, and its mouth in the Baltic Sea (EnvBa).

Sampling. Sampling was carried out on 29 May 2012, towards the end of the spring flood. Two samples were taken at each site: one for bacterial abundance and community composition and one for water chemistry analyses. Samples for bacterial abundance and community composition were collected in sterile 1-litre polypropylene bottles (Nalgene, Rochester, MN, USA), whereas water chemistry samples were collected in acid-washed (p.a. quality HCl; Sigma-Aldrich, St Louis, MO, USA) and Milli-Q (ion- and nuclease-free water) -rinsed polyethylene bottles (Mellerud Plast, Mellerud, Sweden). EnvBa, EnvHL and EnvVA were sampled taking grab samples, whereas EnvGW was sampled from a shallow, perforated groundwater well.

Samples were kept cold and in the dark during transportation to the laboratory. In the laboratory, water chemistry samples were stored at −20 °C until further processing, while samples for bacterial abundance and community composition were first pre-sieved (225 μm; nylon net filter) and filtered with a GF/F filter (0.7 μm, pre-combusted at 400 °C for 6 h; Whatman, Maidstone, UK) to avoid capturing larger particles and remove grazers, respectively.

Experimental set-up

The experiment was performed as a common garden experiment, applying a batch culture approach in which a medium derived artificially from soil was inoculated with bacterial cells from the four environments (henceforth called experimental treatments: Ba, GW, HL, and VA). A control, consisting of medium only (that is, no bacterial inoculum added), was run alongside the four experimental treatments.

Batch cultures were prepared in 1-litre glass bottles with a sealing constructed as follows: a polybutylene terephthalate screw-cap with aperture, holding a silicone rubber seal pierced with two holes; one for a metal needle connected to a sterile 60-ml syringe to enable the withdrawal of sample material, the other one for a sterile venting filter to
avoid the creation of a vacuum when withdrawing sample material. Batch cultures were filled without headspace and stirred continuously throughout the experiment. Stirring was performed using magnetic stir bars in combination with magnetic stirrers. Magnetic stir bars were acid-washed (p.a. quality HCl; Sigma-Aldrich), rinsed with Milli-Q and heat-sterilised at 120 °C before usage in batch cultures.

The medium was prepared from soil collected from the topsoil layer within the riparian zone 50 m downstream of the groundwater-sampling site. The soil was kept cold and in the dark during transportation to the laboratory, where it was stored at −20 ºC until further processing. In the laboratory, ~200 g of soil were added to 0.8 l of Milli-Q and shaken on a rotary table in the dark for 3 h. The soil–water mixture was then subjected to a stepwise filtration, starting from filters with a pore size of 225 down to 0.2 μm. Coarse filters (225, 150, 75, and 50 μm; nylon net filters) were acid-washed (p.a. quality HCl; Sigma-Aldrich), rinsed with Milli-Q and heat-sterilised at 120 °C, whereas filters of smaller pore size were either combusted at 400 °C for 6 h (20 and 8 μm; Cellulose Filters Ashless Grades; Whatman) or heat-sterilised at 120 °C (0.7 and 0.2 μm; Supor PES Membrane Disc Filters; Pall Corporation, Port Washington, WI, USA) before utilisation. In a final step, tangential flow filtration (50 kDa; Pellicon XL Filter; Merck Millipore, Billerica, MA, USA) was performed to obtain sterile medium with regard to bacteria.

BCC were, moreover, also taken from the four original environments (from the respective GF/F-filtrate; Supplementary Methods S1 and Supplementary Figure S1).

DOM analyses
Samples analysed for DOC and optical properties were pre-filtered with a 0.2-μm syringe filter (Puradisc PES; Whatman).

Dissolved organic carbon. The concentration of DOC was recorded using a Sievers 900 Laboratory Total Organic Carbon Analyzer (UV/persulfate oxidation; GE Analytical Instruments, Manchester, UK). The manner in which the medium was prepared ensured negligible concentrations of particulate organic material; hence, total organic C is comparable to DOC.

UV–visible absorbance and fluorescence. Absorbance spectra were measured from 200 to 700 nm at 1-nm intervals, with a Lambda 35 UV–visible spectrometer (Perkin Elmer, Waltham, MA, USA). Samples were measured in a 1-cm quartz cuvette and distilled water was used as a blank measurement.

Excitation–emission matrices (EEMs) were collected with a FluoroMax-2 spectrofluorometer (Horiba Scientific, Edison, NJ, USA), using a 1-cm quartz cuvette. Excitation wavelengths (λE) spanned from 250 to 445 nm in 5-nm increments, whereas emission wavelengths (λEm) ranged from 300 to 600 nm at increments of 4 nm. Excitation and emission slit widths were set to 5 nm and the integration time was 0.1 s. Blank subtraction, correction of EEMs and calibration to Raman units was carried out according to Murphy et al. (2010). Four individual fluorescing components in the EEMs were identified and validated with parallel factor (PARAFAC) analysis, using the MATLAB and Statistics Toolbox (R2013a; The MathWorks, Inc., Natick, MA, USA) in combination with the DOMFluor toolbox (Stedmon and Bro, 2008). The components were derived from the EEMs of 95 samples and their fluorescence characteristics are depicted as insets in Figure 3b.

Electrospray ionisation mass spectrometry. DOM was first isolated via solid-phase extraction (SPE) as described by Dittmar et al. (2008).
any presently available DOM isolation method—only retains a certain fraction of the total DOM (that is, polar compounds of low to moderate molecular weight), yet extraction efficiency is generally higher compared with other isolation methods (Green et al., 2014). In brief, experimental samples were filtered (0.2 μm; Puradisc PES Syringe Filter; Whatman), acidified with HCl (p.a. quality; Sigma-Aldrich) to pH 2.5 and stored at 4 °C until SPE. SPE cartridges (Bond Elut-PPL, 1 g, 6 ml; Agilent Technologies, Santa Clara, CA, USA) were soaked overnight in methanol (LC-MS CHROMASOLV; Sigma-Aldrich), rinsed in Milli-Q, re-rinsed with methanol, and then rinsed with acidified Milli-Q (pH = 2, p.a. quality HCl; Sigma-Aldrich). Immediately before SPE, samples were acidified one more time with HCl (p.a. quality; Sigma-Aldrich) to pH 2. Acidified sample aliquots (600 ml) were allowed to pass through the SPE cartridges by gravity. Cartridges were subsequently rinsed with acidified Milli-Q (pH = 2, p.a. quality HCl; Sigma-Aldrich) and dried with gaseous N₂. DOC was eluted with 4 ml acidified sample aliquots (600 ml) were allowed to pass through the SPE cartridges by gravity. Cartridges were subsequently rinsed with acidified Milli-Q (pH = 2, p.a. quality HCl; Sigma-Aldrich) and dried with gaseous N₂. DOC was eluted with 4 ml acidified Milli-Q and stored at −20 °C.

Mass spectra were collected on a quadrupole time-of-flight mass spectrometer, operating in scan mode with negative ESI. Blank injections of the mobile phase and two selected samples (henceforth called reference samples) were each measured four times throughout, with the latter to check for instrument drift, measurement reproducibility and analytical precision. A more detailed description can be found in Supplementary Methods S2.

Data reduction of mass spectra was conducted as follows: mass to charge ratios (m/z) were binned to integers, which resulted in 1900 m/z ranging from 100 to 1999 (see Figure 5a for an example). Subsequently, sample representative mass spectra were obtained by combining spectra across the injection profile. An average blank measurement was calculated and subtracted from all samples. The four separate measurements from each of the two selected reference samples were thereafter used to estimate and test for analytical precision. At first, the s.d. for each m/z for both reference samples were calculated. Next, the highest s.d. from either of the two was selected at each m/z and multiplied by 2. Finally, this recombined spectra was adopted to define a threshold for indicating significant changes in DOM mass spectra during the experiment; only changes >2 s.d. for each respective m/z were considered significant and included in subsequent analyses.

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**Bacterial abundance**

Bacterial cells were preserved in sterile-filtered, borax-buffered formaldehyde at a final concentration of 4% w/v. Bacterial abundance was enumerated by flow cytometric determination (CyFlow space; Partec, Münster, Germany) of SYTO 13- (Invitrogen, Carlsbad, CA, USA) stained cells, following the method described by del Giorgio et al. (1996).

**Nucleic acid extraction, PCR and pyrosequencing**

Bacterioplankton cells were collected onto 0.2 μm membrane filters (Supor-200 Membrane Disc Filters; Pall Corporation), filtering 0.2 l of water. Filters were placed into sterile cryogenic vials (Nalgene) and finally kept at −80 °C until further processing.

**Nucleic acid extraction.** Nucleic acid extraction was performed following the protocol 3 of the Easy-DNA kit (Invitrogen) with an extra 0.2 g of 0.1 mm zirconia/silica beads. Extracted nucleic acids were sized and yields quantified by means of agarose (1%) gel electrophoresis, GelRed staining (Biotium Inc., Hayward, CA, USA) and UV transillumination before PCR amplification.

**PCR amplification and template preparation.** The bacterial hypervariable regions V3 and V4 of the 16S rRNA gene were PCR amplified, using bacterial forward and reverse primer 341 (5’-CCTACGGGNGGCWGGCAG-3’) and 805 (5’-GACTACHVGGGTATACTAATCC-3’), respectively (Herlemann et al., 2011). The primers were modified before employment according to the final configuration: Adaptor B-341F and AdaptorA-MID-805R (AdaptorA and B are 454 Life Sciences adaptor sequences; 454 Life Sciences, Branford, CT, USA). Multiplex identifiers were seven-nucleotide long, sample specific and developed following recommendations by Engelbrektsen et al. (2010). PCR reactions were performed in a 20-μl reaction volume comprising 0.4 U Phusion high-fidelity DNA polymerase (Finzymes, Espoo, Finland), 1× Phusion HF reaction buffer (Finzymes), 200 μM of each dNTP (Invitrogen), 200 nM of each primer (Eurofins MWG, Ebersberg, Germany), 0.1 mg ml⁻¹ T4 gene 32 protein (New England Biolabs, Ipswich, UK) and finally 5–10 ng of extracted nucleic acid. Thermocycling (DNA Engine (PTC-200) Peltier Thermal Cycler; Bio-Rad Laboratories, Hercules, CA, USA) was conducted with an initial denaturation step at 95 °C for 5 min, followed by 27 cycles of denaturation at 95 °C for 40 s, annealing at 53 °C for 40 s, extension at 72 °C for 1 min and finalised with a 7-min extension step at 72 °C. Four technical replicates were run per sample, pooled after PCR amplification and purified using the Agencourt AMPure XP purification kit (Beckman Coulter Inc., Brea, CA, USA). Nucleic acid yields were checked on a fluorescence microplate reader (Ultra 384; Tecan Group Ltd, Männedorf, Switzerland), employing the Quant-iT PicoGreen dsDNA quantification kit (Invitrogen). Finally, PCR amplicons were pooled in equimolar proportions to obtain a similar number of 454-pyrosequencing reads per sample.

**Pyrosequencing.** The final pooled amplicon was sequenced unidirectionally (Lib-L chemistry) on a
454 GS-FLX system (454 Life Sciences) at the Norwegian High-Throughput Sequencing Centre (NSC, Oslo, Norway; http://www.sequencing.uio.no), using GS-FLX Titanium reagents.

**Sequence analyses**
The 454-pyrosequencing errors, PCR single base errors and chimeric sequences were removed from the 454-pyrosequencing amplicon library employing AmpliconNoise (v1.26; Quince et al., 2011) followed by Perseus (Quince et al., 2011). Pyrosequencing reads not matching multiplex identifier and/or primer sequences were removed just as were reads shorter than 200 bp. Reads were further truncated at 450 bp, eliminating additional noise (Mardis, 2008), and finally trimmed off multiplex identifier and primer sequences.

Denoised 454-pyrosequences were clustered into operational taxonomic units (OTUs) at a level of 97% sequence identity (AmpliconNoise, v1.29; Quince et al., 2011) and classified based on the RDP naive Bayesian rRNA Classifier (RDP Classifier, v2.6; Wang et al., 2007). Representative sequences were aligned based on the SILVA alignment (release 102; Quast et al., 2013) using mothur (v1.33.2; Schloss et al., 2009). Finally, pyrosequences that could neither be aligned nor assigned, or were assigned as Archaea or Eukaryota (for example, chloroplasts) were further removed. The 454-pyrosequencing reads of both experimental (Ba, GW, HL, and VA) and environmental (EnvBa, EnvGW, EnvHL, and EnvVA) samples have been deposited at the NCBI Sequence Read Archive under accession number SRP021096.

**Data analyses**
Pyrosequencing sampling efforts (that is, the number of pyrosequences obtained per sample) were normalised for statistical data analysis. Normalisation was done across samples through sub-sampling and analyses are based on 29 206 reads randomly drawn from each experimental sample. To analyse differences in BCC among experimental treatments, a multivariate generalised linear model (Wang et al., 2012; Warton et al., 2012) was applied. The model that is fitted is log-linear and assumes a negative binomial distribution of data. Relationships between bacterial assemblages at the end of the experiment were visualised employing non-metric multidimensional scaling (Bray–Curtis distance) ordination.

To investigate whether bacterial abundances or DOC concentrations were significantly different between treatments at the end of the experiment, a one-way analysis of variance (ANOVA) was carried out. Repeated-measures ANOVAs were performed for bacterial abundances, DOC concentrations and fluorescent intensities of PARAFAC components between experimental treatments, to test for treatment and time effects as well as for an interaction of the two throughout the experiment. Permutational ANOVAs (Euclidean distance) were computed to examine differences between the experimental treatments with respect to fluorescence, fluorescent (PARAFAC) components and m/z at the end of the experiment. The relationships between experimental treatments regarding m/z were, in addition, analysed by principal component analysis.

Finally, associations between bacterial OTUs and change in m/z were examined via Mantel’s test and correlation analysis. Mantel’s testing was carried out between distance matrices derived from the final relative abundances of bacterial OTUs (Bray–Curtis distance) and change in m/z from the beginning to the end of the experiment (Euclidean distance). Correlation analysis tested for co-variation between the final relative abundance of the most abundant bacterial taxa and change in m/z. The most abundant taxa were arbitrarily defined as OTUs containing >100 reads per OTU across all experimental samples (35 in total). To correct for multiple correlations, P-values were adjusted according to the false discovery rate (Benjamini and Hochberg, 1995).

All statistical data analyses were conducted using R (2015), in particular the vegan (Oksanen et al., 2008) and the mvabund (Wang et al., 2012) packages, and P-values were opposed to an α-value of 0.05.

**Results**

**BCC analysis**
Environmental and experimental samples contained on average 1259 and 64 OTUs, respectively (Supplementary Table S2 and Supplementary Figure S2). Bacterial communities from the four environmental sites were distinct from one another in composition (Supplementary Figure S3). The triplicate experimental bacterial assemblages were more similar in composition to each other than to such from other experimental treatments as both non-metric multidimensional scaling ordination (Figure 1) and multivariate generalised linear model (Wald = 35.88, P = 1.00E−03) show.

**Bacterial abundance analysis**
Bacterial abundances of the four treatments increased considerably over the course of the experiment from on average 1.6 × 10^4 in the beginning to between 4.1 × 10^7 and 1.8 × 10^8 cells ml^−1 in the end (Figure 2a). Abundances recorded in the controls were at, or marginally above, the detection limit throughout the experiment. Overall, bacterial abundances changed significantly over time and differently as to treatments over the course of the experiment (Table 1). Yet, only HL differed significantly from the other treatments in terms of bacterial abundance at the end of the experiment (ANOVA; F = 21.67, P = 3.39E−04; Figure 2a). The growth curves more or less resemble the growth of a single
species batch culture (with an initial lag, an exponential and the onset of a stationary phase).

**Analyses of DOM**
The concentration of DOC in all treatments decreased by approximately two-thirds over the course of the experiment from on average 17 mg C l$^{-1}$ in the beginning to 6 mg C l$^{-1}$ in the end (Figure 2b). The control samples also experienced a decline, albeit a considerably less pronounced one. On the whole, DOC concentrations changed significantly over time, although only VA significantly differed from the other three treatments over the course of the experiment (Table 1). DOC concentrations measured at the end of the experiment did not differ among the four treatments (ANOVA; $F = 0.85$, $P = 0.51$).

The changes in fluorescence in the controls were minimal compared with the four experimental treatments (Figure 3a). Ba, GW and HL showed a high degree of similarity in qualitative (spectral) change with a distinct removal of fluorescence at $\sim \lambda_{Em}$ 460 and 300 nm. VA, on the other hand, exhibited a notable difference from the other experimental treatments in that a loss of fluorescence at $\sim \lambda_{Em}$ 360 nm and an increase in fluorescence at $\sim \lambda_{Em}$ 470 nm could be observed (Figure 3a). Furthermore, the four treatments differed significantly from each other with regard to fluorescence at the end of the experiment (permutational ANOVA; $R^2 = 0.83$, $P = 2.00E-03$). PARAFAC analysis identified four distinct fluorescent components for which the molecular structures are unknown. Components one and two (C$_1$ and C$_2$) showed locations of maximum peak intensities typical of what is referred to as humic like, whereas component 3 (C$_3$) exhibited fluorescence properties similar to that of the amino acid tryptophan (also called protein like). Component 4 (C$_4$) depicted intermediate characteristics. The controls, in general, showed no change in fluorescence intensities for all four components (Figure 3b). Compared with the other two components, C$_1$ and C$_2$, remained more or less unaltered in fluorescence throughout the experiment, with only a slight systematic removal of C$_1$ in all treatments but VA. C$_3$ showed a substantial decrease in intensity, whereas C$_4$ experienced a marginal increase for all four experimental communities (Figure 3b). PARAFAC components predominantly changed significantly over time but only C$_3$ differed significantly throughout the experiment among all treatments (Table 1). Permutational ANOVA, furthermore, identified significant differences in PARAFAC components among the four treatments at the end of the experiment ($R^2 = 0.97$, $P = 9.99E-04$).
Table 1 Results from repeated measures ANOVA, testing for differences in bacterial abundances, DOC concentrations and fluorescent intensities of the four components identified by PARAFAC analysis between the experimental treatments and over time

|                  | Bacterial abundance | DOC | C1   | C2   | C3   | C4   |
|------------------|---------------------|-----|------|------|------|------|
|                  | df                  | F   | P    | df   | F    | P    | df   | F    | P    | df   | F    | P    |
| Treatment        | 3                   | 176.72 | <2.00E<sup>-16</sup><sup>a,b</sup> | 3 | 12.64 | 2.22E<sup>-6</sup> | 3 | 160.98 | <2.00E<sup>-16</sup><sup>c,d</sup> | 71.54 | <2.00E<sup>-16</sup><sup>x</sup> | 58.18 | <2.00E<sup>-16</sup><sup>d</sup> | 19.35 | 1.48E<sup>-08</sup><sup>x</sup> | 9.77 | 1.80E<sup>-06</sup><sup>x</sup> |
| Time             | 8                   | 2012.42 | <2.00E<sup>-16</sup><sup>*</sup> | 6 | 600.25 | <2.00E<sup>-16</sup><sup>*</sup> | 6 | 75.39 | <2.00E<sup>-16</sup><sup>*</sup> | 3.48 | 2.73E<sup>-04</sup> | 2.81 | 2.19E<sup>-03</sup> | 5.24 | 1.83E<sup>-06</sup> |
| Treatment:time   | 24                  | 21.54 | <2.00E<sup>-16</sup><sup>*</sup> | 18 | 10.06 | 1.78E<sup>-11</sup> | 17 | 4.12 | 4.03E<sup>-05</sup> | 3.48 | 2.73E<sup>-04</sup> | 2.81 | 2.19E<sup>-03</sup> |
| Residuals        | 70                  | 54   |      | 52   |      |      |      |      |      |      |      |      |      |

Abbreviations: ANOVA, analyses of variance; BA, Baltic; DOC, dissolved organic carbon; GW, groundwater; HL, headwater lake; PARAFAC, parallel factor; VA, Vindelälven River.

*Indicate significant P-values.

Note that experimental treatments HL and VA did not significantly differ in bacterial abundances from each other throughout the experiment (assessed by linear mixed-effects model and Tukey’s post-hoc test).

Note that only VA significantly differed from the other treatments throughout the experiment with regard to DOC concentrations (assessed by linear mixed-effects model and Tukey’s post-hoc test).

Note that experimental treatments GW and HL did neither significantly differ in C1 nor C2 from each other throughout the experiment (assessed by linear mixed-effects model and Tukey’s post-hoc test).

Note that linear mixed-effects modelling and subsequent Tukey’s post-hoc testing with respect to PARAFAC components C1, C2, C3 and C4 could only be performed starting from the second time point, as linear mixed-effects models do not accept missing data (data for the first time point was not available for VA).

Note that BA and GW did not significantly differ in C4 from HL or VA and HL, respectively, throughout the experiment (assessed by linear mixed-effects model and Tukey’s post-hoc test).
Figure 3  Net changes in DOM fluorescence (a) and fluorescent intensities of components identified by PARAFAC analysis (b).  
(a) Excitation–emission matrices (EEMs) at the start of the experiment (n = 1) together with the mean change and s.d. in fluorescence from the beginning to the end of the experiment across the three replicates for each treatment (n = 3). Excitation (λEx) and emission (λEm) wavelengths are given on the x and y axis, respectively. (b) Pictures changes of fluorescent intensities of PARAFAC components: C₁, C₂, C₃ and C₄ (mean ± s.e., n = 3; except for time point 0 and time point 2 for VA only, where n = 1). All components were normalised to zero for time point zero, except the ones in VA, which were normalised to zero for the second time point, as the measurement at time point zero had to be discarded owing to an erroneous reading. Insets visualise the respective spectral properties of the four fluorescent components identified by PARAFAC analysis. Abbreviations: Ba, Baltic; C, control; GW, groundwater; HL, headwater lake; VA, Vindelälven.
A major goal in ecology is to link the composition of biological communities with processes occurring in an ecosystem. Given the entwined nature of microbial community composition, the environment and ecosystem processes, one of the greatest challenges is to test for direct effects of composition on functioning. Common garden experiments allow for precisely that by standardising environmental parameters and, therefore, enabling the teasing apart of the effects of the environment from the composition of microbial communities on functioning (Reed and Martiny, 2007). The downside of incubating microbial communities under batch growth conditions, however, is that the resulting community will differ from the composition of its original inoculum (for example, Christian and Capone, 2002). Indeed, our analyses identified a change from environmental to compositional differences among the bacterial communities.

Our results, thus, show a close link between BCC and function. Going beyond a mere identification of a link between BCC and DOM degradation, our results further highlight that the four experimental communities degraded different components of the DOM pool. Although fluorescence analyses illustrate that certain DOM components were commonly more bioavailable than others, both fluorescence and ESI-MS analyses demonstrate that the four different bacterial communities differed in which DOM components were degraded preferentially. Most importantly, ESI-MS analysis uncovered that community composition was of little importance regarding the degradation of LMWC, whereas the utilisation of masses of greater size differed among communities. This means that the ability to use LMWC is a functional property (trait) rather common
in all of the four bacterial communities, whereas the capability to use C of high-molecular-weight appears to be a trait restricted to particular bacterial communities. An explanation could lie in a finding made by Weiss et al. (1991) that compounds of up to ~600 Da (that is, LMWC) can be taken up readily by microorganisms across the cell membrane (that is, through a variety of transmembranic transport systems), whereas larger ones require extracellular cleavage by means of enzymatic hydrolysis (that is, via individual or interacting ectoenzymes), an ability that indeed not all bacterial taxa possess (for example, Berlemont and Martiny 2013). Yet, bacterial members within a community vary not only with regard to the ability to produce ectoenzymes but also in their capability to express transmembranic transport systems that allow the uptake of compounds exceeding 600 Da (for example, Teeling et al., 2012). However, a microbial community’s toolbox of traits is more than the sum of its parts; on the one hand, some bacterial taxa may be needed to actually facilitate the degradation process, allowing other micro-organisms to either hydrolyse substrates further or take them up, on the other the process may only continue when some microbes act in concert. Pedler et al. (2014), for instance, demonstrated that the readily available fraction of a coastal DOM pool could be completely removed by a single taxon, whereas decomposition of the less bioavailable portion required additional members of the community. Hence, it becomes apparent that not only the chemical composition of DOM (that is, quality) but also the distribution of traits within microbial communities are important when it comes to whether or not DOM evades microbial remineralisation and transformation. As such, bioavailability can be perceived as an ongoing interaction between the chemical composition of DOM and a microbial community’s metabolic capacity rather than merely an inherent property of DOM (Nelson and Wear 2014). Although the degradation of either low- or high-molecular-weight carbon was not restricted to a single phylogenetic clade, our results illustrate that bacterial taxa of similar phylogenetic classification differed substantially in their association with the degradation of DOM compounds (both at a 97% and 99% sequence identity level; results for the latter are not shown). This may be an indication for high variation in the functional, and thus ecological,
potential among closely related populations within microbial communities (that is, micro-diversity; see Zimmerman et al., 2013); for example, the two as *Herminimonas* classified bacterial taxa C3 and C836 were generally associated with the degradation of low- and high-molecular-weight carbon, respectively. Hence, our results demonstrate that the capacity of a community to degrade DOM compounds cannot easily be predicted from phylogenetic information alone, at least not from information derived from the 16S rRNA gene (see also Covert and Moran, 2001; Fuhrman and Hagström, 2008 and Martiny et al., 2013).

Considering the associations observed between the relative abundances of the most abundant bacteria and the degradation of DOM compounds the question arises ‘Why do the observed degradation patterns not look more similar, given that these bacterial taxa were generally present in all four communities?’ One explanation could be that the functional gene repertoire of these bacteria varied between experimental communities as a result of adaptation to their original environments. Another could be that these abundant bacteria depend on other taxa with a different set of traits fundamental to the degradation of certain DOM compounds (see Pedler et al., 2014); taxa that are rarer and may not be present in all communities. Such interplay will, though, not be detectable via correlation analysis. In fact, caution has to be exercised when interpreting the results from the correlation analysis in that it does not allow drawing conclusions about the cause and effect, and, as such, cannot be used to unambiguously link a specific bacterial taxon to the degradation and utilisation of a particular DOM compound. In addition, size (for example, \( m/z \)) represents only one property of DOM; correlating other properties with bacterial taxa may yield more nuanced and different associations, as well as trait-specific insights. Once associations have been established, they may guide researchers to conduct studies more non-generic in character, such as controlled experiments in which the degradation capacities of a single bacterial population are investigated. Moreover, identifying functional genes involved in the degradation of DOM along with assigning the chemical composition to individual DOM compounds via ultrahigh-resolution MS (for example, Fourier transform ion cyclotron resonance MS; see Hertkorn et al., 2008) could potentially provide insight into microbial traits that may or may not be phylogenetically constrained. Combining such trait-based information with knowledge of the regulation of microbial activities, the monitoring of functional genes (metatranscriptomics or metaproteomics; for example, Moran, 2009; Teeling et al., 2012, respectively) and/or metabolic features (single cell genomics; for example, Rinke et al., 2013) may offer a way to explore the use of individual organic matter compounds by specific microbial taxa in complex communities to an even greater depth and improve our understanding of how microbial community composition may affect the cycling of C in the biosphere.

**Conflict of Interest**

The authors declare no conflict of interest.

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**Author contributions**

ESK conceived the study with contributions from CAS and ESL. JBL designed the study with contributions from CAS, ESL and ESK. JBL collected environmental samples with assistance of HL. AMK carried out the SPE and NIN ran the ESI-MS, while CAS performed PARAFAC and ESI-MS analyses. JBL collected all experimental data, performed 454-pyrosequencing analyses, analysed output data in close collaboration with AFA and wrote first draft of the manuscript to which all authors contributed in subsequent revisions.

**References**

Azam F, Fenchel T, Field JG, Gray JS, Meyerreil LA, Thingstad F. (1983). The ecological role of water-column microbes in the sea. *Marine Ecol Prog Ser* **10:** 257–263.
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Battin TJ, Luysaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ. (2009). The boundless carbon cycle. *Nat Geosci* 2: 598–600.

Benjamin Y, Hochberg Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J R Stat Soc B Met* 57: 289–300.

Berlemont R, Martiny AC. (2013). Phylogenetic distribution of potential cellulases in bacteria. *Appl Environ Microbiol* 79: 1545–1554.

Bertilsson S, Eiler A, Nordqvist A, Jørgensen NOG. (2007). Links between bacterial production, amino-acid utilization and community composition in productive lakes. *ISME J* 1: 532–544.

Christian RR, Capone DG. (2002). Overview of issues in aquatic microbial ecology. In: Hurst CJ, Crawford RL, Knudsen GR, Mcinerney MJ, Stetenbach LD (eds), *Manual of Environmental Microbiology*. 2nd edn. ASM Press: Washington, pp 323–328.

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Judd KE, Crump BC, Kling GW, Hobbie JE. (2003). Variation in bacterial plankton community structure along a range of freshwater ecosystems. *Environ Microbiol* 11: 1704–1716.

Jansson M, Persson L, De Roos AM, Jones RI, Tranvik LJ. (2007). Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends Ecol Evol* 22: 316–322.

Kritberg ES,コレ JJ, Pace MM, Granelli W. (2006). Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: Results from whole-lake C-13 addition experiments. *Ecosystems* 9: 489–499.

Kujawinski EB. (2011). The impact of microbial metabolism on marine dissolved organic matter. *Annu Rev Mar Sci* 3: 567–599.

Landa M, Cottrell MT, Kirchman DL, Kaiser K, Medeiros PM, Tremblay L et al. (2014). Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. *Environ Microbiol* 16: 1668–1681.

Langenheder S, Lindström ES, Tranvik LJ. (2005). Weak coupling between community composition and functioning of aquatic bacteria. *Limnol Oceanogr* 50: 957–967.

Langenheder S, Lindström ES, Tranvik LJ. (2006). Structure and function of bacterial communities emerging from different sources under identical conditions. *Appl Environ Microbiol* 72: 212–220.

Laudon H, Taberman I, Ågren A, Futter M, Ottosson-Löfvenius M, Bishop K. (2013). The Kryckelan Catchment Study—a flagship infrastructure for hydrology, biogeochemistry, and climate research in the boreal landscape. *Water Resour Res* 49: 7154–7158.

Lindström ES, Feng XM, Granelli W, Kritberg ES. (2010). The interplay between bacterial community composition and the environment determining function of inland water bacteria. *Limnol Oceanogr* 55: 2052–2060.

Logue JB, Lindström ES. (2008). Biogeography of bacterioplankton in inland waters. *Freshwater Rev* 1: 99–114.

Mardis ER. (2008). Next-generation DNA sequencing methods. *Ann Rev Genomics Hum Genet* 9: 387–402.
Martiny AC, Treseder K, Pusch G. (2013). Phylogenetic conservatism of functional traits in microorganisms. *ISME J* 7: 830–838.

Moran MA. (2009). Metatranscriptomics: eavesdropping on complex microbial communities. *Microbe* 4: 329–335.

Murphy KR, Butler KD, Spencer RGM, Stedmon CA, Boehme JR, Aiken GR. (2010). Measurement of dissolved organic matter fluorescence in aquatic environments: an interlaboratory comparison. *Environ Sci Technol* 44: 9405–9412.

Nelson CE, Wear EK. (2014). Microbial diversity and the lability of dissolved organic carbon. *Proc Natl Acad Sci USA* 111: 7166–7167.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB et al. (2008). vegan: Community Ecology Package. R package version 2.2-1.

Pedler BE, Aluwihare LI, Azam F. (2014). Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. *Proc Natl Acad Sci USA* 111: 7202–7207.

Pomeroy LR. (1974). The oceans food web, a changing paradigm. *Bioscience* 24: 499–504.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41: D590–D596.

Quince C, Lanzén A, Davenport RJ, Turnbaugh PJ. (2011). Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* 12:38: 1–18.

R. (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria. http://www.R-project.org/.

Reed HE, Martiny JBH. (2007). Testing the functional significance of microbial composition in natural communities. *FEMS Microbiol Ecol* 62: 161–170.

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF et al. (2013). Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499: 431–437.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75: 7537–7541.

Shabarova T, Villiger J, Morenkov O, Niggemann J, Dittmar T, Pernthaler J. (2014). Bacterial community structure and dissolved organic matter in repeatedly flooded subsurface karst water pools. *FEMS Microbiol Ecol* 89: 111–126.

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averty KB et al. (2007). *Climate Change 2007: The Physical Science Basis*. Cambridge University Press: Cambridge.

Stedmon CA, Bro R. (2008). Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnol Oceanogr Methods* 6: 572–579.

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Bennke CM et al. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336: 608–611.

Tranvik LJ, Downing JA, Cotner JB, Loisel SA, Striegl RG, Ballatore TJ et al. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol Oceanogr* 54: 2298–2314.

Wang Q, Garrity GM, Tiedje JM, Cole JR. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73: 5261–5267.

Wang Y, Naumann U, Wright ST, Warton DI. (2012). mvabund - an R package for model-based analysis of multivariate abundance data. *Methods Ecol Evol* 3: 471–474.

Warton DI, Wright ST, Wang Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods Ecol Evol* 3: 89–101.

Weiss MS, Abele U, Weckesser J, Welte W, Schiltz E, Schulz GE. (1991). Molecular architecture and electrostatic properties of a bacterial porin. *Science* 254: 1627–1630.

Zimmerman AE, Martiny AC, Allison SD. (2013). Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. *ISME J* 7: 1187–1199.

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