

Aging of allochthonous organic carbon regulates bacterial production in unproductive boreal lakes

Martin Berggren,^{a,*} Hjalmar Laudon,^b and Mats Jansson^a

^aDepartment of Ecology and Environmental Science, Umeå University, Umeå, Sweden

^bDepartment of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden

Abstract

We calculated average aquatic dissolved organic carbon (DOC) age (the time span from soil discharge to observation) in water from the inlets and outlets of two unproductive Swedish lakes at different times during an annual cycle. Bacterial production (BP) and bacterial growth efficiency (BGE) determined during 7-d bioassays decreased with increasing average aquatic DOC age. Parallel to the declines in BP and BGE there was a rise in specific ultraviolet absorbance at the wavelength of 254 nm (SUVA₂₅₄), which indicates that decreasing BP and BGE were connected to a shift to a more aromatic and recalcitrant DOC pool. The relationships between bacterial metabolism and DOC age were stronger after a Q_{10} correction of the DOC age, showing that temperature affected rates of DOC quality changes over time and should be taken into account when relating lake bacterial growth to substrate aging in natural environments. We propose that hydrological variability in combination with lake size (water renewal time) have a large influence on pelagic BP in lakes with high input of terrigenous DOC.

After the introduction of the microbial loop concept (Azam et al. 1983), heterotrophic bacteria have been recognized as a significant component of aquatic food webs, channeling energy from dissolved organic carbon (DOC) to higher trophic levels (e.g., via phagotrophic grazers). The growth of aquatic bacteria is mostly considered to be controlled by the supply of phytoplankton-derived substrates, suggested by empirical relationships between bacterial production (BP) and primary production (Cole et al. 1988) and by laboratory observations of rapid bacterial exploitation of phytoplanktonic exudates (Chen and Wangersky 1996). However, experimental work has shown that BP can also be based on allochthonous DOC (Tranvik 1988; Moran and Hodson 1994; i.e., humic substances and other organic compounds originating from the catchment soils). For unproductive humic systems, typical for the boreal region, recent literature stresses the importance of terrestrial DOC control of BP (Jansson et al. 2007). A main concern is that as much as 75–90% of bacterial carbon contents in humic systems can be of terrestrial origin, as assessed by indirect balance calculations (Hessen 1992; Jansson et al. 1999) as well as direct radiotracer techniques (Kritzberg et al. 2006). Accordingly, BP and bacterial biomass in humic unproductive lakes are often positively correlated to the concentrations of allochthonous DOC rather than to primary production (Jones 1992; Bergström and Jansson 2000; Jansson et al. 2000).

Laboratory studies indicate declines in bacterial metabolism with aging of allochthonous DOC in the aquatic environment. Dark bioassays have shown that ~10% of the organic carbon pool can be readily consumed by bacteria within weeks (Moran and Hodson 1990; Tranvik 1998), while an additional ~50% can be consumed at low rates on

the time scale of years (Raymond and Bauer 2001). Parallel to the decrease in bacterial carbon consumption rate with allochthonous DOC aging, there is a decrease in the bacterial growth efficiency (BGE; i.e., in the share of the carbon consumption that supports BP [and not respiration]). BGE on fresh allochthonous DOC from a variety of different forest sources can be as high as 50% (Lennon and Pfaff 2005), but aged allochthonous DOC is usually metabolized with BGE lower than 10% (Eiler et al. 2003). The drop in BGE tends to be rapid and takes place in days (Berggren et al. 2007), possibly as a result of depletion of labile low molecular weight fractions of DOC (Weiss and Simon 1999; McArthur and Richardson 2002). Most of the nonrecalcitrant terrigenous substrates that enter boreal headwaters (supporting high BP and BGE) can be consumed by natural bacterial communities within 7 d of processing in 20°C (Ågren et al. 2008). Additional labile bacterial substrates may be formed by photochemical processes (Bertilsson and Tranvik 1998), but it is not known if such formation can compensate for the loss of bioavailable carbon from bacterial consumption. In highly humic lakes, light attenuation is generally too high for photochemical processes to be of major quantitative importance for bacterioplankton use of DOC (Granéli et al. 1996; Jonsson et al. 2001; Moran and Covert 2003).

Thus, previous experience shows that allochthonous DOC and not primary production may control BP in unproductive humic freshwaters and that rates of BP tend to decrease with the processing time of allochthonous DOC. Combining these two prerequisites at an ecosystem level, we hypothesize that BP can be a function of, and decreases with, natural DOC aging in unproductive humic freshwater systems. We tested this hypothesis during an annual cycle, by comparing the potential of DOC for supporting bacterial production and respiration in water flowing into a lake system and in water which had spent

* Corresponding author: martin.berggren@emg.umu.se

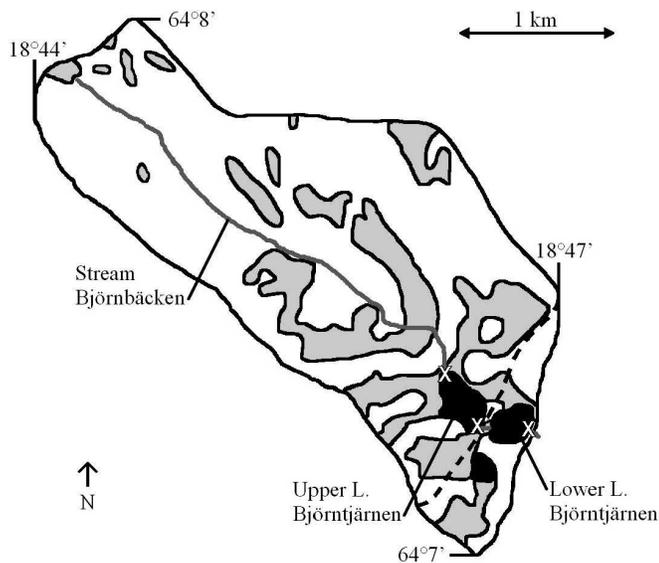


Fig. 1. Study catchment area. Mires and streams are in grey, forests in white, and lakes in black. Sampling stations are labeled with a white 'X'. The dashed line shows sub-catchment border.

different time periods within the lake system. We, thereby, assessed how aging of DOC under the influence of natural abiotic and biological processes in lakes affected the potential of DOC to support bacterial metabolism. For that purpose, we conducted bacterial bioassays coupled to average aquatic DOC age calculations (the time span from soil discharge to observation) in two nested lakes in Northern Sweden, receiving water and DOC mainly via a humic headwater stream. Our results show that the support for BP was strongly linked to the hydrological turnover of allochthonous organic carbon.

Methods

Study site—The Björntjärnarna catchment (3.5 km²) in northern boreal Sweden was selected for this study (Fig. 1). A 3-km headwater stream, Stream Björnbäcken, channeled terrestrial drainage from the catchment into two nested (92% shared catchment area) lakes, Upper Lake Björntjärnen (64°7'N, 18°46'E) and Lower Lake Björntjärnen (64°7'N, 18°47'E), connected by a small stream channel of 70 m in length. The drainage area consisted of coniferous forest (80%) and mires (20%). Anthropogenic influences on water quality were minimal, except some forestry activities throughout the catchment. The lakes were relatively deep (8–10 m) in relation to their surface areas (0.03–0.05 km²) and littoral zones were virtually lacking. A stable thermal stratification developed soon after the ice break in spring and lasted until autumn. There was no clear reversed thermal stratification in winter. Earlier studies showed that the lakes were dominated by allochthonous DOC (~15 mg L⁻¹ or more) and that they were naturally unproductive with epilimnetic primary production of 1–3 μg C L⁻¹d⁻¹ (Jansson et al. 2001; Bergström et al. 2003).

The flux of water through the system is believed to be dominated by superficial runoff (Laudon et al. 2007). The

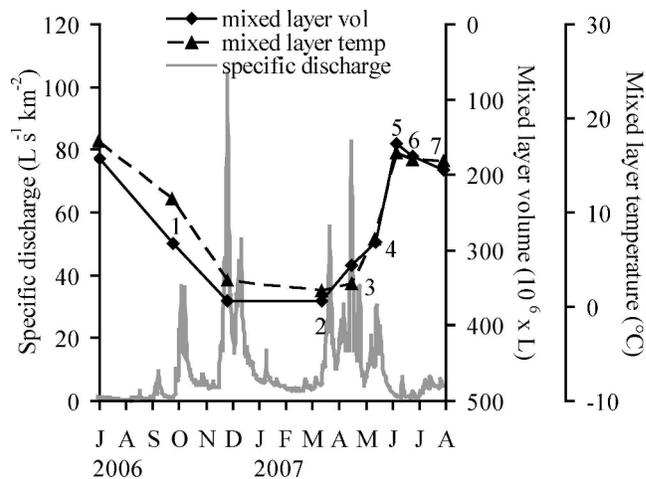


Fig. 2. Total mixed-layer volume (sum of the two study lakes), average mixed-layer temperature, and specific discharge from July 2006 to August 2007. Temperature and mixed-layer data are shown for the seven dates of bioassay sampling, indicated by numbers, plus for two additional dates.

catchment is mainly comprised of till soils known to consist of an exponentially decreasing hydrological conductivity with depth (Rodhe 1989). Thus, most water that was discharged from the catchment moved along a unidirectional flow path via Stream Björnbäcken through the mixed surface layers of Upper Lake Björntjärnen and Lower Lake Björntjärnen. We considered that water at the inlet to Upper Lake Björntjärnen, draining >90% of the lake catchment area, was representative to all water that reached the lake. In theory, there may be quality differences between streamwater and groundwater DOC inflow. However, as the catchment area drained by diffuse flow was of similar character as the catchment of the inlet stream (spruce forest mixed with peat mire) and the transit time in the stream was short (<1 d), such differences were probably small. Similarly, water at the Lower Lake Björntjärnen inlet was considered representative to all water reaching that lake. This inlet water had passed an upstream lake but so had a major part of the diffusive groundwater inflow into Lower Lake Björntjärnen, by its transit through the small headwater lake without visible inlets or outlets at the far south part of the catchment (Fig. 1).

Sampling and analyses—At seven dates (Fig. 2), water from the outlets of Stream Björnbäcken, Upper Lake Björntjärnen, and Lower Lake Björntjärnen (Fig. 1) was filtered in the field through 50-μm filters in order to remove metazoan zooplankton and collected in 2-liter high-density polyethylene bottles. Temperature was measured on each sampling occasion plus on two additional dates (Fig. 2) at the inlet, outlet, and at the deepest part of each lake, from 0-m depth down to the lake bottom, with 0.5-m or 1-m increments. Water samples were equilibrated with standard air (78% N₂, 21% O₂, and 0.03% CO₂) to remove supersaturation of CO₂ and then directly subsampled into 22-mL gas-tight glass bottles, leaving a 12-mL headspace

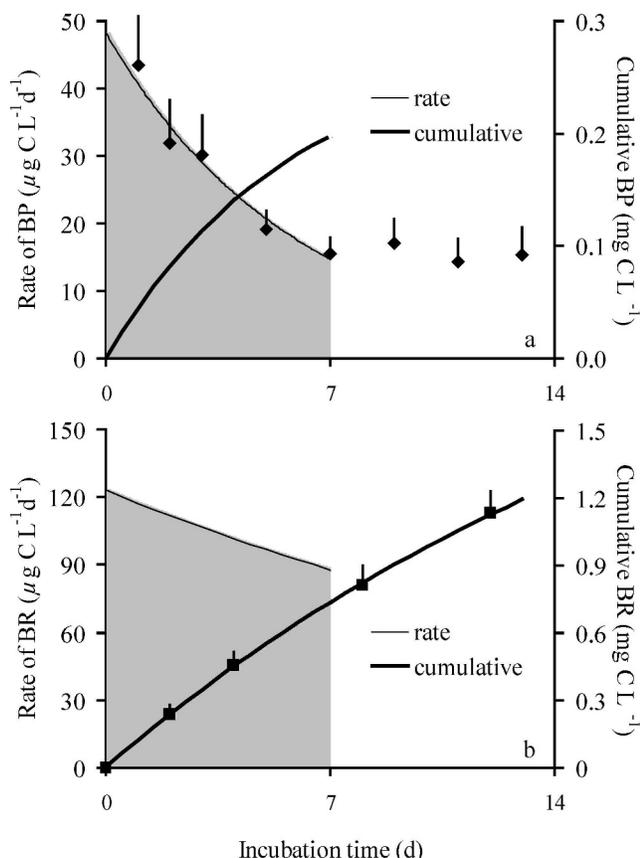


Fig. 3. The definitions of BP_7 and BR_7 (the gray areas) as the 7-d cumulative bacterial production and respiration, assessed by exponential regression. Although BP_7 and BR_7 were calculated separately for each bioassay, the figure is based on the mean values of all analyses for illustrative purposes. (a) Observed average rates of bacterial production (BP; $\mu\text{g C L}^{-1}\text{d}^{-1}$) over the incubation time, with an exponential regression line for the first 7 d ($y = 48.62e^{-0.17x}$, $R^2 = 0.98$, $p < 0.01$) and its primitive function (converted to $\text{mg C L}^{-1}\text{d}^{-1}$) showing cumulative BP. (b) Observed cumulative bacterial respiration (BR; $\text{mg C L}^{-1}\text{d}^{-1}$) over the incubation time, with an exponential regression line ($y = 0.28 - 0.28e^{-0.00017x}$, $R^2 = 1.00$, $p < 0.01$) and its derivative (converted to $\mu\text{g C L}^{-1}\text{d}^{-1}$) for the first 7 d showing rate of BR.

flushed with standard air. Within 24 h from sampling, all bottles were incubated in the dark at 20°C. The reduction of initial dissolved inorganic carbon (DIC) concentrations made it possible to accurately measure the production of DIC during incubation, in spite of high in situ concentrations.

Unique triplicates of the 22-mL incubation bottles were analyzed for BP and bacterial respiration (BR) repeatedly during a period of 13 d (Fig. 3). BR was measured as DIC production on a Perkin-Elmer GC-FID, with a headspace auto-sampler that operated directly on the incubation bottles. Separation was carried out on a Haysep Q column using N_2 (40 mL min^{-1}) as carrier gas. Before analysis, the samples were acidified to potential for hydrogen (pH) 2.5 and shaken. It was assumed that potential respiration of protozoa was negligible due to the vast dominance of bacteria in dark bioassays using humic water (Daniel et al.

2005). The rate of BP was measured with the leucine incorporation method described by Smith and Azam (1992), slightly modified by Karlsson et al. (2002). We applied an isotopic dilution factor of 2 (Simon and Azam 1989), which is the most widely used dilution factor in freshwater systems. The samples were exposed to $[^3\text{H}]$ -leucine concentrations between 30 nmol L^{-1} and 70 nmol L^{-1} during 1 h in 20°C. Blanks were pretreated with 5% w/v of TCA (trichloroacetic acid). Leucine incorporation into protein was determined by precipitation with TCA and centrifugation, followed by scintillation counting (Beckman LS 6500). The leucine incorporation was converted into carbon units according to Simon and Azam (1989).

Unfiltered samples for measurements of total phosphorus (TP) and total nitrogen (TN), plus filtered samples ($0.45 \mu\text{m}$ Millipore) for DOC, absorbance, and fluorescence analyses, were frozen directly after arrival to the laboratory. Later, DOC was analyzed with a Shimadzu TOC- V_{CPH} analyzer, TN with an Antek 9000 Nitrogen Analyzer and TP after persulphate oxidation followed by the standard molybdenum blue method. Filtering procedures should not have had any pronounced effects on observed concentrations, because particulate organic matter concentrations usually are negligible relative to the dissolved fractions in Swedish boreal surface waters (Ivarsson and Jansson 1994; Laudon and Bishop 1999). Absorbance spectra were measured in 1-cm quartz cuvettes with a Hewlett Packard 8452A diode array spectrophotometer. Fluorescence was analyzed at an excitation wavelength of 370 nm on a Perkin-Elmer LS 45, as described by McKnight et al. (2001). This analysis included acidifying of samples, scanning of spectra and correction of data for absorbance and blank curves.

We calculated the daily flux of water through the study lakes by using daily means of specific discharge from the nearby Krycklan catchment (50 km northeast), where stream water levels were recorded continuously using a pressure transducer and a 90° V-notch weir housed in a heated shed (Buffam et al. 2007). Discharge was given from the stream water level and established height-discharge rating curves. Detailed discharge curves reported for Upper Lake Björntjärnen inlet for the period 1996–1998 (Jansson et al. 2001) had about the same mean specific discharge ($\sim 10 \text{ L s}^{-1} \text{ km}^{-2}$) as recorded for the Krycklan catchment during the same period of time (Laudon et al. 2004). In addition, manual registration of the water level at the inlet to Upper Lake Björntjärnen on the sampling dates of this study demonstrated a strong correlation with the water height in the Krycklan stream ($R^2 = 0.90$, $n = 7$, $p < 0.01$). In another study that used the same Krycklan stream for assessing the flow in other nearby streams (Ågren et al. 2007), the inter-site differences in annual discharge (e.g., due to differences in sub-catchment evapotranspiration) were calculated to be no $> \pm 12\%$ (based on discrete discharge measurements at the other sites, which were compared to the continuous measurements in the shed). An additional uncertainty due to inter-site differences in flow regime ('flashiness') was calculated as $\pm 1\%$ maximum error during the snow-free season and $\pm 12\%$ during the spring.

Calculation of aquatic DOC age—To be able to compare BP to the time during which the carbon had been subject to aquatic processing, the average aquatic DOC age (i.e., the time period between soil discharge and sampling), was assessed. Exchange of dissolved substances between epilimnion and hypolimnion in thermally stratified lakes is very low (Wetzel 2001; Kalff 2002). Due to the stable stratification, fluxes of DOC were assumed to pass only through the mixed superficial layers of the system. The assumption is supported by a previous study in the nearby Lake Örträsket, which showed that hypolimnetic bacterial production was stable at low values, with no coupling to surface mixed-layer dynamics (Bergström and Jansson 2000). This lake, at only 10-km distance, is similar to the study lakes in several ways. It has a catchment composed of the same major landscape components, is equally dominated by allochthonous DOC and has a similar water renewal time.

The average DOC age (A) in a lake is a function of fluxes, to and from the lake, of DOC of different ages and of the amount of DOC in the lake. The age also changes with '+1' per unit of time, as the DOC pool gets 1 d older per day. Hence, the change in A over time can be described by the derivative

$$\frac{dA}{dt} = A_{in} \times \frac{C_{flux\ in}}{C_{stock}} - A \times \frac{C_{flux\ out}}{C_{stock}} + 1, \quad (1)$$

where 'A_{in}' is the age of inflowing DOC, 'C_{flux}' the mass flux of DOC in or out, and 'C_{stock}' the total amount of DOC in the system. In a discrete form, the equation can be rewritten as

$$A(t+1) = A(t) + A_{in}(t) \times \frac{C_{in}(t)}{C_{stock}(t)} - A(t) \times \frac{C_{out}(t)}{C_{stock}(t)} + 1, \quad (2)$$

where 't' is day (in whole numbers) and 'C_{in}(t)' and 'C_{out}(t)' are the amounts of DOC imported and exported during day t.

We first calculated 'A' according to Eq. 2 on Upper Lake Björntjärnen, iteratively for each day in sequence, starting from an assumed value at day one of the study period (Fig. 2). 'A_{in}' was given from the turnover of DOC in the inflowing Stream Björnbäcken (*see below*). 'C_{in}' and 'C_{out}' were estimated from the daily flux of water through the system multiplied by DOC concentrations for the Stream Björnbäcken and the lake outlet, respectively. The water flux was calculated as drainage area multiplied by specific discharge. 'C_{stock}' was calculated as the mixed-layer volume of the lake multiplied by the lake concentration of DOC, measured at the outlet. After finishing the calculations for Upper Lake Björntjärnen, we repeated the procedure for Lower Lake Björntjärnen. Here, 'A_{in}' was given from the average DOC age in the upstream Upper Lake Björntjärnen. Analogously, 'C_{in}' for Lower Lake Björntjärnen was the same as 'C_{out}' for Upper Lake Björntjärnen.

The mixed-layer volumes of the two lakes were calculated from thermocline depths and established depth-volume curves. Thermocline depth was calculated as the mid-depth of the metalimnion, defined as the water layer where temperature (ΔT) changes were $>1^\circ\text{C m}^{-1}$

(Wetzel 2001). Continuous depth profiles for ΔT were obtained by linear interpolation between values representing the middle of the measured depth transects. Daily values of mixed-layer volumes and DOC concentrations were obtained by linear interpolation between available measurements over time. The assumed initial values of 'A' (at day 1) were given by running the iteration from an arbitrary 'A' value several years back in time, using the continuous record of discharge and assuming that previous years had the same seasonal patterns of mixed-layer depths and DOC as the study year.

In contrast to the lakes, the full volume of Stream Björnbäcken was always rapidly flushed (in fractions of a day) and, thus, the turnover of DOC in this stream was not important for the overall aging of DOC in the surface waters of the catchment. Due to the rapid flushing, the DOC aging in Stream Björnbäcken could be calculated on a daily basis by dividing daily discharge by stream volume. The stream volume was roughly estimated from depth measurements by the outlet (linearly interpolated between sampling dates) multiplied by stream length (3000 m) and average width (0.4 m). This volume was three orders of magnitude smaller than the mixed-layer volumes of the lakes.

Temperature correction of aquatic DOC age—We used the average aquatic DOC age as a basic measure of the time period during which allochthonous DOC was processed in the aquatic system. Bacterial degradation is the single most important DOC-consuming process in humic lakes (Jansson et al. 2001). A problem with this approach is that the bacterial carbon consumption rate is closely coupled to temperature, with higher rates at high temperatures (in summer) compared to at low temperatures (in winter). Therefore, a certain value of DOC age represents a higher degree of processing (more rapid aging) at high temperature than at low temperature. Consequently, a straightforward comparison of BP and age can only be made if the temperature of the lake was constant. For this reason, we made a Q_{10} correction to a normalized temperature of 20°C at which the laboratory incubations were conducted. The metric Q_{10} states how many times faster a process goes when the temperature increases with 10°C . For example, if $Q_{10} = 2$, 2 d in 10°C corresponds to 1 d in 20°C . An extensive data set (Apple et al. 2006) suggests that Q_{10} for bacterial carbon consumption (BP + BR) is in the range of 1.8–3.0 for normal temperature intervals. We used the intermediate value 2.5 in our calculations and regarded 2 and 3 as extremes covering all probable outcomes.

The temperature correction was performed by repeating the DOC age calculations, using a modified version of Eq. 2. The '+1' at the end of the expression, which states that the DOC age increases by one during a day, was changed to '+1 $\times Q_{10}^{(T_{lake} - 20)/10}$ ', where T_{lake} is the average above thermocline temperature. Similarly, the DOC age of Stream Björnbäcken was multiplied by $Q_{10}^{(T_{stream} - 20)/10}$, where T_{stream} is the Stream Björnbäcken outlet (i.e., Upper Lake Björntjärnen inlet) temperature. T_{lake} and T_{stream} were estimated for each day by linear interpolation between measurements.

Table 1. Pearson r correlations ($n = 21$) between bacterial metabolic measures during 7-d incubations and average aquatic DOC age, corrected DOC age, dissolved organic carbon (DOC), Specific ultraviolet absorbance at 254 nm (SUVA₂₅₄), total phosphorus (TP), and total nitrogen (TN).

Variable	BP ₇	BGE ₇	BR ₇
DOC age, uncorrected	-0.62**	-0.52*	n.s.
DOC age, corrected	-0.79**	-0.68**	n.s.
DOC	n.s.	n.s.	0.72**
SUVA ₂₅₄	-0.73**	-0.54*	n.s.
Fluorescence index	-0.68**	-0.63**	n.s.
TP	n.s.	n.s.	n.s.
TN	n.s.	n.s.	n.s.

Significance: * $p < 0.05$, ** $p < 0.01$, n.s. not significant.

Analyses of DOC in situ characteristics—Carbon-specific ultraviolet absorbance (SUVA₂₅₄) was calculated by dividing absorbance at the wavelength of 254 nm (m^{-1}) with DOC ($mg L^{-1}$). A higher value of SUVA₂₅₄ indicates higher contents of aromatic carbon (Weishaar et al. 2003) that, in general, has lower bioavailability compared to the aliphatic compounds (Perdue 1998). A fluorescence index was calculated as the emission intensity at a wavelength of 450 nm to that of 500 nm, obtained at an excitation wavelength of 370 nm. This index has been suggested to reflect the source of the organic carbon, with an autochthonous signal close to 1.90 and an allochthonous signal close to 1.40 (McKnight et al. 2001).

Results

The in situ water temperatures varied from a couple of degrees above 0°C in winter to about 15°C in summer (Fig. 2), with very small differences between the two lakes (<1°C for all sampling dates). The mixed-layer volume was tightly related to average mixed-layer temperature (Fig. 2). Uncorrected average aquatic DOC age, from catchment discharge to sampling, ranged from fractions of a day at the inlet of Upper Lake Björntjärnen to a maximum of 173 d at the outlet of Lower Lake Björntjärnen. Temperature-corrected DOC age (days in 20°C), assuming Q_{10} of 2.5, covered a gradient up to 68 d.

Both BP and BR decreased exponentially during the first 7 d of the bioassays (Fig. 3). The half-life of BP was on average 3.6 times shorter than the half-life of BR, reflected in a much steeper relative slope of the BP curve. After 7 d, BP stabilized at a seemingly constant level, at values considerably lower than the initial values. We denote the cumulative BP during the first 7 d as BP₇ (Ågren et al. 2008), which we use as a measure of the potential of the organic carbon for support of efficient bacterial growth. Correspondingly, cumulative BR during the first 7 d is denoted as BR₇ (see Fig. 3 for detailed definitions of BP₇ and BR₇).

BP₇ was 0.09–0.37 $mg C L^{-1}d^{-1}$, with the highest values in samples with low DOC age and the lowest values in samples with high DOC age. There was a significant negative Pearson r correlation between BP₇ and uncorrected DOC age as well as between BP₇ and the corrected DOC

age, although the relationship was stronger after the correction (Table 1). An even stronger negative relationship ($R^2 = 0.70$, $n = 21$, $p < 0.01$) was obtained when modeling BP₇ as an exponential function of corrected DOC age (Fig. 4a). BR₇, on the other hand, with values of 0.45–1.1 $mg C L^{-1}d^{-1}$, showed no significant patterns with DOC age (Table 1; Fig. 4b). Bacterial growth efficiency [BGE₇ = BP₇ / (BP₇ + BR₇)] was 0.11–0.36 and demonstrated similar patterns as those of BP₇, with negative Pearson r correlations with DOC age (Table 1). As in the case of BP₇, BGE₇ was best described ($R^2 = 0.54$, $n = 21$, $p < 0.01$) as an exponential function of the corrected DOC age (Fig. 4c).

BP₇ and BR₇ differed markedly in their relations to in situ chemical properties of the lakes (Table 1). BP₇ showed significant negative linear relationships with carbon-specific ultraviolet absorbance at wavelength of 254 nm (SUVA₂₅₄; $R^2 = 0.53$, $n = 21$, $p < 0.01$) and with the fluorescence index ($R^2 = 0.46$, $n = 21$, $p < 0.01$), indicating that BP₇ was coupled to the character of the organic carbon (Fig. 5). BR₇, on the other hand, was explained by the quantity of the organic carbon (i.e., the DOC concentration [$R^2 = 0.52$, $n = 21$, $p < 0.01$]), and was not significantly related to other chemical properties (Table 1).

There were significant changes in the character of the organic carbon with increasing DOC age. Both SUVA₂₅₄ ($R^2 = 0.35$, $n = 21$, $p < 0.01$) and the fluorescence index ($R^2 = 0.25$, $n = 21$, $p < 0.05$) were positively correlated to the uncorrected DOC age. Considerably more variance, however, was explained when correlating SUVA₂₅₄ ($R^2 = 0.55$, $n = 21$, $p < 0.01$) and the fluorescence index ($R^2 = 0.40$, $n = 21$, $p < 0.01$) to the corrected DOC age (Fig. 6). DOC, TP, and TN were similar between the different sampling stations (Table 2) and showed no overall relationships to DOC age, BP₇ or BR₇. However, during the falling limb of the spring flood 2007 (samplings 3–7 in Fig. 2), there was a negative linear trend between DOC and corrected DOC age in the lakes (Fig. 4d; $R^2 = 0.69$, $n = 10$, $p < 0.01$).

Discussion

This study demonstrates that the BP in unproductive humic lakes can be controlled by the hydrological turnover of allochthonous organic carbon. BP and growth efficiency during 7-d bioassays (BP₇ and BGE₇) decreased with the average aquatic DOC age, calculated as the time between catchment discharge and lake sampling. These relationships were stronger after a Q_{10} correction of the DOC age (Table 1; Fig. 4), suggesting that the temperature should be taken into account when relating lake bacterial growth to substrate aging.

In aquatic systems, a number of conceivable processes, including biological or photochemical degradation (Moran and Covert 2003) and interactions with the plankton community (Azam et al. 1983), may affect DOC (in situ aging). The quality of DOC in the Björntjärnarna lakes was clearly related to DOC aging, as reflected by the positive correlation between SUVA₂₅₄ and DOC age (Fig. 6a). This relationship indicated that the DOC was deprived of labile substrates during its residence in the lakes, leading to a

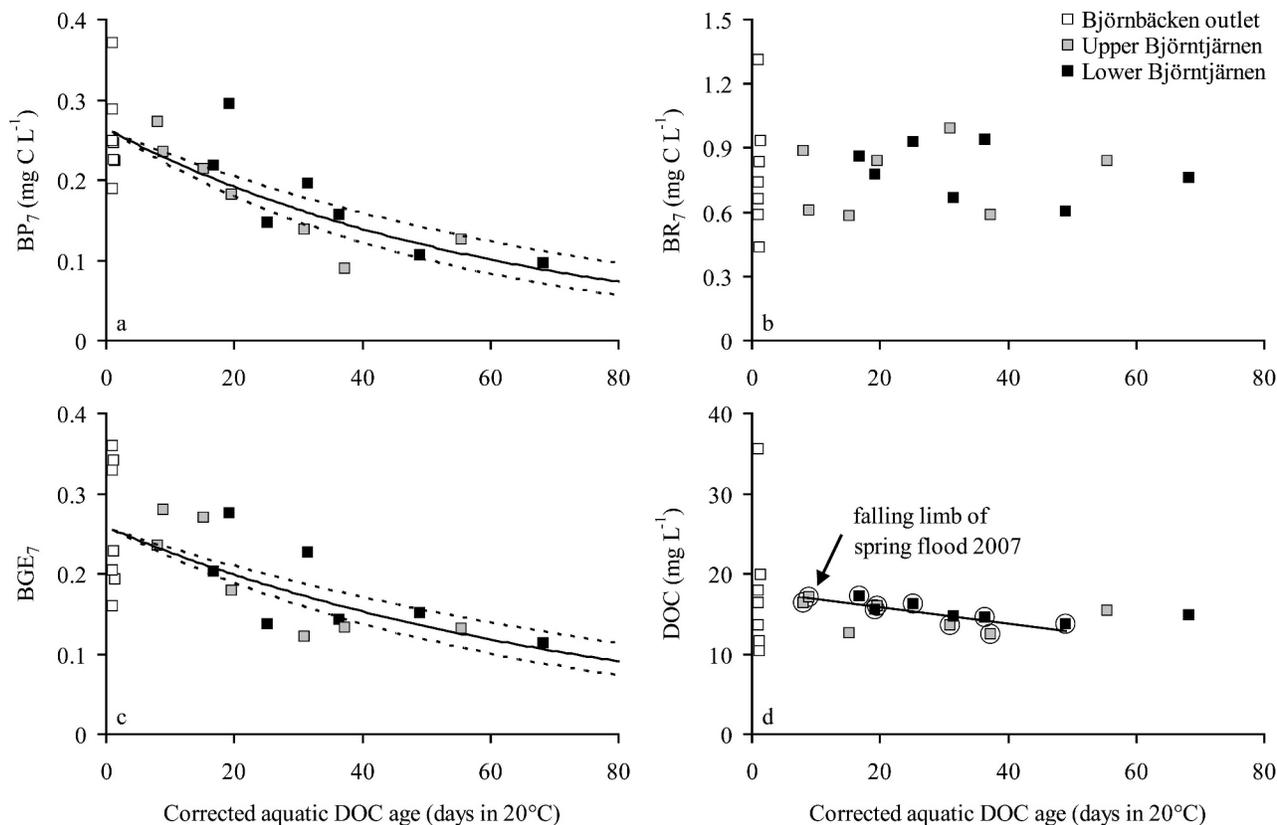


Fig. 4. (a) BP_7 , (b) BR_7 , (c) BGE_7 , and (d) in situ DOC over a gradient of average aquatic DOC age, which has been normalized to a temperature of 20°C using the Q_{10} value of 2.5. (a) The relationship between BP_7 and corrected DOC age ($y = 0.26e^{-0.016x}$, $R^2 = 0.70$, $p < 0.01$). Upper ($y = 0.26e^{-0.013x}$, $R^2 = 0.65$, $p < 0.01$) and lower ($y = 0.26e^{-0.019x}$, $R^2 = 0.74$, $p < 0.01$) dashed lines illustrate the shape of this relationship using Q_{10} values of 2 and 3, respectively. (b) Absent relationship between BR_7 and corrected DOC age. (c) The relationship between BGE_7 and corrected DOC age ($y = 0.26e^{-0.013x}$, $R^2 = 0.54$, $p < 0.01$). Again, the upper ($y = 0.26e^{-0.010x}$, $R^2 = 0.50$, $p < 0.01$) and lower ($y = 0.26e^{-0.016x}$, $R^2 = 0.56$, $p < 0.01$) dashed lines represent relationships with Q_{10} correction values of 2 and 3. (d) Development of DOC concentration after the spring flood 2007 in circles ($y = -0.010x + 17.83$, $R^2 = 0.69$, $p < 0.01$; samplings 3–7 in Fig. 2).

more aromatic and recalcitrant DOC pool. Parallel incubations with nutrient-enriched water at four of the sampling dates (M. Berggren unpubl.) showed no patterns of increasing inorganic N or P limitation with increasing DOC age. Therefore, the response of BP to aquatic DOC aging can be interpreted as a result of successive depletion of labile substrates from the DOC pool.

With higher DOC age, there was also a small but significant increase in the fluorescence index, possibly reflecting a higher share of autochthonous organic carbon. As suggested by linear regression (Fig. 6b), the index increased by 0.06 units from 1.42 to 1.48 at maximum DOC age, which corresponds to an autochthonous contribution in the order of 10% of DOC (McKnight et al. 2001). It could be assumed that such a contribution would stimulate BP, as bacterial growth efficiency generally is high on phytoplankton exudates (Del Giorgio and Cole 1998). Still, the conditions for bacterial growth did not improve. Instead, $SUVA_{254}$ rose (Fig. 6a) and decreasing BP_7 was coupled to this increasing aromaticity (Fig. 5a). Thus, although phytoplankton may have contributed to the DOC pool, the data suggest that this contribution was too small to balance the negative effects on BP due to aging and

processing of labile allochthonous substrates. The fact that there was a reversed (negative) relationship between fluorescence index and BP_7 (Fig. 5b) is a further indication of strong allochthonous control of bulk DOC properties and its use for BP. Compared to other types of aquatic systems worldwide (Cole et al. 1988), the study lakes are extremely unproductive with mean summer epilimnetic primary production not exceeding $3 \mu\text{g C L}^{-1}\text{d}^{-1}$ (Jansson et al. 2001; Bergström et al. 2003).

There were no overall correlations between DOC concentration and DOC age (corrected or not corrected for temperature). The inlet DOC concentration was variable (Fig. 4d) and the DOC was flushed into the lakes at different concentrations in distinct pulses (Fig. 2). Fortunately, the data allowed us to follow the DOC development in the lakes after a single major hydrological pulse followed by low-flow conditions. Looking at field dates 3–7 (Fig. 2), which cover the time span from peak flow in spring 2007 to low-flow conditions during summer, there was a strong ($R^2 = 0.65$) negative correlation between DOC concentration and temperature-corrected aquatic DOC age (Fig. 4d; circles). Indicated by the regression, the DOC loss in 20°C was $0.10 \text{ mg L}^{-1} \text{ d}^{-1}$, corresponding

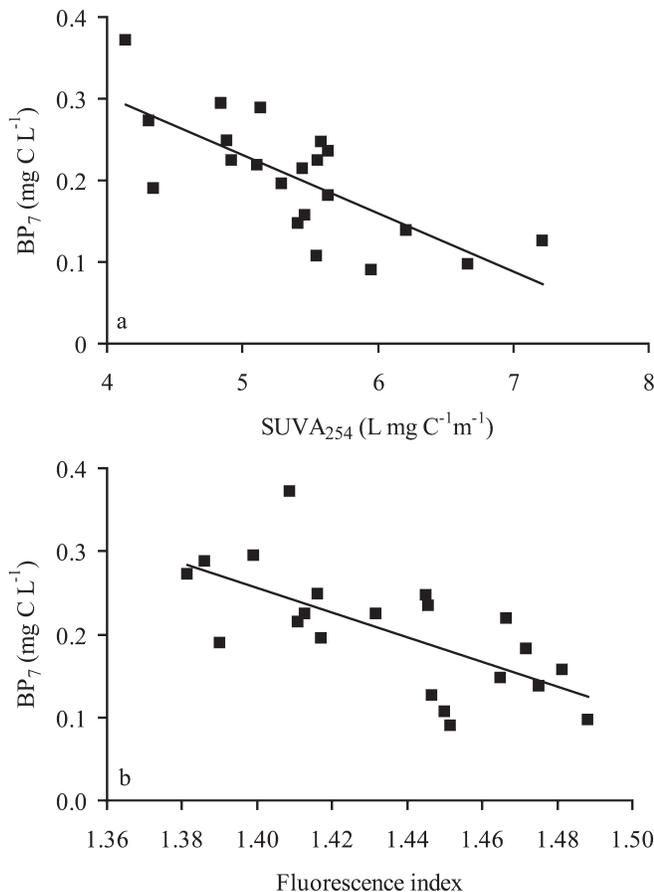


Fig. 5. BP_7 as a function of in situ values of (a) carbon-specific ultraviolet absorbance at wavelength of 254 nm ($SUVA_{254}$; $y = -0.071x + 0.59$, $R^2 = 0.53$, $p < 0.01$) and (b) a fluorescence index calculated as the emission intensity at a wavelength of 450 nm to that of 500 nm, obtained at an excitation wavelength of 370 nm ($y = -1.49x + 2.34$, $R^2 = 0.46$, $p < 0.01$).

to a weekly mineralization of 0.71 mg L^{-1} . This value fits the range of measured bacterial mineralization in 20°C (i.e., BR_7 [Fig. 4b]).

Thus, bacterial respiration alone was sufficient for explaining the decrease in mixed-layer DOC after the spring flood of 2007. Other DOC-consuming processes, such as photomineralization or net transformation of DOC to particulate organic carbon, can have contributed to DOC depletion, but our results suggest that they probably were not of major importance. A study in the nearby humic Lake Öträsket, at 10-km distance from Björntjärnarna, showed that bacterial respiration was the dominant component of total water-column DOC mineralization, and that biological mineralization in the epilimnion was five times greater than photomineralization (Jonsson et al. 2001).

In our bioassays we measured the potential of naturally aged DOC to support BP and BGE. There were, however, analogies between the incubation patterns of bacterial metabolism (Fig. 3) and the patterns of BP_7 over the in situ DOC age gradient (Fig. 4). In both cases, the decline in bacterial production over time was much more pronounced

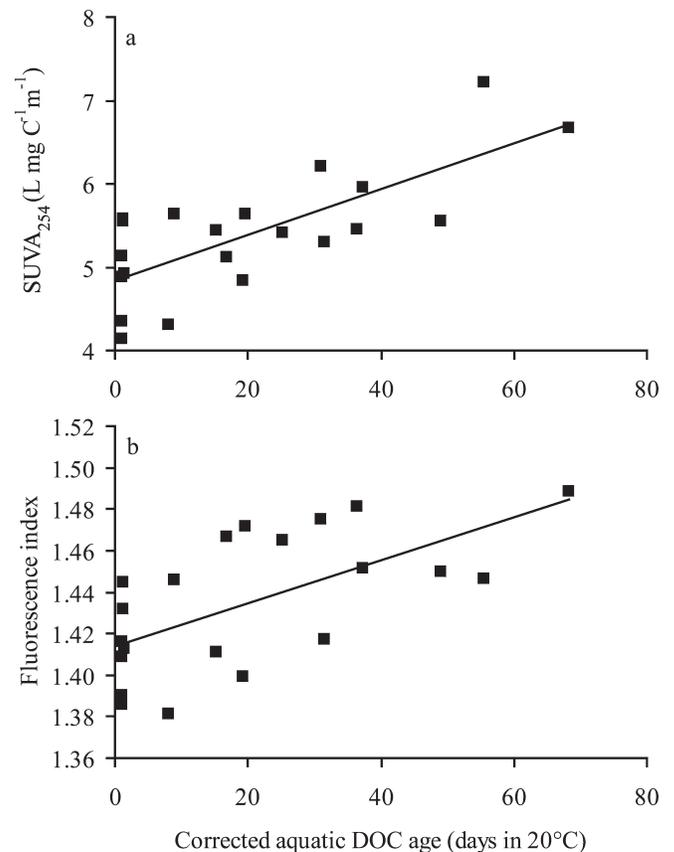


Fig. 6. (a) $SUVA_{254}$ ($y = 0.027x + 4.83$, $R^2 = 0.55$, $p < 0.01$) and (b) fluorescence index ($y = 0.0010x + 1.41$, $R^2 = 0.40$, $p < 0.01$) as functions of temperature-corrected DOC age.

than the decline in bacterial respiration. By definition, these patterns imply that the BGE also decreased. This result fits the general picture of BP being more variable and resource-dependent than BR (Grossart and Ploug 2001), and that BP and BGE, therefore, co-vary over resource gradients (Del Giorgio and Cole 1998). A difference, however, was that it took only 7 d in 20°C bioassays for BP to reach a low, stable, level (Fig. 3a) while it took months of DOC aging in 20°C to reach the corresponding decrease in BP_7 in the lake system (Fig. 4a). There are several possible explanations to this discrepancy. First of all, contrary to laboratory incubations, the change in aquatic DOC age in lakes is not a plain function of time. As long as there is runoff from the catchment, there is a continuous input of new DOC to the lake, and as indicated by this study, such recent DOC might have an un-proportional effect on BP. Secondly, contribution of labile organic carbon from photochemical processes (Bertilsson and Tranvik 1998) or from phytoplankton activities (Chen and Wangersky 1996) may have dampened the descent in BP, by balancing parts of the microbial consumption of imported 'high quality' organic substrates. Finally, there is the possibility of unknown effects of the laboratory treatment, because the bioassay samples were filtered for removal of larger organisms, oxygenated, and temperature-controlled.

Table 2. Dissolved organic carbon (DOC), total phosphorus (TP), total nitrogen (TN) and water temperature at the three sampling stations. Mean values of seven sampling dates are shown along with standard deviation in brackets. Shared index letters within columns identify variables not statistically different from each other ($p < 0.05$) as determined by pair-wise comparisons (2-tailed paired t -tests).

Site	DOC (mg C L ⁻¹)	TP (μ g P L ⁻¹)	TN (mg N L ⁻¹)	Temperature (°C)	df
Stream Björnbäcken outlet	17.9(7.9) ^a	24(6) ^a	0.76(0.15) ^a	5.9(3.9) ^a	6
Upper Lake Björntjärnen outlet	14.9(1.7) ^a	22(4) ^a	0.73(0.12) ^a	9.8(7.0) ^b	6
Lower Lake Björntjärnen outlet	15.1(1.2) ^a	23(3) ^a	0.75(0.13) ^a	9.8(6.4) ^b	6

The relationships between aquatic DOC age and BP or BGE (Table 1; Fig. 4) highlight the importance of seasonal and between-lake variations in flushing rates for bacterial growth in unproductive humic waters. On spatial scales, BP can be expected to increase with increasing discharge to lake-volume ratio (i.e., with smaller lakes and larger catchments). However, the position of the lake in the landscape may also be as important. It could be assumed that if the DOC that reaches a certain lake has passed upstream lakes, it has been subject to aquatic processing during a considerably longer time compared to if it was directly channeled via headwater streams. In our study, the headwater Stream Björnbäcken channeled organic carbon with a much higher potential for supporting bacterial growth compared to the Upper Lake Björntjärnen outlet water (Fig. 4a,b). However, there were no clear differences in BP or BGE between the two lakes, which had a similar span of DOC age (Fig. 4). Therefore, our results suggest that the position of the lakes will be of dominant importance only in systems with considerably longer DOC residence times in each lake compared to in the two-lake Björntjärnen systems.

In a seasonal perspective, high-flow events are important as they can replace a significant fraction of the DOC pools with labile DOC of low age. For example, contributing with >50% of the annual DOC export from boreal catchments (Ågren et al. 2007), the snow-melt period alone replaces most of the water and DOC in the vast majority of Swedish lakes (Lindström et al. 2006). Summer and autumn storms represent another major cause of rapid DOC turnover in highly colored boreal systems (Ivarsson and Jansson 1994). High-flow events are not only important due to the resulting flushing rates, but also because the DOC that is exported with high-flow water in boreal areas has a higher share of labile organic forest substrates, which supports higher BP and BGE (Berggren et al. 2009). Consequently, spring flood periods and other high-flow events can be followed by increased BP in humic lakes (Bergström and Jansson 2000). In this study, the aging of DOC during its downstream transport through the lakes resulted in much larger variation in bacterial growth compared to the effects on BP of the seasonal variation in DOC quality of the headwater source. Therefore, flushing rates and their effects on DOC aging can be as important as or even more important for determining lake BP than quality differences in the DOC exported from terrestrial soils.

In an ecosystem perspective, the aquatic DOC age might be of importance for the structure of lake food chains. With sufficient supply of young 'high quality' allochthonous

DOC, bacteria might outcompete phytoplankton for limiting inorganic nutrients and replace phytoplankton as the main channel for entry of energy into the food web (Jansson 1998; Jansson et al. 2007). Thus, lakes with a low aquatic DOC age could be expected to be dominated by heterotrophic food chains.

To conclude, aging of DOC is an important regulator of BP, which means that hydrological temporal and spatial variation in combination with lake size (water renewal time) have large influence on pelagic BP in lakes with high input of terrigenous DOC. The role of BP for energy mobilization in unproductive lakes (Jansson et al. 2007) implies that conditions that determine aquatic DOC age can have considerable spin-off effects on the structure and function of pelagic food webs.

Acknowledgments

We thank Anna Nilsson, Lars-Inge Karlsson and Anders Lövgren for laboratory and field assistance, Fredrik Lidman for mathematical guidance and two anonymous reviewers for constructive criticism. This study was financed by the Swedish Research Council (VR) and the Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning (FORMAS).

References

- ÅGREN, A., M. BERGGREN, H. LAUDON, AND M. JANSSON. 2008. Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshw. Biol.* **53**: 964–972.
- , I. BUFFAM, M. JANSSON, AND H. LAUDON. 2007. Importance of seasonality and small streams for the landscape regulation of dissolved organic carbon export. *J. Geophys. Res. Biogeosci.* **112**: G03003, doi:10.1029/2006JG000381.
- APPLE, J. K., P. A. DEL GIORGIO, AND W. M. KEMP. 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. *Aquat. Microb. Ecol.* **43**: 243–254.
- AZAM, F., T. FENCHEL, J. G. FIELD, R. A. MEYER-REIL, AND T. F. THINGSTAD. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**: 257–263.
- BERGGREN, M., H. LAUDON, AND M. JANSSON. 2007. Landscape regulation of bacterial growth efficiency in boreal freshwaters. *Glob. Biogeochem. Cycles* **21**: GB4002, doi:10.1029/2006GB002844.
- , ———, AND ———. 2009. Hydrological control of organic carbon support for bacterial growth in boreal headwater streams. *Microb. Ecol.* **57**: 170–178.
- BERGSTRÖM, A. K., AND M. JANSSON. 2000. Bacterioplankton production in humic Lake Örtasket in relation to input of bacterial cells and input of allochthonous organic carbon. *Microb. Ecol.* **39**: 101–115.

- , ———, S. DRAKARE, AND P. BLOMQUIST. 2003. Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. *Freshw. Biol.* **48**: 868–877.
- BERTILSSON, S., AND L. J. TRANVIK. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. *Limnol. Oceanogr.* **43**: 885–895.
- BUFFAM, I., H. LAUDON, J. TEMNERUD, C. M. MÖRTH, AND K. BISHOP. 2007. Landscape-scale variability of acidity and dissolved organic carbon during spring flood in a boreal stream network. *J. Geophys. Res. Biogeosci.* **112**: G01022, doi:10.1029/2006JG000218.
- CHEN, W. H., AND P. J. WANGERSKY. 1996. Rates of microbial degradation of dissolved organic carbon from phytoplankton cultures. *J. Plankton Res.* **18**: 1521–1533.
- COLE, J. J., S. FINDLAY, AND M. L. PACE. 1988. Bacterial production in fresh and saltwater ecosystems—a cross-system overview. *Mar. Ecol. Prog. Ser.* **43**: 1–10.
- DANIEL, C., K. GUTSEIT, A. M. ANESIO, AND W. GRANÉLI. 2005. Microbial food webs in the dark: Independence of lake plankton from recent algal production. *Aquat. Microb. Ecol.* **38**: 113–123.
- DEL GIORGIO, P. A., AND J. J. COLE. 1998. Bacterial growth efficiency in natural aquatic systems. *Annu. Rev. Ecol. Syst.* **29**: 503–541.
- EILER, A., S. LANGENHEDER, S. BERTILSSON, AND L. J. TRANVIK. 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Appl. Environ. Microbiol.* **69**: 3701–3709.
- GRANÉLI, W., M. LINDELL, AND L. TRANVIK. 1996. Photo-oxidative production of dissolved inorganic carbon in lakes of different humic content. *Limnol. Oceanogr.* **41**: 698–706.
- GROSSART, H. P., AND H. PLOUG. 2001. Microbial degradation of organic carbon and nitrogen on diatom aggregates. *Limnol. Oceanogr.* **46**: 267–277.
- HESSEN, D. O. 1992. Dissolved organic carbon in a humic lake—effects on bacterial production and respiration. *Hydrobiologia* **229**: 115–123.
- IVARSSON, H., AND M. JANSSON. 1994. Temporal variations in the concentration and character of dissolved organic matter in a highly colored stream in the coastal zone of northern Sweden. *Arch. Hydrobiol.* **132**: 45–55.
- JANSSON, M. 1998. Nutrient limitation and bacteria–phytoplankton interactions in humic lakes, p. 177–196. *In* L. J. Tranvik and D. O. Hessen [eds.], *Aquatic humic substances: Ecology and biogeochemistry*. Springer-Verlag.
- , A. K. BERGSTRÖM, P. BLOMQUIST, AND S. DRAKARE. 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology* **81**: 3250–3255.
- , ———, ———, A. ISAKSSON, AND A. JONSSON. 1999. Impact of allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Ötrasket. *Arch. Hydrobiol.* **144**: 409–428.
- , ———, S. DRAKARE, AND P. BLOMQUIST. 2001. Nutrient limitation of bacterioplankton and phytoplankton in humic lakes in northern Sweden. *Freshw. Biol.* **46**: 653–666.
- , L. PERSSON, A. M. DEROOS, R. I. JONES, AND L. J. TRANVIK. 2007. Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends Ecol. Evol.* **2**: 316–322.
- JONES, R. I. 1992. The influence of humic substances on lacustrine planktonic food-chains. *Hydrobiologia* **229**: 73–91.
- JONSSON, A., M. MEILI, A. K. BERGSTRÖM, AND M. JANSSON. 2001. Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ötrasket, N. Sweden). *Limnol. Oceanogr.* **46**: 1691–1700.
- KALFF, J. 2002. *Limnology: Inland water ecosystems*. Prentice-Hall.
- KARLSSON, J., M. JANSSON, AND A. JONSSON. 2002. Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes. *Ecology* **83**: 2902–2910.
- KRITZBERG, E. S., J. J. COLE, M. M. PACE, AND W. GRANÉLI. 2006. Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: Results from whole-lake C-13 addition experiments. *Ecosystems* **9**: 489–499.
- LAUDON, H., AND K. H. BISHOP. 1999. Quantifying sources of acid neutralisation capacity depression during spring flood episodes in Northern Sweden. *Environ. Pollut.* **105**: 427–435.
- , S. KÖHLER, AND I. BUFFAM. 2004. Seasonal TOC export from seven boreal catchments in northern Sweden. *Aquat. Sci.* **66**: 223–230.
- , V. SJÖBLOM, I. BUFFAM, J. SEIBERT, AND M. MÖRTH. 2007. The role of catchment scale and landscape characteristics for runoff generation of boreal streams. *J. Hydrol.* **344**: 198–209.
- LENNON, J. T., AND L. E. PFAFF. 2005. Source and supply of terrestrial organic matter affects aquatic microbial metabolism. *Aquat. Microb. Ecol.* **39**: 107–119.
- LINDSTRÖM, E. S., M. FORSLUND, G. ALGESTEN, AND A. K. BERGSTRÖM. 2006. External control of bacterial community structure in lakes. *Limnol. Oceanogr.* **51**: 339–342.
- MCCARTHUR, M. D., AND J. S. RICHARDSON. 2002. Microbial utilization of dissolved organic carbon leached from riparian litterfall. *Can. J. Fish. Aquat. Sci.* **59**: 1668–1676.
- MCKNIGHT, D. M., E. W. BOYER, P. K. WESTERHOFF, P. T. DORAN, T. KULBE, AND D. T. ANDERSEN. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol. Oceanogr.* **46**: 38–48.
- MORAN, M. A., AND J. S. COVERT. 2003. Photochemically mediated linkages between dissolved organic matter and bacterioplankton, p. 243–262. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems: Interactivity of dissolved organic matter*. Academic Press.
- , AND R. E. HODSON. 1990. Bacterial production on humic and nonhumic components of dissolved organic carbon. *Limnol. Oceanogr.* **35**: 1744–1756.
- , AND ———. 1994. Support of bacterioplankton production by dissolved humic substances from 3 marine environments. *Mar. Ecol. Prog. Ser.* **110**: 241–247.
- PERDUE, E. M. 1998. Chemical composition, structure, and metal binding properties, p. 41–61. *In* D. O. Hessen and L. J. Tranvik [eds.], *Aquatic humic substances*. Springer-Verlag.
- RAYMOND, P. A., AND J. E. BAUER. 2001. Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. *Nature* **409**: 497–500.
- RODHE, A. 1989. On the generation of stream runoff in till soils. *Nordic Hydrol.* **20**: 1–8.
- SIMON, M., AND F. AZAM. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* **51**: 201–213.
- SMITH, D. C., AND F. AZAM. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. *Mar. Microb. Food Webs* **6**: 107–114.
- TRANVIK, L. J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microb. Ecol.* **16**: 311–322.
- . 1998. Degradation of dissolved organic matter in humic waters by bacteria, p. 259–283. *In* L. J. Tranvik and D. O. Hessen [eds.], *Aquatic humic substances: Ecology and biogeochemistry*. Springer-Verlag.

WEISHAAR, J. L., G. R. AIKEN, B. A. BERGAMASCHI, M. S. FRAM, R. FUJII, AND K. MOPPER. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* **37**: 4702–4708.

WEISS, M., AND M. SIMON. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquat. Microb. Ecol.* **17**: 1–12.

WETZEL, R. G. 2001. *Limnology: Lake and river ecosystems*, 3rd ed. Academic Press.

Associate editor: Peter G. Verity

Received: 16 September 2008

Accepted: 16 March 2009

Amended: 26 March 2009