Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems

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Abstract

   We explored various aspects of freshwater dissolved organic carbon (DOC) lability by comparing short-term (< 2 d) bacterial C consumption (STCC; derived from bacterial respiration measurements) with long-term (28 d) C consumption (LTCC) in DOC bioassays in lakes, rivers, and marshes located within the same complex drainage basin in southern Québec. We also combined STCC and LTCC measurements to estimate the proportion of DOC removed, and to derive a first-order decay constant (k). STCC rates were, on average, 25% higher than LTCC, and both parameters showed distinct patterns, reaching their lowest and highest values in lakes and marshes, respectively. STCC and LTCC were correlated to DOC concentration across these freshwater ecosystems, whereas in lakes, STCC was positively correlated to chlorophyll and LTCC to terrestrial C inputs. k showed no ecosystem-specific patterns but was negatively correlated to chlorophyll across systems. The size of the DOC pools supporting STCC and LTCC, as well as k, were related to distinct components of the DOC pool, as revealed by a parallel factor analysis of fluorescent dissolved organic matter excitation–emission spectra. Short- and long-term lability and C consumption, and the resulting k, are shown to be complementary facets of DOC bioavailability, which may play very different roles on aquatic ecosystem functioning.

Freshwater ecosystems, including lakes, streams, and marshes, process large amounts of organic carbon that is exported from the terrestrial environment, in addition to that generated in the aquatic system itself. Inland waters do not act as a passive pipe transporting this organic material toward the sea but are sites of intense biogeochemical activity (Cole et al. 2007). The flow of dissolved organic carbon (DOC) from the terrestrial environments to inland waters and finally to the ocean margins is influenced by processes that remove DOC, such as sedimentation or degradation of the organic matter by a combination of abiotic and biological processes (Cole et al. 2007; Holmes et al. 2008). In particular, the consumption of DOC by bacteria represents one of the major sinks of DOC in the biosphere (Raymond and Bauer 2001; Cole et al. 2007), and there has thus been much interest in exploring factors that govern this DOC consumption. It has now been well established that bacterial consumption of DOC is influenced by a number of environmental factors, including temperature, nutrient availability, and ultraviolet (UV) radiation (Zweifel et al. 1993; Marschner and Kalbitz 2003; Anesio et al. 2005).

The intrinsic chemical properties of the DOC also exert a major influence on the availability of DOC to bacteria (Fellman et al. 2009; Roehm et al. 2009; Berggren et al. 2010) and, in this regard, it is clear that bacterial DOC consumption is intimately linked to the concept of DOC lability. DOC in natural systems is composed of a complex mixture of organic compounds of different origins (Benner 2003), and this chemical heterogeneity results in variations in reactivity within the bulk DOC. It is common to find mention in the literature of two or three discrete pools of DOC, defined on the basis of their degree of reactivity (i.e., time frame of consumption), often referred to as "labile,"

"semi-labile," and "recalcitrant" (Kirchman et al. 1993; Middleburg et al. 1993; Kragh and Søndergaard 2004).

However, there are several problems associated with the concept of DOC lability. The term is generally used to denote the proportion of DOC removed by bacteria over a certain period of time, but it has also been used as a synonym of total amount of DOC removed (Davis and Benner 2007; Lønborg and Søndergaard 2009) and of rate of C removal (Cherrier et al. 1996; del Giorgio and Pace 2008); these are far from being synonyms, and in fact, refer to very different properties of the DOC. Also, while the concept of discrete pools can be useful from a modeling point of view, DOC most likely comprises a continuum of reactivity (Middleburg 1989; Boudreau and Ruddick 1991; Amon and Benner 1996). In addition, there is ambiguity from an experimental point of view. The term "lability" is operational because the apparent labile DOC, whether defined as a proportion, absolute amount, or rate, depends greatly on experimental conditions (such as temperature, nutrient limitation, nature of the inoculum), and most importantly, on the experimental timescales. This becomes critical for study comparisons for example, because microbial consumption of DOC, and the associated DOC lability, have been quantified using very different approaches.

In this regard, some studies have taken a whole-ecosystem mass balance approach to determine DOC losses using inflow and outflow data (Dillon and Molot 1997). Most studies, however, have used an in vitro approach where the change in DOC is followed over dark incubations (Søndergaard and Middleboe 1995; del Giorgio and Davis 2003). This experimental approach has been extensively used in soil waters (Fellman et al. 2008, 2009), lakes (Søndergaard 1984; Tranvik 1988; Amon and Benner 1996), rivers (del Giorgio and Pace 2008; Holmes et al. 2008), estuaries (Hopkinson et al. 1998; Raymond and

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Bauer 2000), and oceans (Moran and Hodson 1994; Carlson and Ducklow 1996; Zweifel 1999). DOC consumption has also been determined in lakes and marine systems by following changes in dissolved inorganic carbon (DIC) concentration (Obernosterer and Herndl 2000; Langenheder et al. 2006). The changes in DOC or DIC that occur in most samples are small relative to the sensitivity of current analytical techniques and, thus, DOC consumption bioassays typically last weeks to months. Partly because of these limitations, plug-flow biofilm reactors (or bioreactors) were developed in order to measure the bioavailable C fraction within a few hours instead of days (Volk et al. 1997; Søndergaard and Worm 2001). Others have used more sensitive measurements of bacterial metabolism as proxies for DOC consumption, including bacterial growth (Middleboe and Søndergaard 1993; Søndergaard et al. 1995; Middleboe and Lundsgaard 2003), production (Bergström and Jansson 2000; Ågren et al. 2008; Berggren et al. 2009), and respiration (del Giorgio and Pace 2008; McCallister and del Giorgio 2008). These metabolic measurements are also carried out at much shorter time frames, in the order of hours to days.

When long- and short-term approaches have been carried out in parallel, they have yielded very different apparent levels of DOC lability. For example, del Giorgio and Pace (2008) found that short-term (6–8 h) bacterial consumption rates in the Hudson River were one order of magnitude higher than the rates of DOC utilization in long-term (3 weeks) incubations system. This apparent discrepancy is not surprising because the short-term metabolic measures target a highly reactive pool that turns over very rapidly, whereas the longer-term measures target a DOC pool that is decreasingly reactive (del Giorgio and Davis 2003). In this regard, Carlson (2002) argued that the turnover of the semi-labile pool in the ocean cannot be predicted from instantaneous measurements of bacterial production (determined by ^{3}H-leucine or thymidine incorporation), as this approach most likely does not capture bacterial consumption of more recalcitrant carbon compounds. Thus, the discrepancies in apparent DOC reactivity are, in fact, to be expected, and we propose that DOC bioavailability and its associated patterns of consumption cannot be fully described or understood by targeting only a portion of the reactivity spectrum or any individual aspect of DOC lability.

In this study, we have quantified both short-term bacterial respiration rates, measured as O_2 consumption over 48 h, and long-term DOC consumption, measured as declines in DOC over several weeks along a water flow path as it traverses different types of freshwater ecosystems (lakes, rivers, marshes) within a complex watershed in southern Québec. We also combined these short- and long-term measurements to estimate the proportion of DOC removed, and to reconstruct and model the complete dynamics of DOC consumption over a period of 4 weeks. We assess the patterns in short-term, long-term, and overall DOC consumption across these freshwater ecosystems, and explore the factors that may regulate DOC bioavailability over these different timescales, including optical properties of the organic pool using parallel factor analysis (PAR-

AFAC) of excitation–emission fluorescence spectroscopy, and a suite of environmental variables.

Materials and Methods

Conceptual approach—In this paper we use the term "bioavailability" to denote the potential for DOC to be consumed by aquatic bacteria. This term is synonymous with "biodegradability" and "bioreactivity," which are often used in the literature (Obernosterer and Herndl 2000; Marschner and Kalbitz 2003; Wickland et al. 2007). Figure 1 shows a conceptual diagram that integrates the different dimensions of DOC bioavailability: in this scheme, we make a clear distinction between the amount and/or proportion of DOC that can be removed by bacteria, which we refer to as "labile DOC," and the rates at which this DOC is removed, which we refer to as "bacterial C consumption rates" (BCC). The total labile pool is the proportion of DOC removed within a given time, 28 d of incubation in the case of our experiments, and we refer to the remaining DOC as the "residual pool." The total labile pool can be further divided into a short-term labile (STL) pool, corresponding to the proportion of DOC removed during the initial phases of the incubation (first 2 d in our case), and the long-term labile (LTL) pool, which is the proportion of DOC removed over the reminder of the incubation (28 d in this study). The removal of DOC is mediated by bacterial C consumption rates, and these can be broadly divided into rates at the initial phases of the incubation, which we refer to as "short-term carbon consumption" (STCC), and the average rates during the remainder of the incubation, which we refer to as "long-term carbon consumption" (LTCC). C consumption rates typically decline over the length of the incubation, and this decline can be modeled using a first-order decay model that contains a decay constant (k) and an estimate of the size of the labile pool. The resulting k represents the shape of the overall DOC decline and provides information on how the initial consumption rates evolve in time and, therefore, on the nature of the DOC reactivity: a high k value implies a strong inflexion in the DOC vs. time curve, suggesting the rapid exhaustion of a highly reactive pool and a rapid transition to refractory carbon characterized by much lower consumption rates. A low k value, on the other hand, implies a much less marked inflexion and relatively constant rates of DOC consumption with time, suggesting a more homogenous composition of the labile pool.

We emphasize that the different facets of DOC bioavailability presented in Fig. 1 may not necessarily be derived or predicted from each other, and that consideration of only a subset of these parameters may lead to biased or incorrect conclusion regarding DOC bioavailability. For example, one could conclude that two DOC samples have the same level of bioavailability based on the fact that bacteria can extract the same amount (or proportion) of C over the incubation period (Fig. 1; total labile). However, this conclusion ignores potential differences in the rate of BCC sustained by these labile C pools: most of the labile carbon could be removed within a few hours in one sample (high STCC and low LTCC) or within
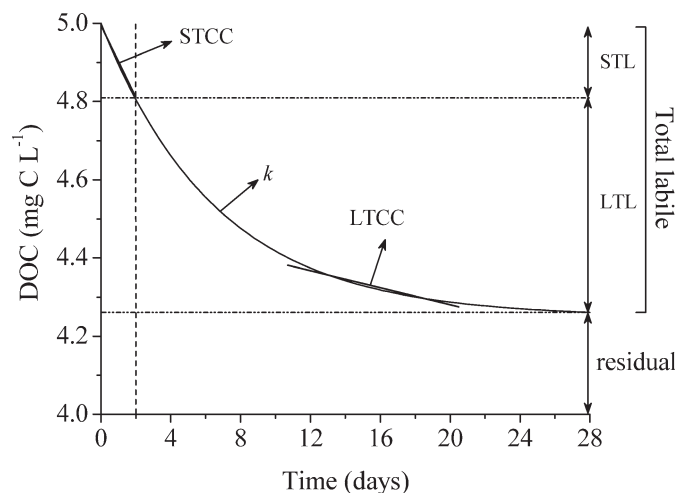
Fig. 1.   Conceptual schematic of the various facets of DOC bioavailability considered in this study. The total labile pool is the proportion of DOC removed within 28 d of incubation, and the remaining pool is considered as residual. The total labile pool can be further divided into short-term labile (STL), corresponding the proportion of DOC consumed during the first 2 d, and long-term labile (LTL), given by the proportion of DOC removed between day 2 and day 28. The short-term bacterial carbon consumption rate (STCC) is given by the initial slope of the consumption curve, and the long-term rate (LTCC) is estimated from the slope between day ~ 8 and day ~ 23. The k constant informs on the overall decay rate of bacterial carbon consumption.

a few days to weeks in another (lower STCC and higher LTCC rates). Measurements of BCC on both short- and long-term are thus important not only to derive the k constant but also to correctly interpret patterns in DOC bioavailability. The k constant is itself the direct result of the interplay between STCC and LTCC. For example, high STCC rates coupled with very low LTCC rates may yield high k values (i.e., early inflexion in the consumption curve), as opposed to time courses characterized by low k, that is, by a linear decline. However, samples that have similarly low k values may still have very different rates of C consumption and, thus, the bioavailability of a DOC sample should not be based solely on its k value. In the following sections, we present an experimental approach that accounts for the various features of DOC bioavailability identified in our scheme and their possible ways of regulation.

Study sites and sampling scheme—We sampled the surface waters of eight lakes, six rivers, and four marshes located within the same drainage basin and interconnected with one another along a water flow path (Fig. 2) in June, July, and August during summer 2005. The drainage basin is located in the Eastern Townships region of southern Québec, Canada, about 100 km east of Montreal (45.24°N, 72.12°W). The watersheds are dominated by temperate mixed forest and low habitation density, and are underlined by the sedimentary geology of the St. Lawrence lowlands. The sampled lakes present a moderate gradient in DOC (2–7 mg C L^{-1}) and chlorophyll *a* (Chl *a*) (1–6 μg L^{-1}) concentrations, and in mean water residence time, varying
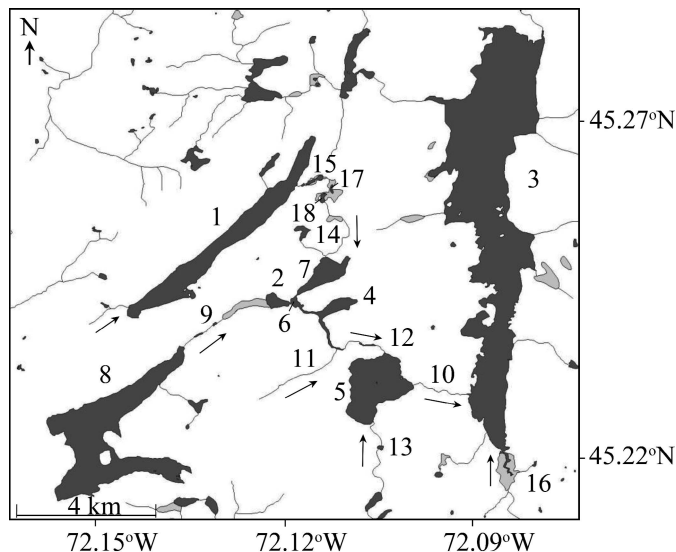

Fig. 2.   Map of the study watershed, showing the interconnected lakes (dark gray), rivers, and marshes (light gray) that were sampled. Black arrows show the direction of the water flow path, and the numbers represent the sampling sites (*see* Table 1 for site descriptions).

from days to years (Table 1). The epilimnia of all these lakes are generally oversaturated with carbon dioxide and, hence, are net sources of C to the atmosphere (del Giorgio and Peters 1994; Prairie et al. 2002). Marshes sampled consist of small (0.003–0.01 km²) and shallow (mean depth of 0.5 m) impoundments, mostly resulting from the presence of beaver (*Castor canadensis*) dams. Macrophytes, namely *Potamogetton* spp. and *Sagittarium latifolia*, cover a significant portion of these marshes, and standing dead trees are present in two of them (Fig. 2; No. 17–18). In a few lakes (Stukely, Bran-de-Scie, Leclerc, and Desmonts), small beds of *Myriophyllum spicatum* were also observed in addition to the two species present in marshes. The sampled lakes and marshes are connected by several rivers varying in size and water flow that were also sampled, and in addition, two headwater streams that do not link lakes or marshes were visited (Fig. 2; No. 11–13).

In addition to in situ temperature and oxygen profiles, 20-liter samples of surface water (< 2 m) were collected in acid-leached (10% HCl), nanopure-rinsed polycarbonate bottles at the deepest point of each lake using a diaphragm pump connected to an acid-washed (10% HCl) plastic hose. In rivers and marshes, water samples were taken at a depth of ~ 15 cm under the water surface to avoid collecting sediments. All the samples were kept cool in the dark and brought back to the laboratory within 2 h of collection.

Metabolic experiments—In order to minimize methodological disparities between STCC and LTCC measurements, we used the same incubation setups and filtration method to conduct both short-term bacterial respiration experiments and long-term DOC lability bioassays. The protocol used to determine bacterial respiration (BR) has been described in detail in a previous study (del Giorgio et al. 2006). Briefly, in the laboratory, 2 liters of unfiltered

Table 1. Biological and chemical characteristic of the lakes, rivers, and marshes sampled in this study. DOC, dissolved organic carbon (<0.2 μm); TP, total phosphorus; TN, total nitrogen; A_{440}, DOC absorbance measured at 440 nm. Mean values of the three sampling dates are shown along with the standard deviation in parentheses. Numbers refer to sampling site positions within the studied watershed (*see* Fig. 1).

Water body	No.	DOC (mg L^{-1})	TP (μg L^{-1})	TN (mg L^{-1})	Chl a (μg L^{-1})	A_{440} (m^{-1})
Bowker	1*	2.46(0.32)	1.0(0.5)	0.12(0.03)	1.7(0.5)	0.38(0.18)
Bran-de-Scie	2*	6.23(0.11)	10.0(2.8)	0.28(0.05)	6.6(3.0)	1.96(0.40)
Brompton	3	6.54(0.18)	6.4(4.4)	0.27(0.07)	5.0(3.2)	1.73(0.90)
Des Monts	4*	6.43(0.77)	8.39(4.6)	0.25(0.06)	3.4(1.6)	1.92(0.11)
Fraser	5*	6.03(0.08)	5.3(1.7)	0.21(0.01)	3.7(0.7)	1.84(0.20)
Leclerc	6*	5.18(0.58)	6.0(3.3)	0.20(0.03)	—	1.67(0.41)
Simoneau	7*	4.56(0.15)	7.6(3.0)	0.18(0.02)	2.9(0.3)	1.38(0.50)
Stukely	8*	4.57(0.10)	7.2(2.7)	0.22(0.05)	1.7(0.8)	1.27(0.30)
Bran-de-Scie stream	9*	5.03(0.68)	13.1(5.4)	0.39(0.11)	—	1.50(0.53)
Brompton stream	10	5.86(0.23)	6.3(4.2)	0.26(0.06)	3.2(0.2)	2.73(1.93)
Fraser stream No. 1	11	4.67(1.16)	8.7(4.3)	0.41(0.05)	—	1.04(0.49)
Fraser stream No. 2	12	6.02(1.15)	9.0(3.4)	0.27(0.06)	1.1(1.2)	1.77(0.07)
Fraser stream No. 3	13	10.22(1.70)	21.6(1.2)	0.45(0.02)	3.9(0.3)	3.68(1.14)
Simoneau stream	14*	5.30(1.80)	8.9(2.3)	0.25(0.05)	3.3(0.03)	2.42(0.98)
Bowker marsh	15	2.79(0.33)	4.7(1.9)	0.16(0.01)	1.9(0.6)	0.50(0.13)
Brompton marsh	16	11.47(1.76)	14.6(4.9)	0.43(0.02)	—	4.43(1.71)
Simoneau marsh No. 1	17*	5.72(1.91)	9.4(2.5)	0.29(0.07)	3.6(3.9)	2.00(0.40)
Simoneau marsh No. 2	18*	5.13(0.46)	7.1(1.5)	0.26(0.02)	—	1.84(0.12)

* Sites selected for the spectrofluorometric characterization of the dissolved organic matter (DOM).

water were set aside for nutrients and chlorophyll analyses as well as DOC characterization (*see* below), and the remainder was filtered to isolate the bacterial community from the other planktonic components in the bulk water. This filtration process may cause significant loss of bacterial cells or may provoke cell breakage, resulting in an overestimation of bacterial respiration, production, and other metabolic parameters (Ferguson et al. 1984; Gasol and Moran 1999). In order to minimize bacterial loss, we used precombusted (500°C) borosilicate glass filters (Pall AD) with a nominal pore size of 3 μm. The large pore size allows most bacteria to go through (average of $92 \pm 4\%$) but also allows passage of some picoeukaryotes, especially flagellates. We quantified the abundance of small flagellates in unfiltered and filtered water samples by filtering 15–20 mL on black 0.2-μm Nuclepore filters, staining with 4,6-diamidino-2-phenylindole (5 μg mL^{-1}), and counting the cells by epifluorescence on an Olympus BX51 microscope (400$\times$). Flagellate abundance in filtered samples was on average $< 10\%$ of that in the ambient water.

Approximately 10 liters of water from each site were gently pushed through precombusted (500°C) Pall AD (15-cm diameter) filters using a filtration tower (Millipore) coupled to a peristaltic pump by acid-washed silicone tubing. Filters were changed halfway to prevent pore clogging and further loss of bacterial biomass. The filtered water was used to fill an acid-washed 4-liter Erlenmeyer flask and an acid-washed 4-liter cubitainer bag. The cubitainer was placed on a stand and connected to the lower flask with acid-washed Tygon tubing so that a siphon could be established by gravity. The Erlenmeyer flask was sealed with a white, acid-washed silicon stopper fitted to a glass tube and connected to a Tygon tube closed with a Teflon pinch-valve that acted as a sampling port. All flow-through systems were placed in a large, dark incubation

chamber and kept at 20°C to standardize temperature for all metabolic experiments. The incubation temperature was within $\pm$ 3°C of the ambient temperature. In order to determine what the upper limit of metabolic activity in these samples and the potential effects of filtration, we also followed respiration in unfiltered water samples using the same flow-through systems.

We determined STCC rates as changes in oxygen concentration in the bottom flasks. Samples were taken at the time when the bottom flasks were first sealed, then at every 2 h for 6 h, and finally at 24 h and 48 h (six time points total); at each time point, triplicate 7-mL glass tubes (Chemglass) were filled by opening the pinch-valve after having allowed 5 mL of water to flow in order to purge the system. All tubes were poisoned with 8 μL of saturated HgCl$_2$ solution and capped with a ground-glass stopper. All samples were kept immersed in a cooler to prevent the ground-glass joint from leaking and stored (< 1 week) at 10°C for the determination of oxygen concentration using a dual-inlet mass spectrometer. In brief, the method is based on the spectrometric determination of the ratio of argon to oxygen in the sample, after the gases in the sample have been allowed to diffuse through a permeable membrane into a high-vacuum system connected to the mass spectrometer (Kana et al. 1994). The oxygen concentration was estimated from this ratio using the solubility of argon corrected for salinity and temperature; the average standard error of oxygen concentration between triplicates was < 2 μg O$_2$ L^{-1}. The rates of oxygen consumption were derived from the slope of O$_2$-vs.-time relationship fitted to a least-squares regression. Most of the time courses were linear within the length of the incubations (48 h); 10 time courses became clearly nonlinear after time 24 h, and for these STCC was calculated over 24 h only. Oxygen consumption rates were converted to CO$_2$ production, in

order to provide short-term estimates of organic carbon consumption, using a respiratory quotient of 1 (McCallister and del Giorgio 2008).

LTCC was estimated by following the decline in DOC concentrations in the same samples that were used to determine short-term bacterial respiration. Sampling for DOC concentration measurements was done every 2–4 d for up to day 28, by collecting 40 mL of filtered water in two acid-washed and 500°C-burned 40-mL vials (replicates) to which 40 μL of 5 mol L^{-1} sulfuric acid had been added, to attain a final pH of $\sim$ 2. To minimize gas exchange between the cubitainer and the flask, duplicate samples of DOC were taken before the respiration setup was sealed (time 0) and after the last sample for oxygen determination 48 h, so the overall water volume taken to determine bacterial respiration was always < 1% of volume contained in the 4-liter flask. DOC samples were kept refrigerated (4°C) for a maximum of 2 months after having been capped with a Teflon-lined septa cap (VWR). At day 2, respiration flow-through systems were dismantled and the filtered water from the respiration incubations was transferred into two acid-washed and 500°C-burned 500-mL culture glass bottles (unscrewed caps to prevent oxygen exhaustion), and samples were taken every 2–4 d to determine DOC concentration. LTCC was calculated from the slope of the DOC-vs.-time relationship fitted to a least-squares regression.

Modeling DOC consumption—In order to reconstruct the complete dynamics of DOC consumption, we combined STCC and LTCC consumption rates to generate a single decay curve. A first-order decay model, based on the multi-G model (Westrich and Berner 1984) with only one reactive member, was applied to this single decay curve. In all DOC consumption experiments there was a relatively large residual portion of the total DOC that was present at the end of the 28-d incubation. The equation for the first-order decay model that accounts for this residual pool is the following:

$$G_T(t) = G_{Lab}[\exp(-kt)] + G_{Res} \qquad (1)$$

where G_T is the total DOC concentration at the beginning of the experiment; G_{Lab} and G_{Res} are the labile and the residual pools estimated by the model, respectively; k is the first-order decay constant; and t is the time of decomposition. Because the model did not provide realistic estimates of the labile and residual pool sizes in some cases (low k values; $n = 15$), and that it could not account for the small labile pool used within 2 d, the size of the short- and long-term labile DOC pools was calculated as the difference between the initial DOC concentration and the concentration after day 2 for STL, and between days 2 and 28 for LTL.

Chemical analyses—Samples for total nutrients (phosphorus and nitrogen) were kept at 4°C in the dark prior to analyses. Phosphorus concentration in unfiltered samples was determined using the molybdenum-blue method after persulfate digestion. Nitrogen concentration was measured

as nitrates after digestion with alkaline persulfate (Cattaneo and Prairie 1995). Colorimetric analyses were carried on a Flow solution IV autoanalyzer (nitrogen) or on a UV-Visible Ultrospec 2100 spectrometer (Biochrom) (phosphorus). DIC and DOC were measured in filtered samples on an OI 1010 total inorganic and organic carbon analyzer that uses a wet persulfate oxidation method and five-point calibration curve using potassium hydrogen phthalate as standard. The analytical precision of the analyzer, based on three injections per sample, was $\pm$ 0.003 to 0.08 μg C L^{-1} for the low and high range of concentrations, respectively, and the detection limit lies at $\sim$ 0.020 μg C L^{-1}. Finally, chlorophyll concentration was determined from ethanol extracts using the same spectrophotometer as for the phosphorus measurements.

DOC characterization—Absorption spectra were measured from 190 to 900 nm with a UV-Visible Ultrospec 2100 spectrometer (Biochrom) using a 2-cm quartz cuvette. The absorption coefficient (A_{440}) was calculated by dividing the optical absorbance at 440 nm by the path length in meters and multiplying by 2.303 (Cuthbert and del Giorgio 1992). The spectrofluorometric characterization of the dissolved organic matter (DOM) pool was performed for 11 selected sites (*see* Table 1) in June and July following the protocol provided in Stedmon et al. (2003). Briefly, fluorescence excitation–emission matrices (EEMs) were determined on a RF-5301PC spectrofluorometer (Shimadzu) with a 150-W xenon lamp at 2-nm excitation wavelength intervals between 240 and 400 nm, and at emissions ranging between 280 and 560 nm with 5-nm increments. Samples were analyzed at 20 $\pm$ 1°C in a temperature-controlled cuvette chamber. Fluorescence spectra were corrected for the inner-filter effect, accounting for the absorption of both emission and excitation light by the DOM sample (McKnight et al. 2001) and calibrated by normalizing to the area under the water Raman peak at excitation wavelength 350 nm of a NanoPure water sample run the same day. Finally, a Raman-normalized NanoPure-water EEM was removed from each spectrum in order to remove the Raman signal. This correction and normalization routine yields EEMs that are expressed as Raman units (R.U.; nm^{-1}).

EEMs were analyzed using the PARAFAC multivariate modeling technique, a trilinear decomposition method analogous to principal components analysis (Stedmon et al. 2003). PARAFAC decomposes the fluorescence spectra of DOM into independent fluorescence groups whose abundance can be related to differences in the composition and source material of the DOM matrix. The modeling of the fluorescence spectra was conducted on a data set of 211 samples, including several samples from other lakes and previous degradation experiments not presented in this study, using the DOMFluo toolbox 1.7 for MATLAB as described in Stedmon and Bro (2008). However, we removed four EEMs from the data set that were collected and analyzed in June (No. 1, 5, 8, 17; Table 1) because the EEMs contained measurement errors. Consequently, the EEMs modeling was conducted on a data set of 207 samples. The model was validated by split-half analysis and

by further examining residuals to ensure no systematic signal was present. All parameters derived from the model were used to estimate the magnitude of the different fluorescence components present in our samples, expressed hereafter as the maximum fluorescence intensity in Raman units (Fmax; R.U.).

Statistical analyses—Differences in terms of BCC and DOC lability between ecosystems were assessed using analysis of variance (ANOVA) in conjunction with a Tukey's pairwise differences test. A mixed stepwise routine (probability to enter or to leave the model was set to 0.05) prior to multiple regression analysis was used to find the best predictive environmental variable(s) describing the variability of STCC, LTCC, and the k constant. While we included all variables presented in Table 1 for the cross-system analysis, we also considered lake size and average water retention time in the stepwise routine for the lakes-only analysis. Finally, linear regression models were used to evaluate the possible relationships between BCC or DOC lability and the fluorescence components. All statistical analyses were conducted with the JMP statistical software version 7.0 (SAS Institute).

Results

Biochemical characterization of the watershed system sampled—There were large variations in DOC and other chemical variables along the water flow path and across the different systems sampled within this watershed. DOC and A_{440} were, on average, higher in rivers and marshes than in lakes, which may indicate a higher contribution of terrestrially derived DOC to the total DOC pool in these systems (Table 1). Lakes showed the widest range of Chl a concentrations (mean values of 1.7–6.6 μg L^{-1}), with the highest values in meso-eutrophic Lake Bran-de-Scie (6.6 μg L^{-1}; Table 1), where the contribution of autochthonous processes to the total DOC pool should be highest.

Patterns in short- and long-term DOC consumption—The metabolic experiments resulted in a data set of 54 individual observations for the short-term aspects of DOC bioavailability, but an instrumental failure resulted in the loss of DOC samples needed for the estimation of the long-term DOC consumption from Simoneau marsh No. 2 in July (site No. 18; Table 1). Accordingly, the final data set for LTCC, LTL, and k comprises 53 individual observations instead of 54.

STCC rates, as estimated in bacterial respiration experiments (filtered water), were significantly lower than total planktonic respiration (unfiltered incubations) in lakes (42%; t-test, $t = -3.89$, df = 46, $p < 0.001$), whereas in rivers and marshes the difference between the unfiltered and filtered incubations was not significant (Fig. 3A). Rates of LTCC were, on average, 27% lower (t-test, $t = -2.37$, df = 105, $p < 0.05$) than STCC rates, indicating that the more labile DOC pool was removed in the first few hours of incubation.

There were distinct between-ecosystem patterns in both STCC and LTCC (Fig. 3A). STCC rates were significantly
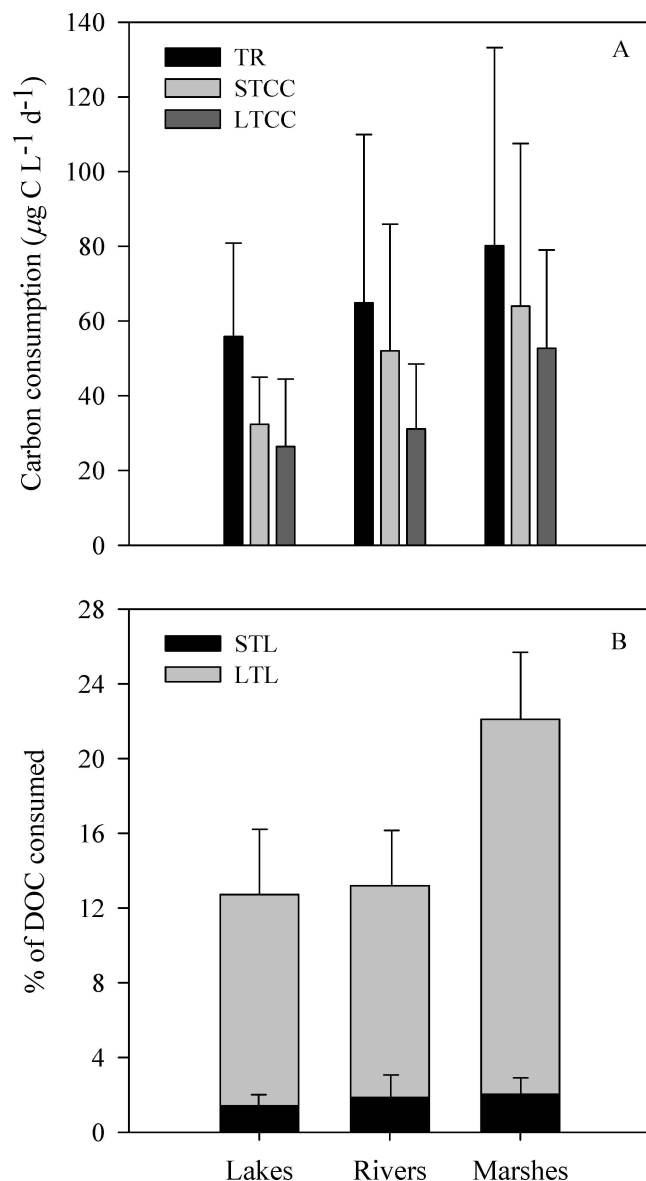

Fig. 3. Average and range, by ecosystem type, in (A) total planktonic respiration (TR), short-term bacterial carbon consumption (STCC) (0–2 d), and long-term bacterial carbon consumption (LTCC) (2–28 d), and (B) the proportion of DOC consumed during both short- and long-term incubations. Each bar represents the average of the June, July, and August carbon consumption experiments, and the error bars correspond to standard deviation.

higher in marshes than in lakes (ANOVA, $F_{53} = 5.20$, $p < 0.01$), rates of STCC in rivers being intermediate between lakes and marshes, whereas LTCC rates were higher in marshes than in both lakes and rivers (ANOVA, $F_{52} = 6.87$, $p < 0.01$). The proportion of DOC consumed during the short-term incubations was relatively constant between ecosystems (overall average 1.7 ± 1%; Fig. 3B). However, there was a significant ecosystem differences in LTL, with marshes showing a larger proportion of DOC consumed over the month of incubation (ANOVA, $F_{52} = 13.4$, $p < 0.0001$; Fig. 3B).

Linking short- to long-term DOC consumption—We first explored the relationship between STCC and LTCC over the entire data set and found that the STCC was a poor predictor of the long-term carbon consumption ($r^2 = 0.27$, $n = 53$, $p < 0.0001$; Fig. 4A): there was roughly an order of magnitude of variation in LTCC for any given STCC estimate. Further, there was no significant relationship between STL and LTL (Fig. 4B). We integrated STCC and LTCC measurements within the same DOC time course (Fig. 5A) and fitted a one-reactant multi-G model as described earlier (*see* Methods). The model performed well over the different time courses, with quotients of determination ranging from 0.90 to 0.99 (mean of 0.98). The k constant varied by over five orders of magnitude between samples, ranging from 5.7×10^{-3} to 0.17 d^{-1} in lakes, from 2.3×10^{-5} to 0.09 d^{-1} in rivers, and from 3.8×10^{-3} to 0.12 d^{-1} in marshes. There was overlap in the k values between the different ecosystem categories, and although k was lower in marshes, the difference was not statistically significant (Fig. 5B).

Environmental regulation of carbon consumption—The mixed stepwise routine showed that STCC and LTCC were both positively correlated to DOC ($r^2 = 0.62$, $n = 18$, $p < 0.0001$, and $r^2 = 0.58$, $n = 18$, $p < 0.001$, respectively; Fig. 6A) across ecosystems. A more detailed analysis of factors influencing STCC and LTCC in lakes showed that the timescale at which organic carbon is consumed may be regulated by different environmental factors: STCC was closely related to Chl a ($r^2 = 0.72$, $n = 7$, $p < 0.05$; Fig. 6B), while water residence time explained a significant portion of the variation in lake LTCC ($r^2 = 0.69$, $n = 8$, $p < 0.05$; Fig. 6C). The stepwise procedure further showed that Chl a was the only significant variable explaining variation in the k constant ($r^2 = 0.73$, $n = 13$, $p < 0.0001$; Fig. 7A).

Fluorescence characterization and links with carbon consumption—A total of five components could be validated following the PARAFAC analysis of the entire data set of fluorescence spectra ($n = 207$), and the model explained 98.7% of the variation. A visual inspection of the residual fluorescence spectra did not reveal systematic patterns in the fluorescence signal. All the components identified by the model have been previously described for aquatic systems (Table 2). Components 2 and 5 were identified as protein-like components because their fluorescence emission resembles that of free tyrosine and tryptophan, respectively. We also identified fulvic-like and humic-like peaks (components 1, 3, 4), which are common feature in most freshwater environments (Stedmon and Markager 2005*a*).

The different fluorescence components did not follow the same distribution among ecosystems: the fluorescence of component 1 was higher in marshes and rivers than in lakes, and followed the same distribution as the DOC concentration (Fig. 8). Component 2 was lowest in lakes and highest in rivers. The fluorescence of components 3 and 4 showed no pattern between ecosystems, whereas the fluorescence of component 5 was highest in lakes compared to rivers and marshes.
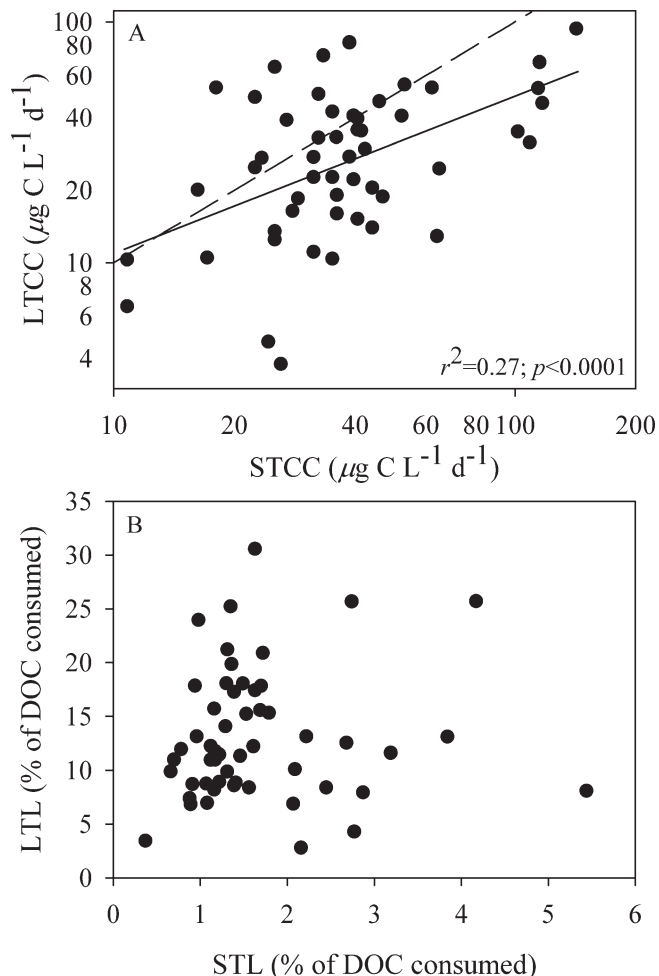

Fig. 4. The relationship between (A) short-term bacterial carbon consumption rates (STCC) (0–2 d) and long-term bacterial carbon consumption rates (LTCC) (2–28 d), and (B) short- and long-term DOC lability (STL and LTL, respectively) determined for the same samples. The metabolic rates are log-transformed to attain normality and homoscedasticity. Data from the three sampling campaigns are shown.

We explored the links between the components of DOC bioavailability and the fluorescence characteristics of the DOM. For this we used the mean values for June and July for the seven sites where data were available for the 2 months and four individual measurements for July for sites No. 1, 5, 8, and 17. We found that only three facets (STL, LTL, and k) showed significant relationships, albeit with different peaks of fluorescence. The strongest relationships were found by expressing the fluorescence of a given component relatively to the ambient DOC concentration. STL was best predicted by the sum of protein-like components 2 and 5 ($r^2 = 0.69$, $n = 11$, $p < 0.01$; Fig. 9A), whereas LTL was best explained by the relative fluorescence of the protein-like component 2 ($r^2 = 0.72$, $n = 11$, $p < 0.01$; Fig. 9B). The k constant was only correlated (negatively) to the protein-like component 5 ($r^2 = 0.76$, $n = 11$, $p < 0.01$; Fig. 9C).
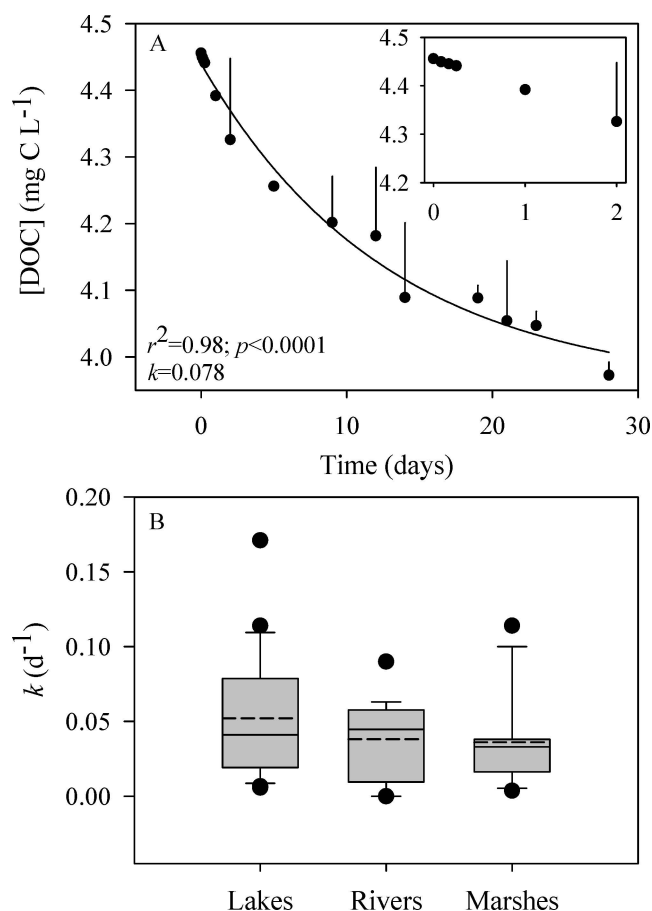
Fig. 5. (A) Example of an integrated carbon consumption time course. The first portion (0–2 d) was derived from a short-term bacterial respiration experiment (inset), the second portion corresponds to DOC consumption in the long-term incubations of the same samples. A first-order decay model was then fitted to the data (black line) to derive a global first-order decay constant, k. The error bars correspond to standard deviation of individual measurements. (B) Box-and-whisker plots of the k constant by ecosystem type. The full and dashed lines show the median and the mean, respectively.

Discussion

Methodological and conceptual considerations—In this study we used a dual approach to assess DOC bioavailability across ecosystems: short-term BR experiments and long-term DOC consumption bioassays. While these two methods address the same general process, they clearly diverge in their estimates of carbon consumption, with rates in the short term being, on average, 25% higher than in the long-term experiments. We argue that the difference between the two estimates of carbon consumption is related to the fact that these two methods do not target the same region of the DOC reactivity spectrum rather than methodological biases: short-term BR targets a smaller, fast-cycling, highly-labile carbon pool, whereas long-term DOC bioassays assess loss rates of more recalcitrant carbon compounds. The short-term pool is difficult to detect based on DOC measurements: in six of our bioassays we followed
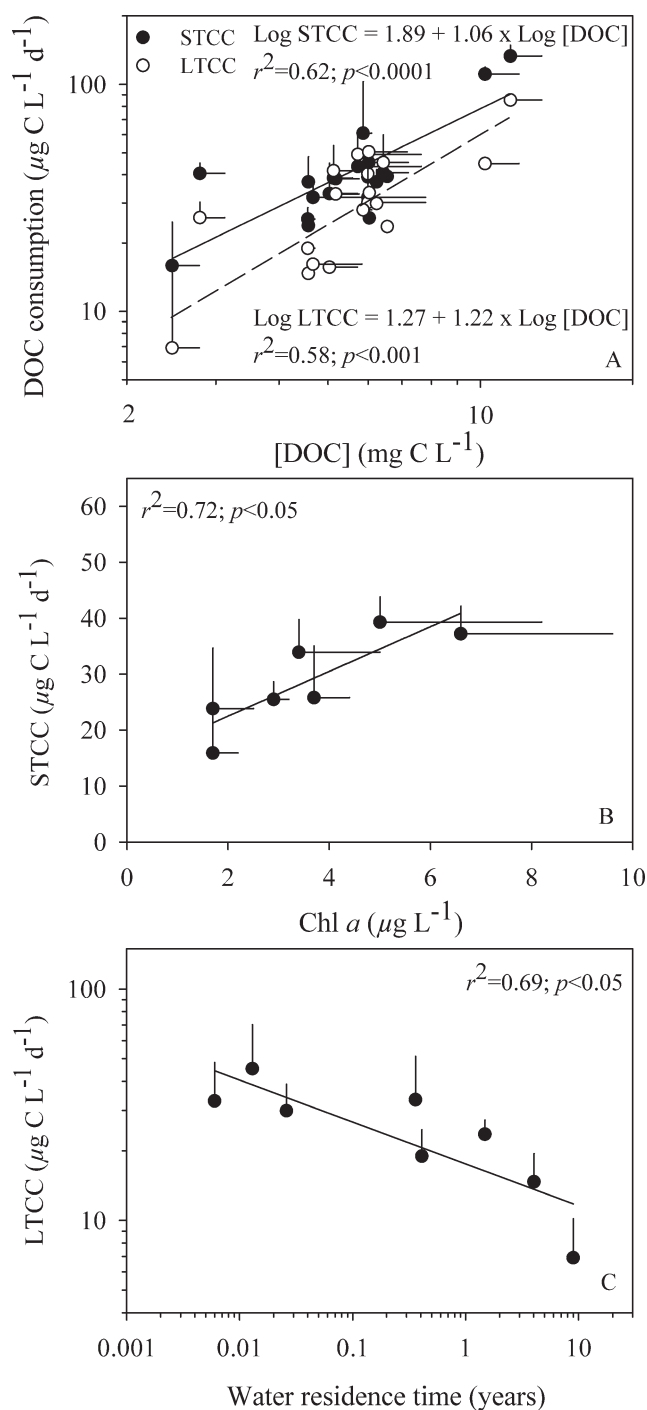

Fig. 6. (A) The relationships between short-term carbon consumption rates (STCC) (0–2 d), long-term bacterial carbon consumption rates (LTCC) (2–28 d), and DOC concentration. DOC was the only selected variable after a mixed stepwise routine of variable selection (*see* text for details). Also shown are the best predictive relationships found for (B) STCC and (C) LTCC for lakes only upon the same routine. Each data point represents the mean of the June, July, and August carbon consumption experiments of a single sampling site, and the error bars denote standard deviation.
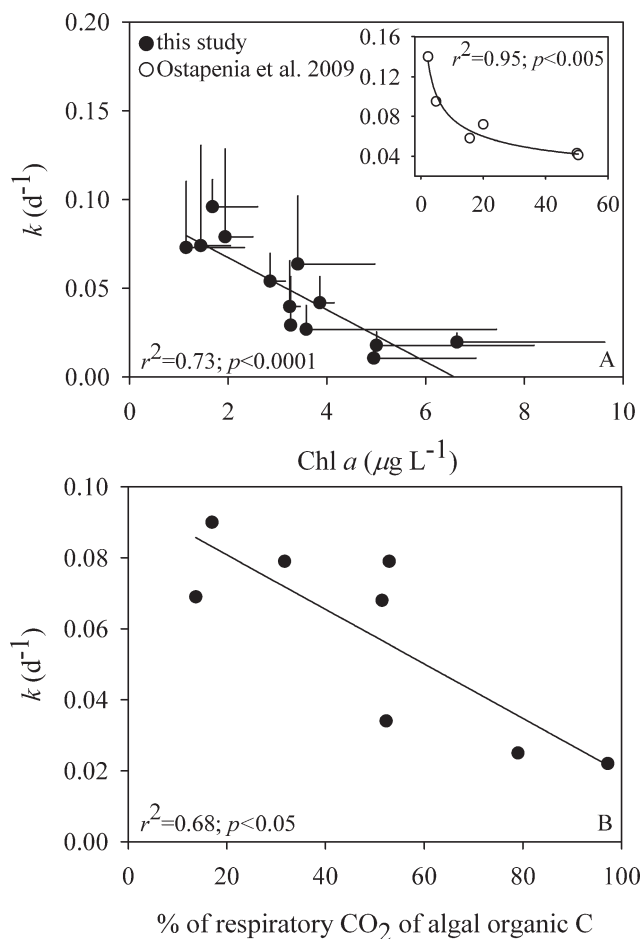
Fig. 7.   (A) The first-order decay constant, k, as a function of Chl a. Also shown is the relationship found between the k constant and Chl a by Ostapenia et al. 2009 (inset). Each data point represents the mean of the June, July, and August carbon consumption experiments of a single sampling site, and the error bars denote standard deviation. (B) The first-order decay constant, k, as a function of the percentage of algal organic carbon (OC) supporting bacterial respiration. Estimations of the percentage algal OC respired were obtained from the study of McCallister and del Giorgio (2008). The k constant values were derived from carbon consumption experiments ran in parallel to the experiments conducted with the respiratory carbon recovery system (ReCReS) for the determination of the percentage of algal C supporting bacterial respiration.

the decrease in the DOC concentration over the initial 24 h (every 2 h for 6 h) but could not detect any significant changes in DOC concentration in this timescale (data not shown). This observation is probably explained by the fact that a very small proportion of the bulk DOC pool is degraded within this short timescale ($< 2\%$; Fig. 3B), and that the total amount of DOC being removed lies within the detection limit of the current techniques (~ 20 μg C L^{-1} using wet persulfate oxidation in our laboratory).

We did, however, observe correspondence between the two methods at intermediate timescales in a sample from Lake Fraser where we continued the BR measurements over 7 d. The BR rates and DOC consumption rates between days 2 and 7 were in good agreement (site No. 5;

Table 1; 27.0 and 25.2 μg C L^{-1} d^{-1} for BR and DOC lability experiments, respectively). In addition, there were a number of time courses where the short- and long-term rates of C consumption were similar (points close to the 1:1 line; Fig. 4A), thus generating quasi-linear patterns of consumption and suggesting that the differences observed in most other samples are not intrinsic to the approaches.

Relationship between short- and long-term components of bioavailability—One of the fundamental questions addressed in this study is whether short- and long-term lability and BCC can be predicted from one another and if they are regulated in a similar manner by environmental factors. We found that while both STCC and LTCC were positively correlated to bulk DOC across these freshwater systems, only a small portion of the variation in LTCC could be explained by STCC. Moreover, there was no relationship between the proportions of C consumed over short- and long-terms. A similar observation was made in the Hudson River, where long-term consumption of the bulk DOC loaded upstream was clearly uncoupled from the short-term bacterial carbon processing along the river (del Giorgio and Pace 2008). These authors further reported that short-term bacterial respiration was very sensitive to local features such as phytoplankton development and particle dynamics, whereas the long-term DOC consumption was more closely linked to external (i.e., terrestrial) inputs of organic carbon. We observed similar differences in the regulation of STCC and LTCC by environmental features in lakes: STCC was positively related to phytoplankton biomass, whereas LTCC was negatively related to the mean water residence time. This last variable has been shown to be negatively related to terrestrially derived humic matter inputs to lakes (Rasmussen et al. 1989; Hessen et al. 1997), suggesting that LTCC is likely positively regulated by terrestrial organic C inputs.

We found further evidence of the differential regulation of these short- and long-term facets of DOC bioavailability in the composition of the organic pool across all systems. The two protein-like components (C2, C5) identified by the PARAFAC analysis were positively related to the overall DOC lability, a relationship also observed by Fellman et al. (2008, 2009) in soil water and streams. Likewise, the tyrosine-like component 2 appeared to be positively related to both short- and long-term lability, whereas tryptophan-like component 5 appeared to enhance STL but not LTL. Stedmond and Markager (2005*b*) noted that the tyrosine-like component 2 remained unaltered upon microbial degradation within 7 d of incubation and only started to decrease after 9 d of incubation, suggesting that this component may be consumed over longer timescales. Mayer et al. (1999) showed that tyrosine fluorescence reaches its highest values when in its monomer form or at low tryptophan concentration, indicating the presence of more degraded peptide material. Consequently, they hypothesized that high tryptophan fluorescence may indicate the dominance of unaltered proteins, probably of recent origin. In addition to our own results, the above evidence suggests that the tryptophan-like component 5 is preferentially consumed by bacteria over the tyrosine-like

Table 2. The peak positions of the five fluorescence components identified by the PARAFAC analysis and their correspondence with previously identified components.

Component	Excitation maxima (nm)	Emission maxima (nm)	Components identified from previous studies	Description
1	350	450	Stedmon and Markager 2005*a*, Component 4	Fulvic-like fluorophore
2	270	295	Stedmon and Markager 2005*a*, Component 8	Tyrosine-like fluorescence
3	260	445	Coble 1996, Component A	Humic-like fluorophore
4	305	400	Stedmon and Markager 2005*b*, Component 3	Humic-like fluorophore
5	285	335	Stedmon and Markager 2005*a*, Component 7	Tryptophan-like fluorescence

component 2 but that both proteinaceous components play a central role in determining the overall DOC lability.

In this regard, none of the fulvic-like or humic-like components appeared to play a role in shaping the overall DOC bioavailability, despite the fact that these fractions overwhelmingly dominated the DOM pool in all of these systems. Our results suggest that a small proteinaceous fraction plays a major role in determining the overall DOC bioavailability and thus bacterial C metabolism. A similar observation was made by Berggren et al. (2010), who reported that a small fraction of the terrestrial DOC pool, composed of simple compounds like amino and organic acids, supported much of the bacterial metabolism in boreal streams. This does not mean, however, that the humic and fulvic fractions are not consumed. Rather, we suggest that these fractions may fuel a low but rather continuous level of bacterial activity, which becomes increasingly important as the other pools are exhausted, and which may have been underrepresented in the time frame of our experimental incubations.

Ecosystem patterns of carbon consumption—Previous cross-system comparisons have reached contrasting conclusions on how DOC bioavailability varies across landscapes. For example, Søndergaard and Middleboe (1995) synthesized published measurements of in vitro bioassays conducted in different aquatic ecosystems (lakes, rivers,

and oceans) and concluded that carbon lability tends to be relatively invariant across natural aquatic systems ($\sim$ 14–19% of the total DOC pool; average of 15%). They noted that the percentage of labile DOC increases with DOC concentration across systems and that, for example, some high-DOC rivers could contain twice the amount in labile DOC than lakes. In contrast, del Giorgio and Davis (2003) did not find a relationship between DOC concentration and percentage of DOC removed and further reported that the lability of river DOC was on average lower than that of lakes. These contrasting results are likely linked to the fact that these meta-analyses of published DOC consumption data combine very different approaches and timescales, such that the resulting patterns may contain strong experimental biases.

Several studies have suggested that bacteria do consume terrestrially derived DOC in lakes (Tranvik 1988; Kriztberg et al. 2004; McCallister and del Giorgio 2008) and that terrestrial organic carbon significantly subsidizes bacterial metabolism in recipient systems (Jansson et al. 2007; Berggren et al. 2010). Nevertheless, it is often assumed that terrestrial carbon transported from the watershed to lakes via runoff and river inputs is recalcitrant in nature, due to features such as high humic and lignin content, or a greater oxidization state as the result of extensive degradation and aging during transport (Sun et al. 1997; Raymond and Bauer 2001; Berggren et al. 2009). Our observation of a relatively high bioavailability of river DOC contrasts markedly with this common assumption and is not unique to this study: Holmes et al. (2008) showed that DOC in Arctic rivers may be highly labile (from 2% up to 30%) as determined by long-term DOC degradation experiments on timescales comparable to ours (30 d). In agreement with Fellman et al. (2008, 2009), we found that rivers and streams could be a significant source of proteinaceous material, especially the tyrosine-like component 2 in our case, and that this protein-like DOM pool positively regulates DOC lability. This supports the notion that a significant fraction of allochthonous DOC may be bioavailable over ecologically relevant timescales and, thus, greatly enhance DOC bioavailability in these terrestrially influenced ecosystems.

Macrophytes have also been shown to contribute substantially to the DOC pool of recipient ecosystems through exudation of DOC (Bertilsson and Jones 2003; Demarty and Prairie 2009) or leaching of plant material (Mann and Wetzel 1996; Lapierre and Frenette 2009), and that this macrophyte-generated DOC may greatly influence water column metabolism (Søndergaard 1983; Rooney and
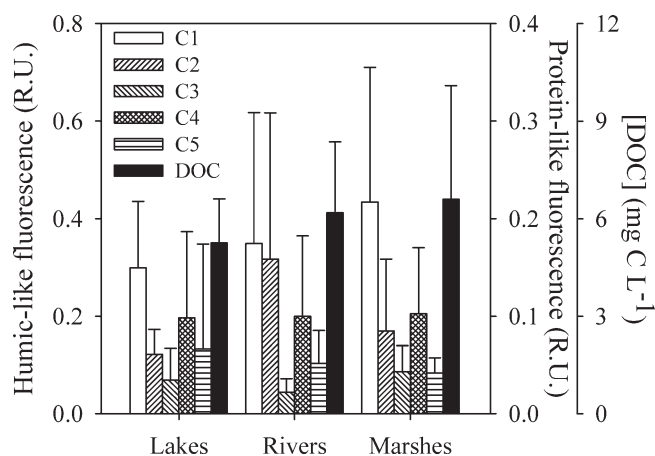

Fig. 8. Ecosystem distribution of the fluorescence components identified by the parallel factor analysis (PARAFAC). Each bar corresponds to the mean fluorescence of a given component for June and July, and the error bar to standard deviation. Also shown is the distribution of the concentration in DOC.
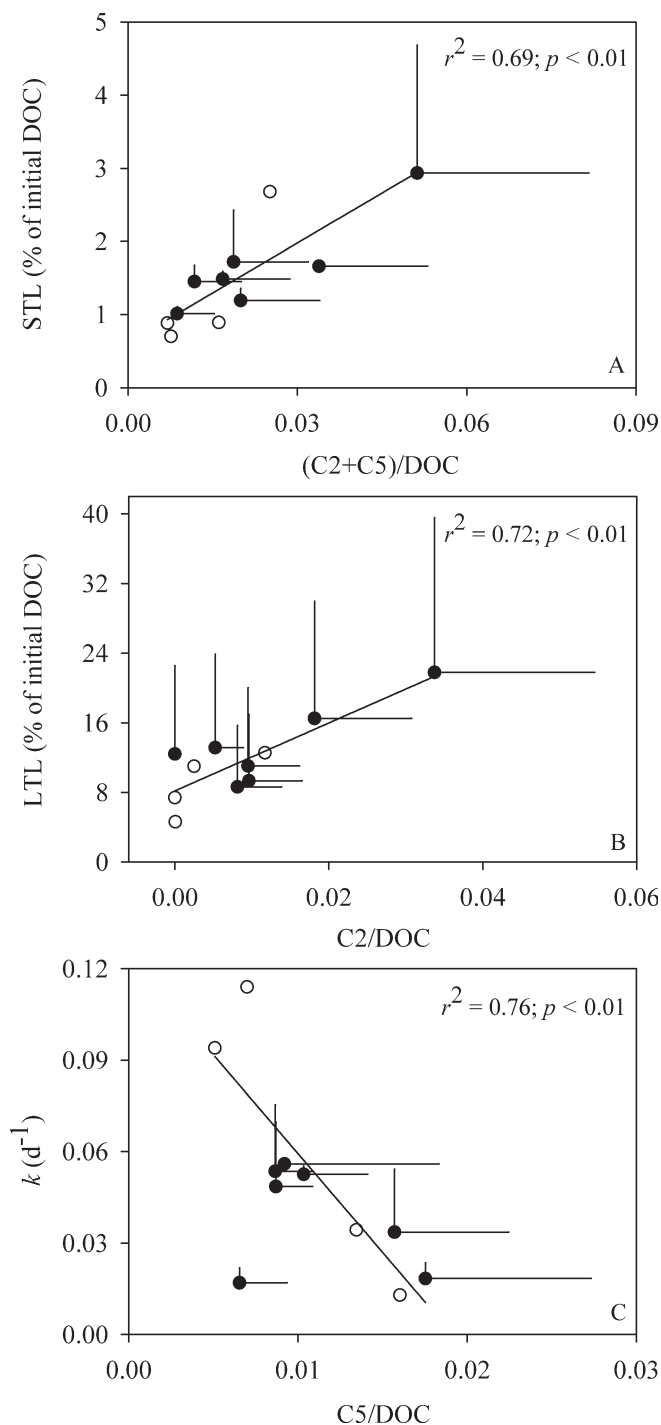
Fig. 9. Regression models describing the relationship between (A) short-term lability (STL), (B) long-term lability (LTL), and (C) the first-order decay constant (k), and the fluorescence components identified by a parallel factor analysis (PARAFAC). Each black dot represents the mean of the June and July carbon consumption experiments for the seven sampling sites where data were available for the 2 months, and error bars correspond to standard deviation. Open circles denote values for the four sites where data were available for July only (*see* Methods for details). The outlier was removed from the regression between the k constant and the fluorescence component 5 because it lies outside the 95% confidence interval (outlier included: $r^2 = 0.45$, $n = 11$, $p < 0.05$).

Kalff 2003; Stets and Cotner 2008). For example, Mann and Wetzel (1996) found that between 22% and 69% of the DOC produced by growing and senescent macrophytes could be used over 24 h, and Søndergaard (1983) showed that up to 30% of the DOC released by the angiosperm *Littorella uniflora* was consumed over longer timescales (10 d). Because macrophytes cover significant portions of the studied marshes, inputs of macrophyte-produced DOC may in part explain the relatively high DOC bioavailability found in these systems. It is also plausible that a significant amount of this bioavailable DOC may have been directly exported to some of the rivers or lakes located downstream, thus contributing to the high level of BCC that we observed in these recipient systems.

Patterns in overall DOC consumption—The approach used in this study to assess the relationship between short- and long-term DOC consumption dynamics involved integrating both measurements into a single time course and fitting a two-pool (labile and refractory) model to this time course of DOC consumption. The k degradation constants estimated by this model are well within the range of what others have found in aquatic ecosystems (Stets and Cotner 2008; Lønborg and Søndergaard 2009; Ostapenia et al. 2009) and even in soil pore waters (Wickland et al. 2007). While we found an ecosystem-specific pattern for both STCC and LTCC, no systematic pattern in the k constant was observed across the different types of ecosystem. This is interesting because both STCC and LTCC were involved in deriving the overall decay curve and estimating the k constant, and further suggests that even if STCC and LTCC vary systematically across ecosystems, they may not vary in a similar manner relative to one another within any system. The resulting overall pattern of consumption, reflected in the k constant, thus represents an emergent property of DOC that cannot be predicted on the basis of either of its components.

The factor that individually explained more of the observed variability in k across ecosystems was Chl a. Ostapenia et al. (2009) also found a negative relationship between the DOC k decay constant and Chl a in six lakes that span a wide range of trophic status (Chl a concentrations up to 50 μg L^{-1}), but their relation was nonlinear with an asymptote approaching a k value of 0.04 d^{-1} (Fig. 7A inset). We did not find a similar pattern in our data set, probably because our Chl a concentrations were all below 8 μg L^{-1}. This result suggests a possible link between the shape of the consumption curve and the origin of the DOC being degraded. To test this idea, we revisited published measurements of the source of organic carbon (OC) supporting bacterial respiration by McCallister and del Giorgio (2008) that were made for the same lakes and two of the streams sampled in this study, and found a negative relationship ($r^2 = 0.68$, $n = 8$, $p < 0.05$; Fig. 7B) between the k constant and the percentage of algal OC supporting bacterial respiration (Fig. 7B). We also found that the k constant was most strongly related to tryptophan-like carbon compounds, and since the k constant was negatively related to both Chl a and the percentage of algal DOC used for respiration by the bacterial community, we propose that

this tryptophan-like material originates mainly from autochthonous aquatic production. This idea is supported by the fact that tryptophan-like material was present in higher concentration in lakes, which also contained higher Chl *a* concentrations, than in the other systems. This would thus suggest that a small, but very reactive fraction of the DOC pool, which appears to be of algal origin, may control in part the overall dynamics of C consumption as reflected in the *k* constant and, in turn, the degree of coupling between STCC and LTCC.

Ostapenia et al. (2009) hypothesized that the low *k* values observed at high Chl *a* concentration could be the result of dominance by cyanobacteria in more eutrophic systems, and further suggested that this may lead to relatively lower rates of decomposition and overall DOC lability. Our observations do not support this hypothesis because Chl *a* concentrations in our study were relatively low and there was no evidence for cyanobacterial blooms. Instead, we propose that as the concentration of highly labile algal-derived DOC increases in the water column, not only STCC increases as a function of Chl *a*, but this rate remains more or less the same over longer timescales, leading to lower *k* values (linear time courses). This idea is partly supported by the fact that we often observed low *k* values associated with high STCC and LTCC rates in lakes, such that the assumption that low *k* values imply low DOC bioavailability is not supported. In other words, a linear time course with an associated low *k* may still present a steep slope and, hence, a high rate of C consumption. It should also be emphasized that it is not possible to predict the size of the labile pool from the *k* value over the different time courses because similar *k* values could be associated with very different levels of DOC lability. Therefore, any conclusions concerning DOC bioavailability in natural systems should not be solely based on an estimate of the *k* value but should also consider both the actual rates of BCC and the amount of DOC removed. In this respect, had they measured BCC rates, Ostapenia et al. (2009) might have also found that their low *k* values in more productive systems still corresponded to high rates of BCC or high overall bioavailability.

The biogeochemical and ecological implications of STCC, LTCC, and k—From a biogeochemical point of view, the timescales of DOC consumption are important to understand the fate of C as it moves across the landscape and its potential to generate greenhouse gases and to fuel linked biogeochemical processes at times and places that may be very different from those where the DOC originated (Cole et al. 2007; Holmes et al. 2008). From an ecological point of view, the consumption of DOC represents the entry point of energy into the microbial food web and fuels a number of processes that are of key importance to the functioning of aquatic systems (Kritzberg et al. 2004; Jansson et al. 2007). Consequently, there has been a wide interest in assessing the regulating factors of DOC bioavailability. Studies have shown that the bulk C consumption by bacteria is modulated not only by the rate of supply of DOC but also by its source and composition (Marschner and Kalbitz 2003; Ågren et al. 2008; Berggren et al. 2010). In this study, we have further shown that the different aspects of DOC bioavailability (short- vs. long-term consumption and lability) are likely to be regulated independently and respond differentially to changes in the ambient DOC pool or to the environment. It is also likely that these different aspects of DOC bioavailability play different roles in terms of ecological and biogeochemical processes.

The shape of the DOC consumption dynamics, reflected in the *k* constant, and in the relative sizes of the short- and long-term labile pools, determines the potential of this C to fuel bacterial metabolism at different temporal and spatial scales. For example, in running waters and connecting systems, such as streams, rivers, and marshes, the shape of the DOC consumption dynamics will determine the amount of bacterial metabolism that this DOC may generate in the receiving systems downstream, thus influencing key aspects of ecosystem function such as trophic interactions, ecosystem respiration, and gas exchange (Cole et al. 2007; Jansson et al. 2007). In systems with longer water residence times, such as lakes, the *k* and the relative sizes of the short- and long-term labile pools will determine the capacity of the DOC pool to support microbial metabolism at different timescales within the system, which will have major implications, among others, on the magnitude of winter (under-ice) and hypolimnetic metabolism in these lakes, and the resulting trophic and gas dynamics. Furthermore, the shape of the DOC consumption curve has implications to ecosystem stability as well, because the relative importance of short- and long-term consumption will influence the capacity of the DOC to act as a buffer to temporal variations in the input of new DOC into the system. We have shown that these key properties of the DOC are not necessarily correlated with total DOC concentration or to any single DOC source. The consequence is that climate- or land-use-driven shifts in DOC export from catchments, or aquatic DOC production, may have vastly different effects in terms of the ecosystem functioning of the receiving aquatic systems, depending not only on the total amount of DOC involved but also on its patterns of biological availability.

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