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ORIGINAL ARTICLE

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

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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, 2005; 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 Kujawinski, 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 Svartberget 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 °C 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 a sterile medium with regard to bacteria. Once the medium was diluted to a final concentration of 17 mg C l^{-1}, nitrogen and phosphorus were added as NH_4–NO_3 (final concentration: 3.26 mg N l^{-1}) and NaH_2–PO_4 (final concentration: 0.97 mg P l^{-1}), respectively, thus preventing nitrogen and phosphorus limitation.

Four inocula were prepared; one each from the respective GF/F-filtrate of the four environmental sites. After having been stored at 4 °C and in the dark for 6 days (that is, from sampling the bacteria in the field to preparing the inocula in the laboratory), the inocula were concentrated to ~ 1×10^6 cells ml^{-1} via tangential flow filtration (Merck Millipore) and subsequently added to the medium (1% of final volume; except to the controls). Finally, at time point zero (that is, the beginning of the experiment), each of the four experimental treatments and the control (5×1, $n = 5$) were divided up into three batch cultures, respectively: that is, treatments and control were each run in three independent triplicates (5×3, $n = 15$) after time point zero (see Supplementary Methods S1 for an in-detail description of inocula preparation, inoculation and start of the experiment).

The experiment was conducted in a constant temperature room at 15 °C and in the dark, and terminated after 5 and a half days on the basis of levelling off DOC concentrations. Samples were taken for bulk DOC concentration, UV–visible absorbance and fluorescence, ESI-MS, bacterial abundance, and BCC. Bulk DOC concentration and UV–visible absorbance and fluorescence were sampled on seven (that is, 0, 30, 78, 90, 102, 114, and 126 h), whereas samples for bacterial abundance were taken on nine occasions throughout the experiment (that is, 0, 10, 30, 54, 78, 90, 102, 114, and 126 h). ESI-MS samples were taken both at the beginning and at the end, whereas BCC was only sampled at the end of the experiment. Samples for BCC were, moreover, also taken from the four original environments (from the respective GF/F-filtrate; Supplementary Methods S1 and Supplementary Figure S1).

All glass- and plastic ware was acid-washed overnight in HCl (p.a. quality; Sigma-Aldrich), extensively rinsed with Milli-Q and combusted at 400 °C for 6 h or heat-sterilised (120 °C), respectively.

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 (λEx) 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). Note that SPE—as

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 N_2. DOC was eluted with 4 ml methanol 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.

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 16 S rRNA gene were PCR amplified, using bacterial forward and reverse primer 341 (5′-CCTACGGG NGGCWGCAG-3′) and 805 (5′-GAC TACHVGGGTA TCTAATCC-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 Engelbrektson *et al.* (2010). PCR reactions were performed in a 20-µl reaction volume comprising 0.4 U Phusion high-fidelity DNA polymerase (Finnzymes, Espoo, Finland), 1× Phusion HF reaction buffer (Finnzymes), 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, $P = 1.00\text{E}-03$) 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, $P = 3.39\text{E}-04$; Figure 2a). The growth curves more or less resemble the growth of a single

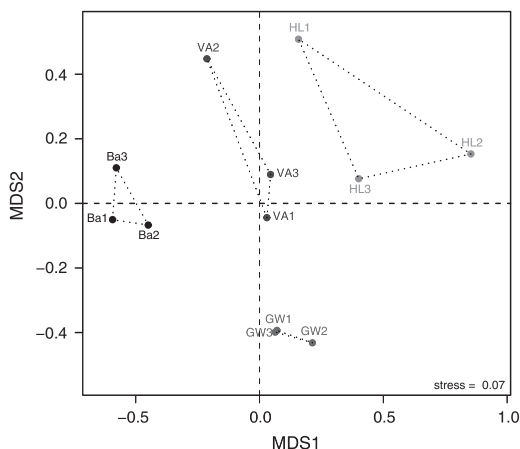

Figure 1 NMDS representation of bacterial communities from the four experimental treatments. NMDS ordination was derived from pairwise Bray–Curtis distances. Numbers depict replicates one, two and three. Hulls were drawn to group replicates within an experimental treatment. Abbreviations: Ba, Baltic; GW, groundwater; HL, headwater lake; VA, Vindelälven.

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 ~λEm 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 ~λEm 360 nm and an increase in fluorescence at ~λEm 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 across all treatments (Table 1). Permutational ANOVA, furthermore, identified significant differences in PARAFAC components among the four treatments at the end of the experiment ($R^2 = 0.97$, $P = 9.99E-04$).

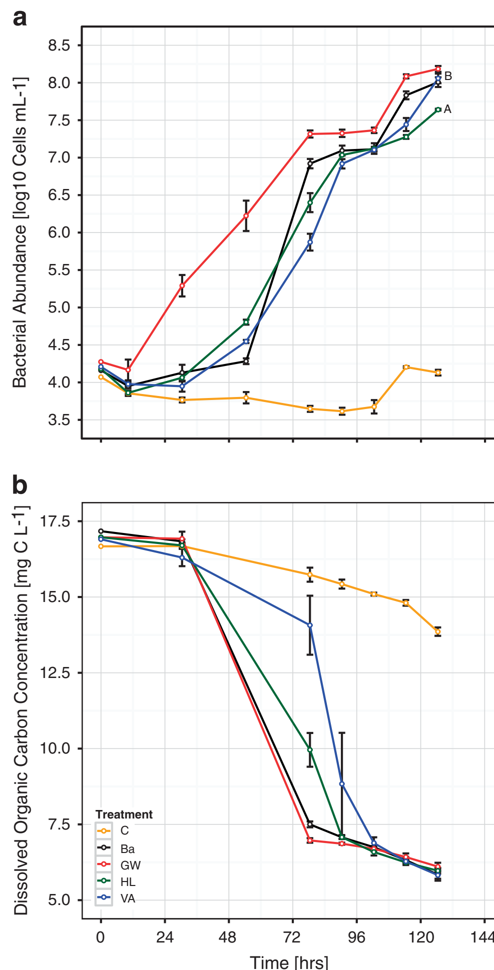

Figure 2 Trends of bacterial abundances (**a**) and DOC concentrations (**b**). Bacterial abundances and DOC concentrations were measured over the course of the experiment for the four treatments and the control (mean ± s.e., $n = 3$ replicates; except for time point 0, where $n = 1$). Letters A and B in **a** denote significant differences between treatments with regard to bacterial abundances at the end of the experiment (A) or not (B), respectively, assessed by means of Tukey's *post-hoc* test on an ANOVA. Abbreviations: Ba, Baltic; C, control; GW, groundwater; HL, headwater lake; VA, Vindelälven.

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*			*df*	*C1*		*C2*		*C3*		*C4*	
	df	*F*	P	*df*	*F*	P		*F*	P	*F*	P	*F*	P	*F*	P
Treatment	3	176.72	<2.00E−16*[,a]	3	12.64	2.26E−06*[,b]	3	160.98	<2.00E−16*[,c,d]	71.54	< 2.00E−16*[,c, d]	58.18	<2.00E−16*[,d]	19.35	1.48E−08*[,d,e]
Time	8	2012.42	<2.00E−16*	6	600.25	<2.00E−16*	6	75.39	<2.00E−16*	26.83	2.66E−14*	103.51	<2.00E−16*	77.89	<2.00E−16*
Treatment:time	24	21.54	<2.00E−16*	18	10.06	1.78E−11*	17	4.12	4.03E−05*	3.48	2.73E−04*	2.81	2.19E−03*	5.24	1.83E−06*
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.
[a]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).
[b]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).
[c]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).
[d]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).
[e]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).

Mass spectra (see Figure 5a for an example) of the four treatments did not differ notably from each other in the beginning of the experiment (Figure 4); however, they did at the end (as seen in Figure 4 and confirmed by permutational ANOVA; $R^2 = 0.42$, $P = 1.70E-03$). Some masses also decreased significantly in intensity in the controls (Figure 5b). Bacterial assemblages from Ba and HL reduced the intensity of masses from a very broad range of *m/z*, whereas bacteria from GW and VA tended to preferentially reduce the intensity of masses of <600 *m/z* (Figure 5b). Further, it is only within a very narrow mass range (~200–600 *m/z*; corresponding to low-molecular-weight carbon (LMWC); Supplementary Methods S2) that bacterial assemblages from all experimental treatments were able to degrade DOM (Figure 5c).

Associations between bacterial taxa and the degradation of DOM

Experimental communities similar in composition disclosed similar trends in the overall change of *m/z* over time (Mantel's test: $R = 0.38$, $P = 0.01$). Correlating the relative abundances of the most abundant bacterial taxa at the end of the experiment (classified predominantly as *Proteobacteria* and, to a far lesser extent, *Bacteroidetes*) with the change in *m/z* throughout the experiment showed a variety of both positive and negative associations, and that OTUs associated with different range intervals of change in *m/z* (Figure 6). With regard to the latter, strong positive associations between relative OTU abundance and the degradation of *m/z* of smaller or larger size could be found for β-proteobacterial taxa (that is, OTUs C1, C430 and C3), and α- (that is, OTUs C3141 and C18) and β-proteobacterial OTUs (that is, C3145 and C836), respectively. Furthermore, high relative abundances of the γ- and α-proteobacteria C812 and C0, respectively, associated strongly with a decrease in *m/z* over almost the entire range of *m/z*.

Discussion

Taking advantage of technological advances in analytical chemistry and molecular biology, we explored how the composition of aquatic bacterial communities affected the degradation of DOM of terrestrial origin. Having adopted an experimental approach in which model communities were exposed to a terrestrially derived DOM substrate, our results highlight that although bacterial communities that differ in composition degraded the same amount of DOM, both the temporal pattern of degradation and, most importantly, the compounds that were degraded significantly differed. Finally, we observed that the most abundant bacterial taxa differed substantially in their association with the degradation of DOM compounds.

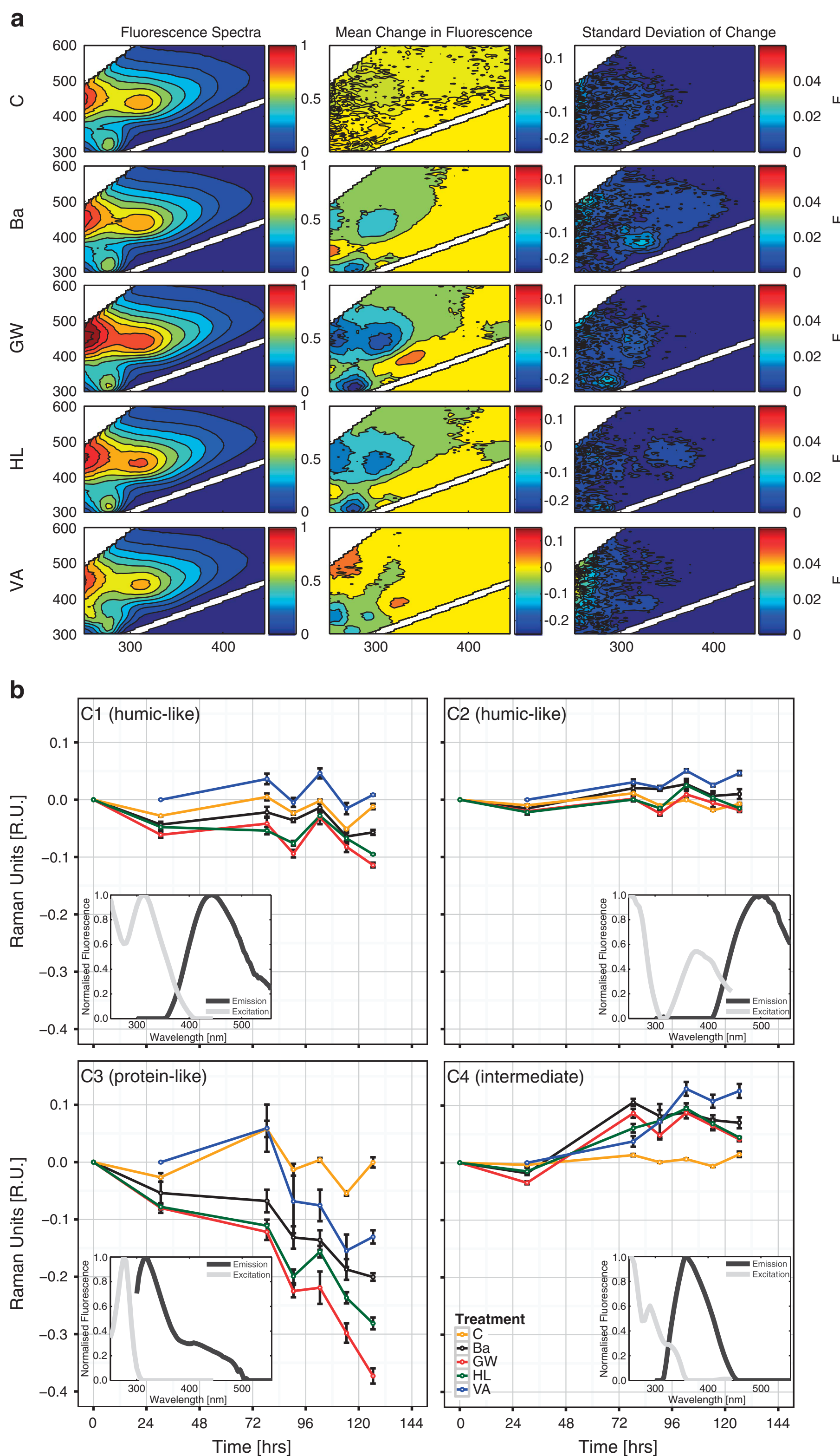

Figure 3 Net changes in DOM fluorescence (**a**) and fluorescent intensities of components identified by PARAFAC analysis (**b**). (**a**) Excitation–emission matrices (EEMs) at the start of the experiment ($n = 1$) together with the mean change and s.d. in fluorescence from the beginning to the end 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: C_1, C_2, C_3 and C_4 (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.

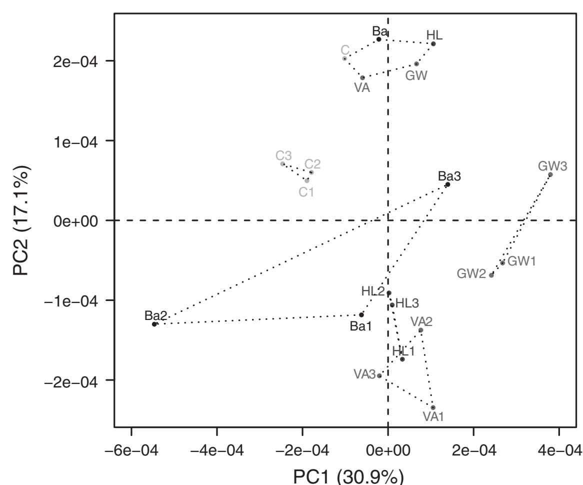

Figure 4 Results from principal component analysis of mass to charge ratios (m/z) that derived from ESI-MS. The first and second principal component explained 30.9% and 17.1% of the variability, respectively. Samples from both the beginning (not numbered) and end (numbered) of the experiment are visualised. Numbers depict replicates one, two and three. Hulls were drawn to group not only the replicates within a treatment but also the samples at the beginning of the experiment. Abbreviations: Ba, Baltic; C, control; GW, groundwater; HL, headwater lake; VA, Vindelälven.

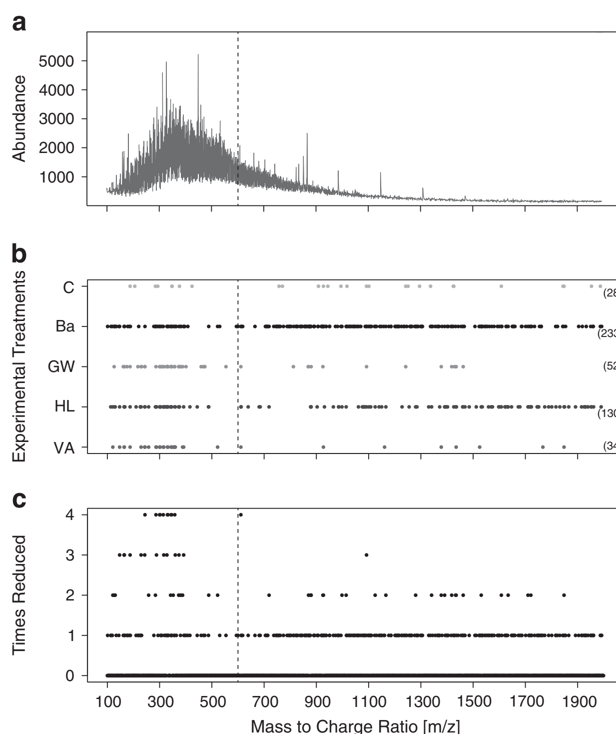

Figure 5 Results from ESI-MS analyses. (**a**) An example for the mass spectra of DOM obtained from an experimental sample (that is, GW) at the beginning of the experiment. Mass to charge ratios (m/z) that were significantly reduced in intensity throughout the experiment are visualised in **b**. Numbers in brackets specify the total number of m/z significantly reduced in intensity per sample by the end of the experiment. (**c**) m/z that were significantly reduced in intensity by none of the four experimental treatments and their respective replicates (Times Reduced 0), all three replicates of just one treatment (Times Reduced 1), all three replicates of only two treatments (Times Reduced 2), all three replicates of three and all four experimental treatments (Times Reduced 3 and 4, respectively) by the end of the experiment. The dashed line visualises the distinction between LMWC ($<600\,m/z$) and high-molecular-weight carbon masses ($>600\,m/z$). 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 experimental bacterial communities in both diversity and composition (Supplementary Figures S2 and S4, respectively). It has been further suggested that such experiments favour micro-organisms rare in nature but featuring opportunistic, copiotrophic qualities that allow for a more rapid adaptation to changes in environmental conditions and, hence, to outcompete others that are originally more abundant. In nature, DOM varies in quality (and quantity) over space and time (for example, Kothawala *et al.*, 2014), variations to which microbes need to adapt. Yet, the exposure of different bacterial communities in our experiment to a freshly prepared, terrestrially derived and, hence, highly bioavailable DOM substrate as C source possibly enhanced growth of such naturally rare bacteria. As the communities still differ in composition at the end of the experiment, it can, however, be assumed that the functional differences observed are the consequence of initial 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

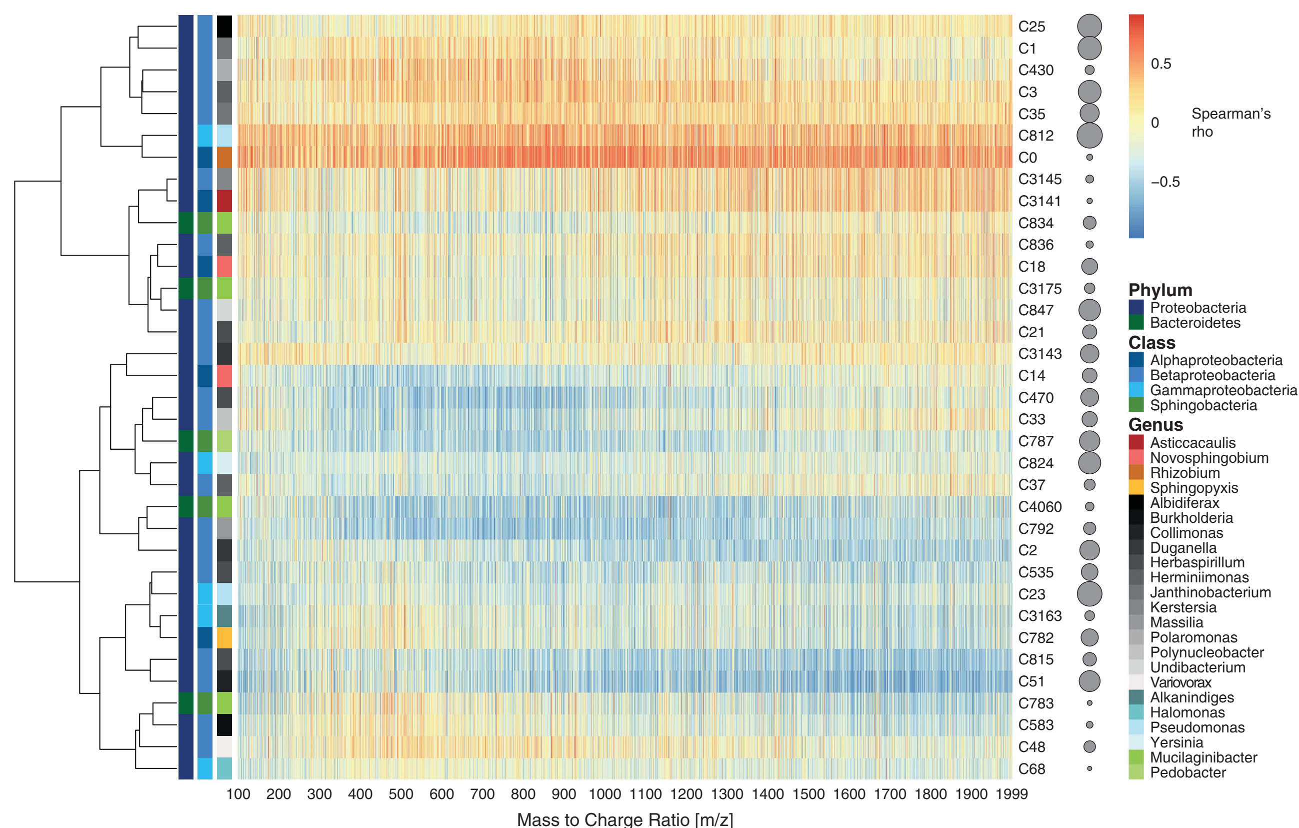

Figure 6 Heatmap visualising the Spearman's rank correlation coefficients (Spearman's ρ) from the correlation analyses between the relative abundance of the 35 most abundant (that is, >100 reads per) OTUs at the end (rows) and the change in mass to charge ratios (m/z) from the beginning to the end of the experiment (columns). A high correlation coefficient (red) stands for a strong positive correlation between an OTU's relative abundance and the decrease in m/z from the beginning to the end of the experiment. The dendrogram clusters OTUs according to Spearman's ρ, whereas the colour columns depict affiliation of OTUs in accordance with taxonomic classification (from left to right: phylum, class and genus; colours match the legend to the right of the heatmap). The size of each bubble is proportional to the OTU's relative abundance.

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 micro-organisms 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 *Herminiimonas* 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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Disclaimer

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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 NJN 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.

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