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RESEARCH ARTICLE

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Key Points:

- Riverine DOC is associated to patterns in microbial metabolic responses
- These metabolic responses are strongly linked to both DOC quality and DOC source
- Changes in DOC source affect aquatic ecosystem functioning

Supporting Information:

- Supporting Information S1

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Distinct patterns of microbial metabolism associated to riverine dissolved organic carbon of different source and quality

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Abstract Dissolved organic carbon (DOC) in rivers contains a wide range of molecules that can be assimilated by microbes. However, there is today no integrated understanding of how the source and composition of this DOC regulate the extent to which the DOC can support microbial growth and respiration. We analyzed patterns in microbial metabolism of DOC from different streams and rivers in Québec, by combining short-term bacterial production and respiration measurements with long-term DOC loss and analyses of bacterial use of different single substrates. We show that distinct metabolic patterns indeed exist across catchments, reflecting the varying nature and sources of the DOC. For example, DOC from forest headwaters systematically supported the highest bacterial growth efficiency (BGE) that was recorded, while in contrast DOC in peat bog drainage was used with significantly lower BGE. The carbon consumption in clear mountain rivers, possibly represented by autochthonous algal DOC, supported the highest bacterial respiration rates and the highest long-term DOC losses. By using principle component analysis, we demonstrate how the major axes of variation in all of the measured metabolic responses are tightly connected to spectrofluorometrical DOC composition indicators and to isotopic indicators of DOC source. If causality is assumed, our results imply that changes in DOC supply from different sources, for example, caused by land use or climate change, should result in dramatic changes in the patterns of aquatic microbial metabolism and thus in altered aquatic ecosystem functioning, with likely consequences for food-web structures and greenhouse gas balances.

1. Introduction

Loading of terrestrially derived dissolved organic carbon (DOC) shapes the functioning of inland water ecosystems, particularly through processes that are controlled by bacteria. For example, the bacterial biomass production (BP) based on DOC is increasingly recognized as a pathway fueling aquatic microbial food webs [Azam *et al.*, 1983; Jansson *et al.*, 2007]. Furthermore, the bacterial respiration (BR) of DOC contributes to globally significant greenhouse gas emissions [Lapierre *et al.*, 2013; Tranvik *et al.*, 2009] and is one of the causes of hypoxia in poorly mixed surface waters [Kerr *et al.*, 2013; Zhang and Li, 2010]. Bacterial DOC degradation processes can partly be predicted from the physical environment in the water, defined by factors such as temperature, inorganic nutrient supply, and ultraviolet (UV) radiation [Anesio *et al.*, 2005; Apple *et al.*, 2006; Tranvik, 1998], but they are also dependent upon the intrinsic chemical properties of the DOC, i.e., the DOC composition [Berggren *et al.*, 2007; Moran *et al.*, 1999].

It has recently been shown that the composition of the DOC strongly influences the overall potential of the DOC to be consumed on different time scales [Koehler *et al.*, 2012]. Furthermore, the DOC composition regulates both absolute and relative microbial C consumption rates [Guillemette and del Giorgio, 2011] and the metabolic allocation of this consumed C to either BP or BR [Berggren *et al.*, 2007]. Thus, the microbial processing of DOC clearly has “various facets” (in the sense of Guillemette and del Giorgio [2011]), yet few studies have simultaneously considered these different aspects of the metabolism [Guillemette and del Giorgio, 2011; Koehler *et al.*, 2012]. Surprisingly, there is today no integrated understanding of how DOC source regulates the patterns in microbial C assimilation, growth, and respiration and how this regulation is mediated by variations in DOC composition.

Most studies that consider the degradability of DOC are centered around the use of a single variable, generically referred to as bioavailable DOC (BDOC) [Servais *et al.*, 1987], measured as the cumulative bacterial DOC consumption in the dark during a fixed time period. However, this approach ignores the

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known reactivity continuum of the DOC, in which different compounds are used on different time scales [Amon and Benner, 1996]. Furthermore, the BDOC concept does not take anabolic (BP) and catabolic (BR) parts of the metabolism into consideration, nor key metabolic parameters such as bacterial growth efficiency ($BGE = BP/(BP + BR)$). These are all aspects of metabolism that have unique effects on the functioning of aquatic ecosystems and which are likely dependent on the chemical nature of the DOC and therefore its source [Berggren *et al.*, 2007; Guillemette and del Giorgio, 2011]. Finally, DOC degradation studies very seldom measure the use of specific groups of compounds, which is a limitation considering the significant importance of amino acid (AA), carbohydrate (CH), and organic acid (OA) metabolism that has recently been demonstrated [Berggren *et al.*, 2010; Salcher *et al.*, 2013]. Thus, there is clearly a knowledge gap in how DOC source and composition regulate these distinctly different aspects of microbial processing of DOC in aquatic ecosystems.

To fill this gap, we performed bioassays that combine short-term bacterial production and respiration measurements with long-term DOC loss and analyses of bacterial substrate consumption profiles. Using this approach, we determine the pattern in bacterial metabolism of DOC from rivers that differ greatly in order, location, and relative catchment cover of forests and peat bogs. Previous studies have shown that the degradation potential depends on the origin of the DOC in the landscape, e.g., peat wetlands, forest soils, or autochthonous algae [Berggren *et al.*, 2007; Guillemette and del Giorgio, 2011]. Differences in DOC metabolism have also been found between headwater sources and downstream aquatic networks [Berggren *et al.*, 2009] and between clear-water and brown-water systems [Koehler *et al.*, 2012]. Therefore, it was predicted that the DOC from our diverse set of streams and rivers in Québec (Canada) would result in contrasting patterns of metabolic responses that reflect the varying chemical nature and sources of the riverine DOC. Using basic ordination techniques and correlative analyses, we examine these metabolic patterns in relation to DOC composition assessed through peaks in excitation-emission fluorescence spectra and in relation to the isotopic composition of both the bulk DOC and that of the CO_2 that result from the degradation of DOC. Our results provide comprehensive support to date for the view that there are distinct microbial metabolic patterns associated to riverine DOC of different sources, suggesting that changes in DOC source have broad consequences on the functioning of the receiving aquatic ecosystems.

2. Materials and Methods

2.1. Study Sites and Sampling Conditions

Twelve streams and rivers in Québec (Table 1) were selected to represent broad gradients in catchment area, peat-land influence, and degree of DOC and water color (Table 1). The rationale for selecting such a diverse set of sites was to maximize the representation of DOC of different terrestrial source (peat bogs versus a range of forest soils) but also DOC of different degradation state (less degraded terrestrial DOC in headwaters, compared to in downstream nonheadwaters) and with different contribution from autochthonous algae. Presumably, the relative contribution of algal DOC can be significant in clear waters and in large catchments with long water retention times. The sites were sampled twice between 31 May and 24 August 2010, during summer low-flow conditions. Catchment discharge was $0.27 \pm 0.47 \text{ mm d}^{-1}$ (mean \pm SD) in 14 out of 24 sampling occasions when in situ flow could be directly measured, using either a handheld acoustic Doppler velocimeter (YSI/SonTek, Inc., San Diego, CA) or a bucket and stopwatch method. As the study was not designed to explore seasonal patterns, the duplicate sampling was performed only to obtain a minimum of replication needed to get representative summer mean values for each catchment.

2.2. Field Sampling

The streams and rivers were sampled at 20–30 cm depths, by hand or with wading suit and a 3 m pole sampler, and all samples were stored in cooling boxes with ice or in laboratory fridges until analysis. Water for analyses of in situ water chemistry (DOC concentrations, isotope ratios, optical properties, and nutrients) was filtered on-site ($0.45 \mu\text{m}$; Sarstedt, Germany) into acid-washed and stream water-rinsed 40 mL glass vials. In addition, one 20 L extensively prerinsed 20 L low-density polyethylene cubitainer was filled for each site to measure the various aspects of microbial metabolism targeted in this study: bacterial production and respiration, DOC consumption and the isotopic composition of DOC consumed during dark incubations, and the substrate utilization profiles using Biolog Ecoplates (as described below). All incubations were initiated on the same day as the sampling, within 2–6 h.

Table 1. Location, Drainage Area, and In Situ Water Chemistry of 12 Stream and River Sites in Québec During Summer Low Flow 2010^{a,b}

| No. | Station Name | Longitude (DD) | Latitude, Longitude (DD) | Headwater | Peat Bogs | Drain Area (km ²) | DOC (mg L ⁻¹) | CDOM _{abs440} (m ⁻¹) | Chl <i>a</i> (μg L ⁻¹) | TP (μg L ⁻¹) | TN (mg L ⁻¹) | Site Category |
|-----|-------------------|----------------|--------------------------|-----------|-----------|-------------------------------|---------------------------|---|------------------------------------|--------------------------|--------------------------|------------------------------|
| 1 | Stream #09 | 48.478 | -79.437 | YES | NO | 0.2 | 10.7 [10.2-11.2] | 2.6 [2.6-2.6] | 0.9 [0.6-1.2] | 28 [28-29] | 0.29 [0.19-0.39] | Headwater forest stream |
| 2 | Petit-Nord Suiuit | 49.678 | -79.019 | YES | YES | 0.5 | 11.9 [8.0-15.7] | 7.4 [4.4-10.5] | 2.2 [1.0-3.3] | 12 [10-14] | 0.24 [0.20-0.28] | Peat-influenced stream/river |
| 3 | Stream #08 | 48.476 | -79.433 | YES | NO | 0.7 | 10.7 [9.6-11.8] | 2.1 [1.3-2.9] | 1.4 [1.3-1.4] | 32 [22-42] | 0.33 [0.31-0.34] | Headwater forest stream |
| 4 | Stream #07 | 48.530 | -79.465 | YES | NO | 0.8 | 17.2 [14.7-19.8] | 9.8 [9.2-10.4] | 0.9 [0.5-1.3] | 18 [16-21] | 0.45 [0.44-0.46] | Headwater forest stream |
| 5 | Petit Aiguebelle | 48.449 | -78.751 | NO | NO | 1.6 | 8.5 [8.0-9.1] | 3.2 [2.4-4.0] | 3.0 [2.4-3.5] | 12 [11-13] | 0.40 [0.35-0.44] | Brown forest river |
| 6 | Stream #32 | 45.749 | -74.689 | NO | NO | 3.0 | 7.8 [5.8-9.8] | 1.2 [0.8-1.6] | 0.4 [0.2-0.5] | 10 [9-11] | 0.27 [0.23-0.32] | Clear mountain river |
| 7 | Ruisseau Brunet | 48.455 | -78.731 | NO | NO | 17.7 | 12.5 [9.8-15.1] | 6.9 [5.0-8.9] | 1.4 [1.2-1.6] | 16 [12-19] | 0.37 [0.30-0.45] | Brown forest river |
| 8 | Inter-Nord Suiuit | 49.745 | -79.036 | NO | YES | 93.0 | 19.3 [17.2-21.4] | 14.9 [14.6-15.2] | 1.1 [1.0-1.2] | 17 [14-19] | 0.46 [0.39-0.52] | Peat-influenced stream/river |
| 9 | Rivière Magousi | 48.436 | -79.428 | NO | NO | 515.4 | 10.5 [8.0-13.0] | 4.7 [2.0-7.5] | 7.4 [5.2-9.6] | 66 [48-85] | 0.42 [0.35-0.48] | Brown forest river |
| 10 | Rivière Kinojevis | 48.432 | -78.662 | NO | NO | 1751.4 | 10.8 [10.2-11.5] | 4.1 [3.5-4.7] | 4.8 [3.1-6.6] | 34 [33-35] | 0.61 [0.55-0.68] | Brown forest river |
| 11 | La Rouge | 45.794 | -74.690 | NO | NO | 5236.7 | 8.6 [6.7-10.5] | 1.3 [1.2-1.5] | 1.5 [0.4-2.6] | 11 [10-11] | 0.25 [0.23-0.27] | Clear mountain river |
| 12 | Harricana | 49.859 | -78.646 | NO | YES | 9651.4 | 13.3 [11.8-14.8] | 7.2 [6.4-7.9] | 1.5 [0.1-3.0] | 41 [36-46] | 0.43 [0.39-0.47] | Peat-influenced stream/river |

^aBecause of missing DOC in situ data in three cases, the underlined DOC values represent DOC concentrations measured early during the bioassays, corrected for the amount of O₂-inferred DOC loss up to the point of measurement.

^bWater chemistry is provided as means of duplicate samplings (low-high range in brackets). Peat bogs covered 10–40% of the peat catchments, which is sufficient to cause dominance of peat-derived DOC in boreal streams during low flow [Laudon *et al.*, 2011].

2.3. Microbial Metabolism

For all microbial metabolic measurements we used water from the 20L cubitainer sample, gently pressure-filtered through 1.0 μm “A/E Glass” filters (PALL Life Sciences, NY, USA; 142 mm diameter) using a peristaltic pump, allowing most bacteria to pass while removing microzooplankton and larger particles [del Giorgio and Pace, 2008]. To avoid influence on bacterial metabolic performance of variable ambient inorganic nutrient concentrations and temperature, which could mask the response to intrinsic DOC properties, the filtered water was spiked with 0.5 mg N L⁻¹ as NH₄NO₃ and 50 μg P L⁻¹ as KH₂PO₄ before initiation of bioassays, and all incubations were carried out at 20 °C in the dark, using sealed containers placed in circulating water baths or climate chambers.

On the same filtered and nutrient-spiked water from the cubitainer, bacterial respiration (BR) was determined as dark O₂ consumption using a Fibox 3 system (PreSens, Germany) following Marchand *et al.* [2009]. Duplicate 500 mL Erlenmeyer flasks, equipped with O₂ optodes, were filled for each sample and sealed with silicone stoppers. The O₂ concentrations were measured 7 times during 72 h incubations. Respiration rates were calculated as the slope of linear regression lines of O₂ versus incubation time assuming a RQ (respiratory quotient) of 1, which is close to the mean bacterial RQ in rivers of the region [Berggren *et al.*, 2012]. In Erlenmeyer flasks of the same type, BP was measured after 0, 12, 36, and 72 h of incubation, following the [³H]-leucine incorporation method by Smith and Azam [1992]. Triplicate aliquots of 1.5 mL water samples were exposed to 40 nM [³H]-leucine during 1 h. Average blank-corrected rates of leucine uptake were converted to carbon production rates by applying the standard conversion factor 1.55 kg C mol leu⁻¹ multiplied by an isotopic dilution factor of 2 [Simon and Azam, 1989]. Finally, integrated average values of BP over time were calculated using the trapezoidal rule and dividing by incubation time. BGE was calculated as BP divided by the sum of BP and BR. BP and BR rates were expressed relative to the DOC, as % d⁻¹. Long-term carbon consumption (LTCC; percentage lost) was assessed by comparing ambient DOC concentration with DOC measured in water that had been incubated for 1 year

(365 days) in 1 L acid-washed Duran glass bottles, with a gas headspace (~100 mL) that theoretically had enough dissolved O₂ to oxidize all DOC in the samples.

A 1.0 μm filtered water sample from each cubitainer was also used to measure the capacity of the riverine bacterial communities to degrade six amino acids, 10 carbohydrates, and nine organic acids (standard substrates; in triplicate) using the Biolog Ecoplate™ (CA, USA), in the dark at 20 °C. Incubations were initiated 2–6 h after field sampling. The respiration by the bacterial community reduces a tetrazolium dye that is included with each substrate, thus inducing a color development that is measured in an optical assay [Garland and Mills, 1991]. The bottom of the Ecoplate wells includes an inorganic nutrient mix by default (together with the C substrate and the color indicator), and thus, we avoided double nutrient additions by not spiking the original sample water with N and P in this case. We calculated the mean color development values for each class of compounds, measured at the overall “average well color development” of 0.5.

2.4. Ambient Water Chemistry and DOC Composition

To characterize the ambient water chemistry (DOC, nutrients, and optical analyses), we used the 0.45 μm filtered 40 mL samples, which had been prepared in field. The DOC was measured on an OI Analytical total inorganic carbon/total organic carbon (TIC/TOC) analyzer (College Station, TX, USA), total phosphorus (TP) using the molybdenum-blue method after persulfate digestion and total nitrogen (TN) as nitrates after alkaline persulphate digestion. The isotopic composition of organic carbon was analyzed as described further below. DOC absorbance were scanned using a UV/visible UltroSpec 2100 spectrophotometer (Biochrom Ltd, Cambridge, UK), and Chl *a* was measured spectrophotometrically in ethanol extracts. Colored dissolved organic matter (CDOM_{Mabs₄₄₀}) was calculated as the absorbance at 440 nm, corrected for turbidity by subtracting the absorbance at 690 nm, and converted to naperian units through division by cuvette length in meters and by multiplying with standard correction factor 2.303.

The DOC fluorescence excitation-emission spectra were measured on nondiluted water samples using a Shimadzu RF5301 PC (Japan), at excitation wavelengths of 250–450 nm in 5 nm increments and emission wavelengths of 280–600 nm in 2 nm increments. The ultrapure water blank-corrected spectra were further corrected for inner filter effects [McKnight *et al.*, 2001], converted to Raman units, and used together with a PARAFAC model to quantify the main fluorescence peaks (shown in Figure S1 in the supporting information). The model was developed using spectra for 1577 samples from lakes, rivers, ponds, and wetlands from boreal Québec [Lapierre and del Giorgio, 2014], using MATLAB DOMfluor 1.7 [Stedmon and Bro, 2008]. Thus, the PARAFAC components reflect fluorescent properties that are widespread across boreal aquatic ecosystems. Nonnegativity constraints were applied and outliers removed to maximize overall representativeness. Among the outliers, three of the samples from this study were not included in the modeling process due to high absorbance (>0.6 cm⁻¹ at 254 nm), which could affect the inner-filter correction and subsequently bias the model [Miller *et al.*, 2010]. The outlier-free model contained 1349 samples and was validated using split-half analysis. The outlier-free model was then reapplied a posteriori on these samples to evaluate the concentrations of the PARAFAC components [Lapierre and del Giorgio, 2014]; the study by Miller *et al.* [2010] suggests that this procedure may slightly bias estimates of the concentrations of humic- and fulvic-like components identified in this study by up to 10% in the highest absorbance sample.

2.5. Isotope Composition of Respired Carbon and Bulk DOC

To determine the δ¹³C of the respired CO₂, the Keeling plot method was used [Karlsson *et al.*, 2007]. For each sampling site, the filtered (1.0 μm) and nutrient-spiked water from the cubitainers (see “Microbial Metabolism” section above) was used to prepare a series of 40 mL top-filled acid-washed borosilicate glass vials with butyl rubber septa. At four different time points, the first three within the initial month and the fourth after 4 months of incubation, samples were poisoned by injection of 5 μL of saturated HgCl₂ and analyzed for dissolved inorganic carbon (DIC) and δ¹³C-DIC. The δ¹³C of the respired CO₂ was calculated as the y axis intercept of the linear regression line for δ¹³C-DIC as a function of 1/[DIC]. Keeling plot results and associated uncertainties are presented in Figure S2 in the supporting information.

In selected stream samples, Δ¹⁴C of DIC was measured on day 0 and day 30 in parallel incubations in 300 mL BOD vials with glass stoppers. Again, the prefiltered (1.0 μm) and nutrient-spiked water was used. These were

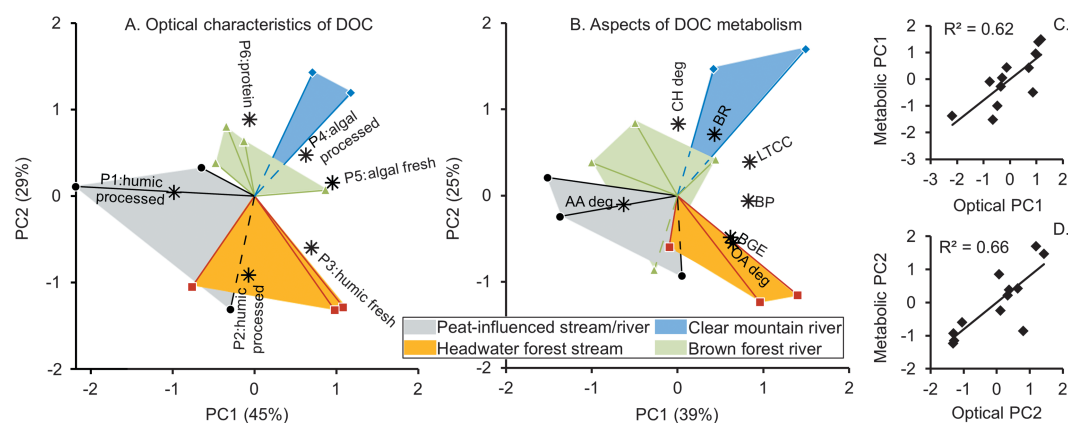


Figure 1. Component scores (asterisks) and site scores (other symbols) from principle component (PC) analyses performed on (a) fluorescence PARAFAC components describing the optical characteristics of DOC and (b) different aspects of DOC metabolism, namely, bacterial growth efficiency (BGE), bacterial production per unit DOC (BP), bacterial respiration per unit DOC (BR), long-term (1 year) carbon consumption (LTCC), degradation of amino acids (AA degr.), carbohydrates (CH degr.), and organic acids (OA degr.), respectively, measured on the Biolog EcoPlate. Mean of two replicate samplings was used as input data (see raw data in Table S2 in the supporting information). The relationships between metabolic and optical PCs are shown for the (c) the first and (d) second PCs from each analysis.

fixed with 40 μL of saturated HgCl_2 and later sparged with ultrahigh purity helium gas after addition of 3 mL of 85% H_3PO_4 , whereby the evolved CO_2 was collected cryogenically, purified on a vacuum extraction line, and collected in break-seal tubes for $\Delta^{14}\text{C}$ analysis. Graphitizing and determination of mass and $\Delta^{14}\text{C}$ of the extracted DIC were performed at the Vienna Environmental Research Accelerator (VERA) laboratory of the University of Vienna. Although these incubations contained only two time points, Keeling plot intercepts were again used, here for $\Delta^{14}\text{C}$ -DIC versus $1/[\text{DIC}]$, to estimate the $\Delta^{14}\text{C}$ of the respired CO_2 . The $\delta^{13}\text{C}$ of DOC and DIC was measured using an OI Analytical (College Station, TX, USA) TIC/TOC analyzer coupled to a DELTA plus XL (Thermo Finnigan, Bremen, Germany) isotope ratio mass spectrometer with a ConFloII system. In addition, DOC samples were freeze-dried, acidified, graphitized, and analyzed for $\Delta^{14}\text{C}$ at VERA.

2.6. Statistics

Patterns in metabolism (bioassay variables) and DOC fluorescence (percent contribution by each PARAFAC component to total fluorescence) between sites were explored through principal component analysis (PCA; nonrotated solutions) using SPSS 19. Component scores were obtained using the “regression” method in SPSS. The same statistical software was also used for basic statistical tests.

3. Results and Discussion

3.1. Patterns in DOC Composition Across Catchments

In the diverse set of streams and rivers studied (Table 1), the character of the DOC varied broadly and systematically, as revealed by the PCA performed on the PARAFAC fluorescence components (see fluorescence characteristics in Figure S1 in the supporting information). Two significant principle components (PCs; eigenvalue > 1) emerged from this analysis, together explaining 83% of the variance in fluorescence components P1–P6. Of these, PC1 was related to increasing algal-like (P4 and P5) and fresh humic fluorescence (P3), but decreasing processed humic-like material (P1), whereas PC2 was related to increasing protein-like fluorescence (P6) and decreasing contributions by the humic-like components P2 and P3 (Figure 1a).

The two clear water rivers from the Laurentian Mountains (sites #6 and #11) with strong positive scores on both of these axes represent a distinct DOC composition category, with large contributions by nonhumic and protein-rich DOC fractions, presumably of algal origin (Figure 1a). In contrast, all peat bog-influenced sites showed negative scores on PC1 and/or PC2, regardless of the size of the catchment, suggesting that the peat-derived DOC was systematically composed of highly processed humus (Figure 1a). Furthermore, all headwater sites were characterized by strong negative scores on PC2, but variable scores on PC1,

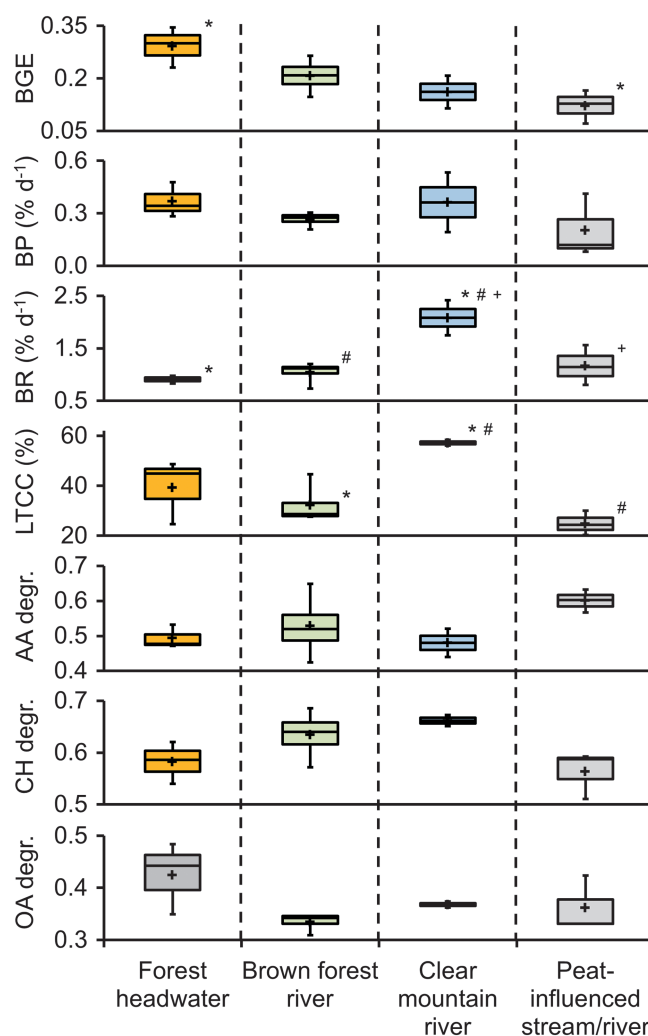


Figure 2. Box and whisker plots of results from DOC bioassays (mean values from two replicate samplings) performed on the four categories of streams/rivers, which emerged from the principle component analysis (see text and Figure 1 for definition of categories and variables). The bars that share the same index symbols represent means that are significantly different from each other (1 factor analysis of variance with Tukey's post hoc test; $p < 0.05$).

indicating that different types of humic DOC, fresh or processed, dominated the DOC. The remaining sites, which represented brown-water forest rivers of intermediate sizes, showed slight positive scores on PC2 (protein-like material), but both negative and positive scores on PC1.

In another study of the same rivers, fluorescence peaks determined using PARAFAC are associated with distinct and extremely diverse families of molecules, as determined using ultrahigh-resolution mass spectrometry (Fourier transform ion cyclotron resonance mass spectrometry) [Stubbins *et al.*, 2014]. The segregation of rivers based on fluorescence properties of the DOC is thus based on fundamental differences in their respective molecular composition.

3.2. Linking DOC Composition to Bacterial Metabolic Responses

According to our prediction, patterns in DOC source and composition reflected in the fluorescence characteristics and their underlying molecular structures should be linked to the various aspects of bacterial DOC metabolism. To test this, we performed a new PCA on all of the metabolic variables obtained from the bioassays and compared the resulting PCs with the PCs for DOC fluorescence characteristics. This time, the PCA procedure extracted three significant PCs that together explained 85% of the variance in the metabolic data. Site scores were again clearly clustered on the first two axes of this model (Figure 1b); PC1 was characterized by positive loadings on variables describing anabolism (BP and BGE), organic acid degradation, and long-term DOC consumption (LTCC); PC2 showed positive loadings for catabolism (BR) and carbohydrate degradation, but negative loading for organic acid degradation.

3.3. Comparisons Between Emerging Categories of Catchment Type

Our results show clear patterns in metabolism across all the rivers studied, but distinct microbial metabolic patterns were also evident when rivers were grouped according to the categories that emerged from the PCA (Figure 2). For example, in line with previous reports [Berggren *et al.*, 2007; Dempsey *et al.*, 2013], BGE

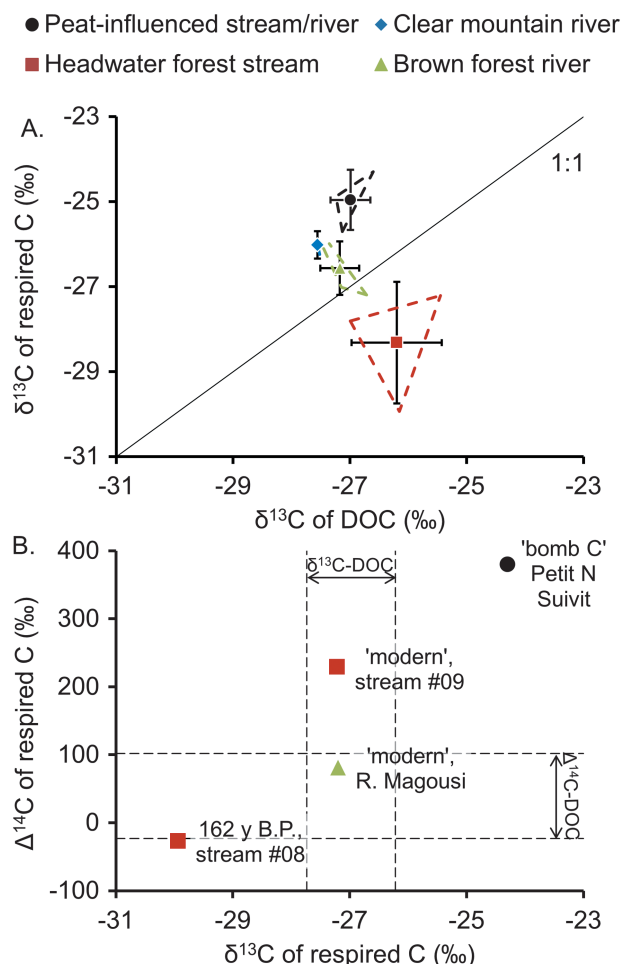


Figure 3. (a) Stable isotope ratio of carbon respired during DOC bioassays, determined with the Keeling plot method, plotted against the carbon stable isotope ratio of ambient DOC. The symbols and error bars show the mean and SD for streams and rivers of each category (12 sites in total), and the corners of the polygons show means of duplicate samples for each individual site. (b) The $\Delta^{14}\text{C}$ of respired carbon in four selected streams, on one sampling date in late summer 2010, plotted against the corresponding values for $\delta^{13}\text{C}$. Data labels indicate the age of the respired carbon in years before present; post-1950 $\Delta^{14}\text{C}$ levels are just denoted “modern” or “bomb C (see text).” The vertical and horizontal dashed lines show $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ (means \pm SD), respectively, in DOC samples from all 12 sites.

that are directly linked to catchment category, and the associated differences in DOC composition. By comparing Figure 1a with Figure 1b, it becomes apparent that (1) the high anabolism and organic acid degradation that is typical of forest headwaters are related to fresh humic DOC, (2) low metabolic rates in peat-influenced river systems is related to processed humic DOC, (3) high catabolism and carbohydrate degradation in clear waters are related to protein- and algal-like fluorescence, and (4) the nonheadwater brown forest rivers show patterns intermediate to all other sites, suggesting that these rivers integrate multiple catchment signatures or in-lake processes (Figure 2).

Some of these patterns are expected from previous knowledge: For example, *Berggren et al.* [2010] showed that high organic acid degradation and high BGE are both characteristic of metabolism of DOC in forest headwater streams. Furthermore, the connection between protein-like fluorescence (P6) and respiration in clear-waters is classical [*Cammack et al.*, 2004], and it is also expected that carbohydrate degradation is significantly positively correlated to the protein-like fluorescence (Table S1 in the supporting information),

was relatively high in all forested brown-water catchments and forest headwaters were associated with significantly higher BGE than peat bog-influenced streams and rivers (Figure 2). BR, on the other hand, was significantly higher in clear nonheadwater mountain rivers than in all other catchments, especially in comparison with the forest headwaters. This difference is shown for standardized BR rates in Figure 2, but also absolute BR was significantly higher (not shown) in the clear forest rivers than in all other sites except in the catchments with peat bogs (see patterns for absolute BP and BR in Tables S1 and S2 in the supporting information). Furthermore, long-term DOC consumption (LTCC) represented a larger fraction of the DOC in clear-waters, compared to brown forest rivers and peat-influenced streams/rivers, but the DOC consumption in forest headwaters was moderate (Figure 2). Although not statistically significant, there were also differences in the substrate uptake capacities, with organic acid uptake being highest in headwater streams, amino acid uptake capacity peaking in peat-influenced streams (Figure 2), and carbohydrate uptake capacity peaking in clear water rivers (Figure 2).

Previous studies have shown links between specific components of aquatic microbial metabolism such as BP to bulk properties of the DOC, such as total concentration [*Lennon and Pfaff*, 2005] or specific ultraviolet light absorption [*Berggren et al.*, 2009], but our results demonstrate for the first time that there are distinct patterns in the ensemble of metabolic variables that we measured,

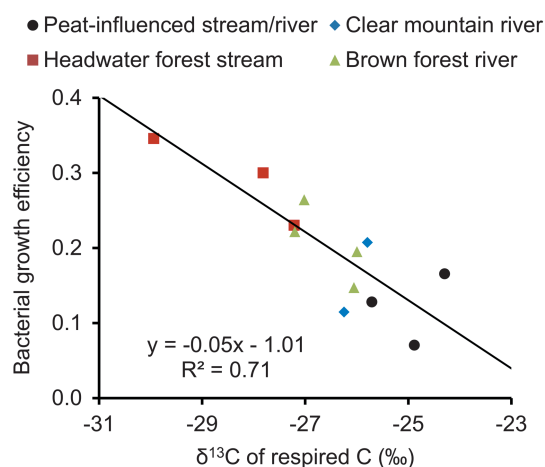


Figure 4. The relationship between bacterial growth efficiency and the stable isotope ratio of carbon respired during DOC bioassays in the 12 sampling sites ($p < 0.01$, $n = 12$).

waters could originate in autotrophic production by benthic algae [Karlsson *et al.*, 2009] or labile microbial-derived DOC from mineral soils [Olefeldt *et al.*, 2013]. However, the fact that we still found a strong connection between the microbial metabolic patterns and the spectrofluorometric properties of bulk DOC suggests that the source of the DOC is one of the master regulators of both bulk DOC properties and properties of the labile DOC.

3.4. Isotopic Evidence of Differences in Source of the Consumed DOC

It could also be argued that the DOC from the peat-influenced water courses comes from a mixture of forest, wetland, and algal sources and that the DOC in the brown forest rivers, owing to their elevated Chl *a* concentrations (Table 1), may include a significant but variable phytoplankton-derived component. Therefore, to test the assumption that differences in metabolic responses in samples from different catchment categories are driven by systematic differences in consumption of DOC from different sources, we used the Keeling plot method [Karlsson *et al.*, 2007] to determine the carbon isotope composition of the metabolized DOC in each river, assessed as the intercepts in $\delta^{13}\text{C}$ -DIC versus $1/[\text{DIC}]$ regressions. The analytical uncertainties resulted in relatively large site-level variability in the $\delta^{13}\text{C}$ of the respired C (1.8‰ SD of replicates on average, see Figure S1 in the supporting information). However, the estimates typically did not vary more than $\sim 1\%$ within each catchment category (Figure 3a), which made between-category comparisons possible.

The $\delta^{13}\text{C}$ of the respired portion of the DOC showed an overall average ($-26.51\text{‰} \pm 1.48\text{‰}$; Figure 3a) that was similar to the $\delta^{13}\text{C}$ of the bulk DOC pool ($-26.95\text{‰} \pm 0.43\text{‰}$; Figure 3b). Nonetheless, the DOC that was consumed often diverged from this bulk signature (Figure 3a). In forest headwater streams, the respired carbon was systematically ^{13}C depleted, on average by 2.1‰ units relative to bulk DOC. In complete contrast, the respired carbon in both clear-water and peat-influenced streams and rivers was systematically ^{13}C enriched relative to bulk DOC. Only in the nonheadwater brown rivers, the Keeling plot results indicated that bulk DOC and respired C had similar stable carbon isotope composition (Figure 3a). We further found that the isotopic composition of the respired DOC was linked to specific aspects of bacterial metabolism (see individual correlations in Table S1 in the supporting information). The clearest example of this was for BGE, which was strongly negatively correlated with $\delta^{13}\text{C}$ of the respired carbon (Figure 4), suggesting a tight relationship between DOC source and its consumption and subsequent allocation by bacteria [Berggren *et al.*, 2007].

In the forest headwater streams, the ^{13}C depleted signal of the respired C probably indicated that isotopically light leaf litter-derived DOC dominated the metabolism [Sebestyen *et al.*, 2008] or that carbohydrates that tend to lead to production of ^{13}C depleted CO_2 were consumed [Blair *et al.*, 1985]. While it is not possible to conclude if the elevated $\delta^{13}\text{C}$ signal in peat catchments is due to the use of isotopically heavy transphilic or protein-like structures within the bulk DOC [Hood *et al.*, 2005; Wickland *et al.*, 2007] or due to

since protein-like fluorescence not only tracks hydrolysable amino acids but also low molecular weight aliphatic compounds such as simple carbohydrates [Stubbins *et al.*, 2014]. However, the significant negative correlation (see Table S1 in the supporting information) between amino acid degradation and the fresh algal fluorescence component (P5) is unexpected and needs further research. The PCAs (Figure 3) suggest that high amino acid degradation is associated with humic DOC from peat bogs, possibly indicating that peat wetlands represent a more important source of bioavailable organic N than previously thought.

It should be emphasized here that the DOC pools in our sampled catchments represent a mixture of different sources and that bacteria selectively consume only a small portion of this bulk mixture.

For example, much of the labile DOC in the clear

something else, it appears beyond doubt that the sources of DOC that were respired in the forest headwater streams and in the peat-influenced running waters were different. Also, in the clear-water streams, the respired carbon was ^{13}C enriched, which is logical as both protein-like DOC (which is high in the clear-water streams) and DOC produced by benthic algae and macrophytes (common autochthonous producers in clear-waters) represent labile components within the DOC pool that are typically enriched in ^{13}C [Hecky and Hesslein, 1995; Tank *et al.*, 2011; Wickland *et al.*, 2007].

We further performed $\Delta^{14}\text{C}$ Keeling plots in a few selected samples to assess the age of the DOC respired. Samples were selected if (1) a relevant amount of DIC (>0.5 mg) could be extracted both at the beginning and end of the incubations and (2) the respired CO_2 added substantially ($>50\%$) to the background DIC, i.e., the DIC that was there from the beginning. There was an overall positive relationship between the $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ of the respired carbon (Figure 3b). In forest headwaters, the respired DOC ranged from approximately 162 years old to modern, while the respired DOC in a peat-influenced stream showed high concentrations of so-called “bomb carbon,” which peaked approximately 60 years ago. Interestingly, although the isotopic signature of the bulk DOC was remarkably similar across sites (Figures 3a and 3b), the DOC consumed was isotopically distinct from this bulk DOC signature, which confirms that bacteria in different categories of catchments selectively remove specific pools of DOC with different sources [Guillemette *et al.*, 2013; Lennon and Pfaff, 2005] and age [McCallister and del Giorgio, 2012].

The summer of 2010, when this study was carried out, was warmer and significantly dryer (50% less precipitation) than the long-term annual average of the region [Campeau *et al.*, 2014]. Thus, it is not surprising that we found traces of a several decades’ old DOC (bomb carbon) in the streams, considering the combination of warm conditions that favor enzymatic release of DOC from peat [Freeman *et al.*, 2001] and dry conditions that lower the water table and potentially make older peat layers relatively more important for the export. It is more surprising that the bacteria appeared to favor the use of such DOC before recently fixed carbon. The mechanisms for the apparent use of bomb-spiked carbon are not clear, but it should be emphasized that selective use of aged DOC (even thousands of years old) has been noted before [McCallister and del Giorgio, 2012]. Our results add to the emerging view that a high radiocarbon-inferred age of the DOC is not indicative of recalcitrance.

4. Summary and Conclusions

Our results empirically confirm the widespread assumption that the composition of DOC (here reflected in its optical properties) strongly influences bacterial metabolism, but we further demonstrate that this metabolic response to the nature of riverine DOC is complex and involves multiple facets. The main PCA axis in the metabolic data, which described anabolism (growth), was positively related to the main DOC composition axis representing algal and fresh humic DOC. The second PCA axis, which described catabolism (respiration), was positively related to the second DOC composition axis representing protein-like fluorescence. From this we can conclude that anabolism and catabolism are two fundamentally different dimensions of the bacterial metabolism that are systematically linked to different DOC components.

The bacterial metabolism is also strongly linked to the DOC source that is utilized, as revealed by the isotopic composition of the metabolized DOC. Our results suggest that bacteria selectively remove specific C fractions that are isotopically distinct from that of the bulk DOC and that these fractions are dramatically and systematically different between rivers belonging to different catchment types. In fact, the data allowed us to group the sites into distinct catchment categories, such as “forest headwater” or “peat bog-influenced,” where each category represents a specific metabolic response, a specific DOC composition and a specific carbon isotope composition of the degraded DOC. For example, fresh humic DOC from forest headwaters had a relatively “isotopically light” degradable fraction, which effectively supported anabolism at a systematically high level of BGE. In contrast processed humic DOC in peat bog drainage had an isotopically heavier degradable fraction, which was used with significantly lower BGE. The carbon consumption in clear mountain rivers appeared to be represented by relatively isotopically heavy autochthonous DOC (possibly produced by benthic algae) that simultaneously supported high anabolism, high catabolism, and also the highest long-term DOC losses.

This study targeted contrasting catchments during low flow, but it could also be expected that the bacterial metabolic responses change seasonally in rivers that receive DOC from different sources during high and low flow, respectively. Considering the fundamentally different ecosystem consequences that are associated with different aspects of bacterial metabolism, we suggest that changes in DOC loading and sources caused by land use or climate change (browning) could result in major changes in aquatic ecosystem functioning. Specifically, by knowing the source of the DOC in the landscape, it is possible to not only predict the microbial anabolism in receiving waters, e.g., with spinoff effects on aquatic food-web structures but also the microbial catabolism that contributes to greenhouse gas production.

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