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Direct measurement of the $\delta^{13}C$ signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism, and CO_2 fluxes

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Abstract

Using a novel method to measure the isotopic signature ($\delta^{13}C$) of respiratory CO_2 produced by bacterioplankton, we determined the proportion of terrigenous vs. algal-derived organic carbon (OC) respired by bacteria in a series of eight lakes in southern Québec (Canada). The lakes are located within the same general basin but span a large range in trophic status, morphometry, and dissolved OC (DOC) concentrations. Isotopic $\delta^{13}C$ values of respired CO_2 ranged from −28.4‰ to −32.5‰ across a gradient of lakes and streams. These values were compared with those of potential OC sources within the lakes (terrigenous and algal) using a mass balance model. The proportion of terrigenous OC respired varied from 3% to >70% and was strongly negatively correlated to lake chlorophyll *a* (Chl *a*) concentrations and weakly positively correlated to DOC : Chl *a* concentrations. While both total plankton and bacterial respiration (BR) increase with lake Chl *a* concentration, the component of BR that is supported by terrigenous OC, which ranges from 0.7 to 1.7 $\mu g\ C\ L^{-1}\ h^{-1}$, stays essentially constant along the trophic gradient, increasing only slightly with DOC concentration. There is a relatively constant baseline BR supported by terrigenous OC, which becomes diluted by the BR of algal OC as the lakes become more productive. The estimated production of CO_2 through BR of terrigenous OC in the epilimnion explains on average 60% of the estimated air–water CO_2 flux calculated for these lakes, suggesting that the processing of allochthonous OC by bacteria is a major component of this flux.

Lakes, and aquatic ecosystems in general, process organic carbon (OC) from a wide variety of sources. These sources may include carbon derived from internal primary production (e.g., phytoplankton, macrophytes) and external OC inputs from the surrounding wetlands and terrigenous ecosystems (Tranvik and Jansson 2002; Pace and Prairie 2005). Multiple lines of evidence suggest that a substantial proportion of these wetland and terrigenous inputs of OC is processed within lakes and rivers and fuels a variable but often significant portion of the total system respiration (del Giorgio et al. 1999; Cole et al. 2002; Pace and Prairie 2005). For example, the net heterotrophy that has been reported for many temperate and boreal lakes and reservoirs, where respiration exceeds gross primary production, can be achieved only if respiration is subsidized by the metabolism of OC derived from the catchment (del Giorgio et al. 1999; Cole et al. 2000). Organic carbon mass balances have further demonstrated that there is significant loss of terrigenous OC loaded into lakes, through a combination of sedimentation, photochemical degradation, and biological consumption (Molot and Dillon 1997; Tranvik and Jansson 2002; Algesten et al. 2004). More recently, whole lake ^{13}C enrichments coupled with C cycling models have shown that a large fraction of total ecosystem respiration, of which most is bacterial, may be derived from allochthonous OC sources (Cole et al. 2002, 2007).

The evidence that terrigenous OC is consumed in lakes and rivers is unequivocal, but because this evidence is derived primarily from indirect approaches (production : respiration measurements, carbon mass balances, air–water gas dynamics, isotope addition experiments, models), the proportion of total bacterial respiration (BR) in the water column of lakes and streams that is actually fueled by these external inputs remains to be empirically determined. Thus, although we know in general that (1) most lakes receive allochthonous organic inputs; (2) at least a portion of this terrigenous OC is respired, primarily by bacteria; and (3) the relative importance of this terrigenous OC supply to lake metabolism likely varies as a function of lake trophy, we still do not know the actual proportion of the various sources of OC that fuel BR in lakes at any given time, nor do we know how this proportion might vary over a seasonal cycle or across a gradient of lake and landscape types. Our inability to directly quantify the relative importance of the sources of OC respired by aquatic heterotrophic bacteria represents a major gap in our understanding of the physiological and environmental factors regulating C cycling across multiple time and space scales.

At the ecosystem level, the processing and ultimate respiration of terrigenous OC influences multiple processes,

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among the most important of which are lake : atmosphere CO_2 fluxes. Many lakes and rivers are characterized by persistent CO_2 supersaturation (Richey et al. 2002; Jones et al. 2003; Algesten et al. 2004), which results in a significant carbon efflux in both tropical and boreal systems (Richey et al. 2002; Algesten et al. 2004). The partial pressure of CO_2 (pCO_2) in lakes and streams determines in part the role of these ecosystems in regional carbon budgets and is influenced by both external (i.e., groundwater dissolved inorganic carbon [DIC], soil-terrigenous DIC) and internal (benthic and planktonic respiration, photochemical production) processes (Kling et al. 1991; Graneli et al. 1996). Lake pCO_2 and C flux to the atmosphere have been shown to be correlated to the dissolved OC (DOC) loading and concentration in lakes (Prairie et al. 2002; Sobek et al. 2003; Sobek et al. 2005), suggesting a strong connection between CO_2 supersaturation and the processing of allochthonous OC. Whether this processing is primarily photochemical or biological (i.e., through bacterial decomposition and ultimately respiration) is still a matter of contention (Graneli et al. 1996; Molot and Dillon 1997; Jonsson et al. 2001). Biologically induced CO_2 supersaturation can only be maintained through the respiration of significant amounts of terrigenous OC. By quantifying the actual rates of bacterial CO_2 production that are supported by terrigenous OC and comparing these rates to the ambient CO_2 flux from these systems, we can begin to resolve the role of bacterial processing of terrigenous OC on lake–atmosphere CO_2 exchange.

This paper is the first attempt to fill this major gap by directly measuring the isotopic signature of bacterial respiratory CO_2 and using this to quantify the proportion of BR that is supported by terrigenous and autochthonous sources in a series of temperate lakes and rivers. Using a newly developed respiratory carbon recovery system (ReCReS) and operating protocol described in McCallister et al. (2006*b*), we have measured the $\delta^{13}C$ isotopic signature of bacterial respiratory CO_2 from a range of lakes and streams in southern Québec. Our objectives were severalfold: (1) to determine the proportion of terrigenous vs. algal OC respired by bacteria and assess how this proportion varies with lake trophy and DOC concentrations; (2) to apportion the total BR that is based on algal vs. terrigenous substrates and to assess how these two key components of total respiration vary among lakes; and (3) to quantitatively establish the potential influence of BR of allochthonous OC on pCO_2 levels and potential C fluxes across the air–water interface.

Materials and methods

Study sites—We sampled the surface waters (0.5 m) of eight lakes and two streams in the Eastern Townships region of Québec, Canada, approximately 100 km southeast of Montréal (45°50′N, 73°58′W). The lakes are dominated by a mix of green algae, diatoms, and cyanobacteria with crysophytes, dinoflagellates, and other mixotrophic flagellates comprising less than 20% of the total biomass (B. Beisner pers. comm.). The watersheds of each lake are dominated by temperate mixed wood forest and low-intensity agriculture and underlain by the sedimentary geology of the St. Lawrence Lowlands. These specific lakes and streams are located primarily within the same drainage basin, chosen such that the major watershed characteristics (i.e., forest and soil types, hydrology and groundwater flow, climate, DOC aromaticity) would remain constant between the sites and only the trophic status of the lakes would vary significantly (chlorophyll *a* [Chl *a*] 1.5–6.9 $\mu g\ L^{-1}$; Table 1). Thus the nature of the allochthonous watershed-derived C should remain comparatively uniform throughout the lakes, although DOC concentrations themselves varied.

Experimental approach—The general approach for this work was to recover the CO_2 produced by bacteria during in vitro incubations for isotopic ($\delta^{13}C$) analysis. The

Table 1. Physical, chemical, and biological data for eight southern Quebec lakes and two streams sampled for this study. Chemical and biological data are from the mixed layer on the date sampled.

Water body	Date	TP ($\mu g\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	TR* ($\mu g\ L^{-1}\ h^{-1}$)	Mixed layer depth (m)	Water retention (yr)	Surface lake (km^2)	Respiratory CO_2 $\delta^{13}C$ (‰)
Fraser Stream	21 Sep 04	21.1	—	6.1	2.7	—	—	—	−29.1
Brome	20 May 04	18.5	5.7	3.4	3.9	12.5	1.0	12.4	−28.9
Bran-de-Scie	03 Aug 04	17.9	6.5	6.1	4.8	8.5	<0.1	0.1	−32.2
Bran-de-Scie	31 Aug 04	16.7	5.1	7.5	4.7	8.3	<0.1	0.1	−32.5
Des Monts	07 Sep 04	12.3	2.5	7.4	3.2	6.0	<0.1	0.3	−28.9
Magog	06 Jun 04	11.3	3.6	3.5	3.3	8.5	0.1	11.5	−29.1
Fraser	10 Aug 04	11.2	6.9	5.9	—†	17.5	0.4	1.6	−31.9
Fraser	27 Sep 04	11.2	1.5	6.9	1.9	19.0	0.4	1.6	−28.4
Bran-de-Scie Stream	21 Sep 04	10.6	—	4.9	2.0	—	—	—	−29.2
Des Monts	13 Jun 04	10.1	4.2	5.7	4.7	6.7	<0.1	0.3	−30.0
Simoneau	04 Jul 04	8.3	2.0	4.5	2.4	24.0	0.4	0.5	−29.2
Stukely	14 Sep 04	6.5	2.5	5.0	2.7	24.0	4.0	4.0	−28.5
Bowker	14 Sep 04	5.8	2.0	2.8	2.0	61.8	9.0	2.5	−29.2
Stukely	20 Jul 04	5.7	1.9	4.2	2.9	28.6	4.0	4.0	−28.9

* Total respiration (TR) as determined by oxygen measurements (*see* Methods).
† Dash designates values that are not applicable or were lost during analysis.

isotopic signature was then compared with potential sources, namely terrigenous- and algal-derived OC, to estimate the relative contribution of each to the respired carbon using a two-source mixing model. Although the $\delta^{13}C$ signature of terrigenous OC is relatively invariant (−27‰, Boschker and Middelburg 2002), the signature of algae is more variable and difficult to assess, and we have devoted considerable effort to effectively constrain this component using several alternative approaches, including the determination of the isotopic signature of zooplankton, DIC, particulate OC (POC), and of the lipid fraction extracted from the POC. In the case of DIC, zooplankton, and POC, we analyzed the samples for both $\delta^{13}C$ and $\Delta^{14}C$ to obtain a more robust index of the algal signature. In parallel incubations we measured total plankton respiration, bacterial growth efficiency, and DOC lability for all the samples for which we investigated the sources of respiratory carbon, in order to determine possible connections between the sources of OC used by bacteria and key aspects of bacterial carbon metabolism. In this paper we focus on the nature of the respiratory CO_2 and its connection to lake trophy, total water column respiration, and lake CO_2 dynamics. We describe each of these methods in the sections below.

Sample collection and processing—Lakes and streams were sampled at least once during summer 2004, with a subset revisited. Throughout sample collection and processing all storage bottles and other materials (forceps, filters, etc.) that contacted the samples were precombusted (525°C for 4 h) and stored in baked aluminum foil and airtight plastic bags prior to use.

Zooplankton biomass (lakes only) and water samples were collected with a diaphragm pump connected to an acid-rinsed (10% HCL) plastic hose at a depth of 0.5–1.0 m. Zooplankton samples were collected by passing a minimum of 200 liters through a screen of 50-μm mesh size. Zooplankton were washed from the screens into precombusted (525°C for 4 h) bottles and stored in deionized (DI) water at 4°C until processing in the laboratory (*see* below).

Approximately 60-liter lake water samples were taken at a depth of 0.5–1.0 m, stored in acid leached (10% HCl) Nanopure-rinsed polycarbonate bottles, and returned to the lab within 2 h of collection. A portion of unprocessed water (8 liters) was set aside for Chl *a*, inorganic nutrients, and bacterial parameters (*see* below). The remaining water sample was then filtered sequentially through a combusted (525°C for 4 h) Millipore AE glass fiber filter (1.0 μm nominal pore size) and an in-line Gelman filter capsule (0.2 μm) to remove particulates and bacteria, respectively. The latter was used because previous work has shown no detectable DOC leaching from these capsules (McCallister unpubl. data). AE filters were saved for particulate analysis, sealed in precombusted foil (525°C for 4 h) and frozen at −80°C until isotopic and lipid analysis, performed within 1 yr of sample collection. The filtered water samples for $\Delta^{14}C$ (300 mL) of DIC and $\delta^{13}C$ of DIC and DOC were poisoned with $HgCl_2$ and analyzed ($\delta^{13}C$) or extracted ($\Delta^{14}C$) within a week of collection (*see* below for analytical details). The remaining filtrate was split, with 15 liters allocated for metabolic measurement (respiration, *see* below) and the 45 liters used to determine the $\delta^{13}C$ of respiratory CO_2 following McCallister et al. (2006*b*).

Respiratory carbon recovery system (ReCReS)—The ReCReS and operating procedure was designed to reduce background DIC values by >98% and then quantitatively recover the CO_2 derived from BR after incubation of freshwater samples (McCallister et al. 2006*b*). The two-component ReCReS consists of an airtight incubation system (20 liters), for both short-term (1–3 d) and long-term (>7 d) regrowth incubation of filtered water samples reinoculated with ambient bacteria, and a harvest system to recover the respiratory CO_2 produced during these incubations. The multistep operating procedure involves the following: (1) filling of incubation system with 0.2-μm filtered sample water; (2) addition of 1 N HCl (pH to ~2.8); (3) sparge with ultra–high-purity (UHP) He to remove DIC; (4) sparge with UHP, volatile organic carbon-free, CO_2-free air to replenish oxygen; (5) neutralization (1 N carbonate free NaOH), reinoculation with the ambient bacterial assemblage and incubation (60–132 h); (6) acidification to pH 2.8; and (7) UHP He sparge for >12 h (McCallister et al. 2006*b*). The evolved CO_2 is sent through two water traps (dry ice slurries) prior to cryogenic trapping in liquid N_2 in a removable dual coil-stainless steel ReCReS trap (details in McCallister et al. 2006*b*). Control incubations were processed concurrently to evaluate extraction efficiencies, potential methodological contamination, and fractionation artifacts. Samples were analyzed for $\delta^{13}C$ as described below within 3 d of CO_2 collection. Preliminary experiments (McCallister et al. 2006*b*) showed little fractionation of respiratory CO_2 relative to the source OC, which is consistent with the conclusions of Hullar et al. (1996), and we have thus not corrected our values.

Sample preparation and isotopic analyses

POC, extracted lipids, and zooplankton—Filters for stable isotope analyses of POC (*see* above for collection details) were thawed, dried at 45°C, acid fumed overnight with HCl, and subsequently dried again at 45°C.

In brief, lipids were extracted from POC samples by accelerated solvent extraction (Dionex ASE 200) using $MeCl_2 : MeOH$ (2 : 1) and partitioned following the procedures of Bligh and Dyer (1959). The aqueous fraction was subsequently reextracted with hexane and the organic fractions combined. The combined organic phases were refrigerated overnight over predried (450°C, 4.5 h) anhydrous Na_2SO_4. The lipid extracts were concentrated to 1 mL, and approximately 50 μL transferred to smooth-walled tin sample cups and blown to dryness under N_2.

Upon return to the lab, zooplankton were stored overnight (4°C) in DI water to void their gut contents prior to isotopic analysis. Previous studies have shown isotopic differences between major zooplankton groups due to differences in diet and, in particular, to their preference for algae, which leads to a more depleted signature. Therefore, rather than combining all zooplankton together,

they were hand picked and separated into Cladocerans and Copepods, and over 100 individuals were collected in each category. Cladocerans were dominated by the genus Daphnia, most notably by *Daphnia mendotae* and *Daphnia catawba*, while copepods were dominated by *Diacyclops bicuspidatus*, *Mesocyclops edax*, and *Letptodiaptomus minutus*. Zooplankton samples for $\delta^{13}C$ (n = 11) and $\Delta^{14}C$ (n = 4) analysis were collected in smooth-walled tin capsules, acidified with 10% HCl, and dried overnight (45°C) prior to analysis. For the purpose of our study, we used the isotopic signature of whichever fraction was the most negative (i.e., algal) for any particular lake.

Stable carbon isotope ratios for POC, extracted lipids, and zooplankton were measured using FinniganMAT Delta^{plus} dual-inlet continuous flow isotope ratio mass spectrometer (IRMS) with on-line sample combustion (G.G. Hatch Lab, University of Ottawa). Selected samples were assessed for analytical precision and run in duplicate (relative standard deviation <0.3‰). Isotopic fractionation of lipids relative to food source and total cellular biomass has been reported to range between 3‰ and 4‰ (i.e., McConnaughey and McRoy 1979; Tieszen et al. 2004; Bouillon and Boschker 2006). We have used an intermediate value of 3.7‰, appropriate for aquatic organisms (Bouillon and Boschker 2006), to correct our data.

Respiratory CO_2—The recovered CO_2 was extracted from ReCReS traps, expanded on a vacuum extraction line, stripped of residual moisture, and quantified manometrically (Baratron, 0.5 μmol sensitivity) prior to transfer to break-seals (Hatch Lab; *see* McCallister et al. 2006*b* for details). Break-seals for $\delta^{13}C$ analysis were transferred into Exetainers and run in continuous flow via GasBench peripheral (Thermo Finnigan) interfaced to an isotope ratio mass spectrometer delta XP (Thermo Finnigan) with an analytical precision of 0.10‰.

$\delta^{13}C$ analysis of bulk DIC and DOC—Isotopic analysis ($\delta^{13}C$) of DIC and DOC were performed on a modified OI Analytical model 1010 wet oxidation total organic carbon (TOC) analyzer interfaced with a Finnegan MAT Delta Plus IRMS with a Conflo III continuous flow interface, as detailed by St-Jean (2003). In brief, addition of phosphoric acid converted DIC into CO_2. Sodium persulfate was subsequently added to oxidize DOC to CO_2. $\delta^{13}C$ of the evolved CO_2 was measured with a typical analytical precision of <0.20‰.

Results for stable isotope values are reported in standard δ notation as

$$\delta^{13}C = \left(\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right) \times 10^3 \qquad (1)$$

where R is $^{13}C:^{12}C$.

Radiocarbon ($\Delta^{14}C$)—Subsamples of acidified POC and zooplankton were transferred to combusted (500°C) quartz tubes (6 mm diameter), sealed under vacuum, and combusted at 900°C using a CuO : Ag metal catalyst to CO_2 (Sofer 1980). Samples for $\Delta^{14}C$ DIC were acidified using 85% H_3PO_4. The sample was sparged using UHP N_2 gas; the evolved CO_2 was collected cryogenically; purified on a vacuum extraction line at G.G. Hatch Lab, University of Ottawa; and collected in a break-seal tube for $\Delta^{14}C$ analysis. The CO_2 from break-seals was subsequently reduced to graphite in an atmosphere of H_2 over cobalt catalyst (Vogel et al. 1987). Graphite targets were analyzed at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. $\Delta^{14}C$ is defined as the (per mil) deviation of a sample from the ^{14}C activity of a 1950 standard, corrected for fractionation using the sample $\delta^{13}C$ (Stuiver and Polach 1977). Total measurement uncertainties for $\Delta^{14}C$ analyses of these samples were typically ±5‰.

Analytical measurement—DIC and DOC were determined using a wet oxidation OI 2010 TIC : TOC analyzer. Analytical precision was determined from multiple injections (n = 3) per sample and ranged from ±0.003 to 0.08 ppm for the low and high range of concentrations, respectively. TP was measured in a FlowSolution OI autoanalyzer, following persulfate digestion. Chl *a* was measured spectrophotometrically from hot ethanol extracts. Alkalinity was measured by Gran titration; pH was measured with a VWR SB301 meter.

Plankton respiration rate measurements—We used the protocol developed by del Giorgio et al. (2006) to determine total plankton respiration in parallel incubations to those carried out in the ReCReS systems. Approximately 10 liters of unfiltered water from each site were used to fill a 4-liter acid-washed Erlenmeyer flask and a 4-liter Cubitainer bag. The bag was placed on a stand and connected by acid-washed Tygon tubing to a lower flask and a siphon established between the two. A Tygon tube allowed sample water to be removed from the bottom flask by gravity, the flow regulated by a pinch-cock valve. These flow-through systems were held in the dark in a constant environment chamber at ambient field temperature. At each sampling time, ~25 mL water sample was collected by opening the two pinch-cock valve, thereby allowing water to gently flow out under gravity to determine BR as described below.

Triplicate water samples for oxygen determination were removed at time zero and then every 2 h for 6 h, poisoned with 8 μL saturated $HgCl_2$ solution, and stored immersed under water prior to analysis by membrane-inlet mass spectrometry (*see* del Giorgio et al. 2006 for further details). This method is based on the spectrometric determination of the ratio of argon to oxygen in the sample, (Kana et al. 1994). The average standard error of triplicate oxygen determinations was <2 μg O_2 L^{-1}, which was far below the changes in oxygen concentration in the flasks. The oxygen concentration was derived from this ratio by determining the solubility of argon corrected for salinity and temperature. The rates of oxygen consumption were calculated from the slope of the O_2 vs. time relationship fitted to a least squares regression, and most of the time courses were linear for the length of the 6-h incubation. Rates of oxygen consumption were converted to CO_2 production using a respiratory quotient (RQ) of 1.

Determination of bacterial respiration (BR)—Bacterial respiration (BR) is generally measured as oxygen consumption in samples that have been prefiltered to eliminate other planktonic components. Our previous work has shown that this approach may considerably underestimate total bacterial respiration in the samples (del Giorgio et al. 2006). For this reason, here we have estimated bacterial respiration from the measured total planktonic rates (as described above) by calculating the contribution of nonbacterial components and subtracting these from the total. Algal respiration was first calculated by estimating the average rates of primary production (PP) for each lake based on the average Chl *a* concentrations, using the empirical model provided by del Giorgio et al. (1999) for these same northern temperate lakes and assuming an average algal respiration 25% of PP. Algal respiration generally ranges from 5% to 10% of PB_{max} (the maximum rate of biomass specific photosynthesis) (del Giorgio et al. 2006), so the above assumption yields a generous estimate of algal *R*. We further assumed that metazoan and microzooplankton respiration was equivalent to that of algae, so the total nonbacterial respiration was estimated as 2× algal respiration. The nonbacterial respiration was subtracted from the total measured *R* to derive total BR. del Giorgio et al. (2006) have shown that this approach yields realistic estimates of BR, based on independent approaches.

Calculation of BR fueled by terrigenous DOC (BR_T)—BR_T was calculated by combining the estimated rates of BR, derived as described above, and the percentage of terrigenous OC respired, determined from the ReCReS incubations based on the isotopic signature of the respiratory CO_2, using a two end member (terrigenous and authochthonous) mass balance model, as described in the results section.

Calculation of CO_2 flux originating from BR_T—Ambient surface water pCO_2 (Pa) for each sampling date was determined from pH and alkalinity, with the appropriate corrections for temperature, altitude, and ionic strength, following Cole et al. (1994). Total ambient CO_2 flux was calculated from the average pCO_2 and an assumed wind speed of 3 km h^{-1}, following Prairie et al. (2002). The total CO_2 flux generated by bacterial respiration of terrigenous OC, BR_T, was calculated by multiplying the volumetric estimates of BR_T (estimated as described above) by the mean depth of the epilimnion of each lake (Table 1), to derive a potential CO_2 flux in mmol m^{-2} d^{-1}.

Results

Characteristics of lakes and streams sampled—Chl *a* concentrations in the lakes sampled ranged from 1.5 to 6.9 μg L^{-1} (Table 1). Lake Bran-de-Scie was characterized by consistently elevated Chl *a* concentrations with a mean of 5.8 ± 0.9 μg L^{-1} relative to the more oligotrophic lakes (Chl *a* < 3.0 μg L^{-1}; Table 1). DOC concentrations varied approximately fivefold with a pattern independent of Chl *a* concentration, suggesting a large component of the DOC pool is catchment derived.

Isotopic values of major C pools—The $\delta^{13}C$ isotopic ranges of inorganic and organic carbon pools and recovered respiratory CO_2 are shown in Fig. 1. Measured $\delta^{13}C$ values of DIC from our subset of lakes and streams in the Eastern Township of Québec ranged from the most depleted (−14.1‰) values in streams to the most $\delta^{13}C$ enriched signature of −2.7‰ in lakes with an overall average of −8.2 ± 3‰ (Fig. 1). Of the three species of DIC ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}), the $\delta^{13}C$ $CO_{2(aq)}$ is comparatively $\delta^{13}C$ depleted (on average 9.3 ± 0.3‰ relative to bulk DIC; *see* Fig. 1 legend for calculation) and considered the preferential inorganic C species taken up by freshwater algae (Goericke et al. 1994). We thus use these latter values in the section below as one approach to estimating the algal signature.

Bulk DOC and POC pools are a composite of both terrigenous and algal-derived sources and reflect the balance between the relative inputs and losses (e.g., sedimentation, consumption, photo-oxidation) in these OC sources for a given lake or stream. Terrigenous C_3 plants typically have a $\delta^{13}C$ enriched OC signature of −27‰ (Boschker and Middelburg 2002) relative to the more depleted $\delta^{13}C$ values generally ascribed to freshwater algae. DOC exhibited the most narrow $\delta^{13}C$ range (average −28.8 ± 0.6‰) and was enriched in ^{13}C by approximately 3‰ relative to the particulate fraction (Fig. 1). The depleted $\delta^{13}C$ values of POC (average −30.8 ± 2.0‰) relative to DOC are consistent with a comparatively greater proportion of more negative algal-derived OC in the

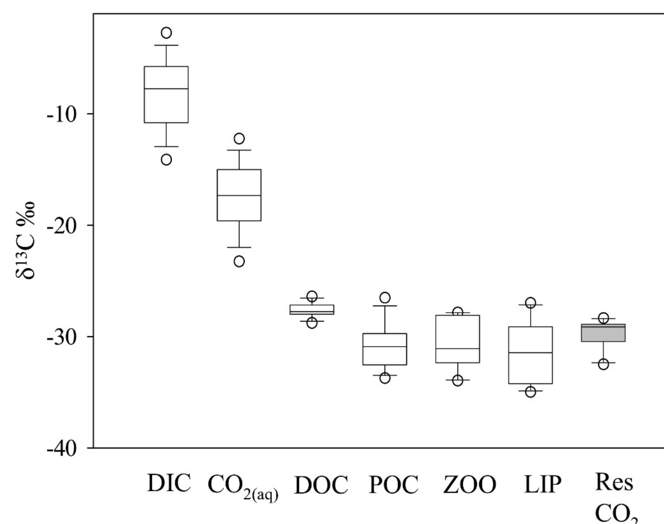

Fig. 1. Box and whisker plot of the $\delta^{13}C$ signatures for inorganic and organic carbon pools from eight lakes and two streams in Southeastern Québec. $\delta^{13}C$ $CO_{2(aq)}$ values are derived from ^{13}C-DIC according to the equations and principles of Mook et al. (1974) and Stumm and Morgan (1996). LIP designates the lipids extracted from POC filter samples (*see* text for details) and are corrected for a 3.7‰ fractionation of lipids relative to total cellular biomass (Bouillon and Boschker 2006). Respiratory CO_2 abbreviated as Res CO_2.

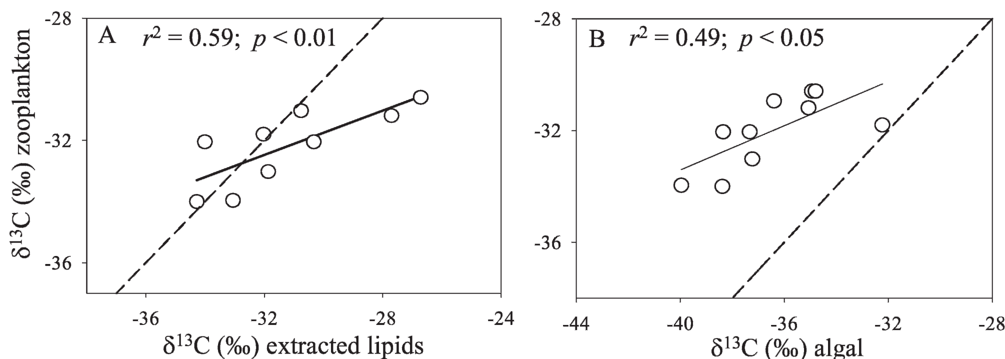

Fig. 2. δ^{13}C of zooplankton as a function of (A) δ^{13}C of extracted lipids from POC and (B) δ^{13}C of algae. Algal δ^{13}C calculated $\delta^{13}C - CO_2$ (*see* Fig. 1 legend) and an assumed 20‰ photosynthetic fractionation. Dashed line is the 1 : 1 line. Lines and regression parameters are the least squares fits to the data.

particulate fraction, an interpretation that is corroborated by lipid biomarker and compound class δ^{13}C analysis (Loh et al. 2006; McCallister et al. 2006*a*).

Isotopic values of respiratory CO_2—The total amount of respiratory CO_2 recovered in the ReCReS experiments ranged from 50 to over 150 μmol C. The δ^{13}C values of respiratory CO_2 in both streams and lakes ranged from −32.5‰ to −28.4‰, with a mean of −29.7 ± 1.4‰ (Table 1). The δ^{13}C values of respiratory CO_2 suggest bacteria must respire a portion of OC derived from both terrigenous and autochthonous OC sources, since the respiratory CO_2 is neither as δ^{13}C enriched as terrigenous sources nor as depleted as proxies for the algal end member (Fig. 2). Prior to apportioning the relative percentages of terrigenous and autochthonous C respired, we first define the algal δ^{13}C signature in the section below.

Constraining the algal $\delta^{13}C$ end member—In this study we have used zooplankton as a proxy for the algal signature, and to assess the robustness of these zooplankton-derived estimates of the algal end member we explore alternate approximations to the algal signature based on the isotopic (δ^{13}C and Δ^{14}C) values of DIC, POC, and extracted lipids (δ^{13}C only). Two independent δ^{13}C approaches were applied: (1) the lipid fraction, which comprises a greater proportion of biomass in algae relative to vascular plants (Killops and Killops 2005), was extracted from the POC (note POC may also contain bacteria) and (2) a −20‰ fractionation was applied to the δ^{13}C of $CO_{2(aq)}$ (Fig. 2B).

The δ^{13}C for the zooplankton and biomass δ^{13}C derived from lipids (*see* Fig. 1 legend for lipid fractionation correction) were in good agreement (mean of −31.8‰ and −30.7‰, respectively) and approximately mirrored those of the POC, with a slight 1–2‰ depletion in the zooplankton δ^{13}C signature relative to the bulk particulate fraction (Fig. 1). Alternatively, the application of a historically accepted 20‰ fractionation by Rubisco yielded comparatively ^{13}C-depleted values for algal biomass in these lakes ranging from approximately −32‰ to −43‰ (Fig. 2B). The zooplankton δ^{13}C varied positively and significantly with both the alternate proxies for algal sources, the δ^{13}C of POC-extracted lipids, which are predominantly algal, and $CO_{2(aq)}$ + assumed fractionation ($r^2 = 0.59$ ($p < 0.01$) and $r^2 = 0.49$ ($p < 0.05$), respectively, Fig. 2). While there was agreement in the pattern of variation of these alternative estimates, there were major discrepancies in the magnitude of the predicted algal signatures based on $\delta^{13}CO_{2(aq)}$ + assumed fractionation and those based on zooplankton, lipid, and POC, with the former being systematically depleted by over 5–10‰ relative to the latter. While it is likely that zooplankton underestimate the true algal signature because there is almost certainly a terrigenous component in the diet of these organisms in most lakes (*see* Pace et al. 2004), an additional explanation for the isotopic discrepancy is that the traditional fractionation value of 20‰ used in many previous studies may overestimate the true fractionation that occurs in situ. The algal signature derived from zooplankton yields a photosynthetic fractionation of −8 to −15‰ relative to $\delta^{13}CO_{2(aq)}$, lending support to recent arguments that algal fractionation may be lower than the generally accepted 20‰ (Cole et al. 2002; Bade et al. 2006).

We further investigated the extent to which zooplankton selectively removed the algal components of the bulk POC pool by combining measurements of Δ^{14}C with δ^{13}C to allow better differentiation of terrigenous and algal OC sources in freshwater given the greater dynamic range of Δ^{14}C (−1,000‰ to +475‰) vs. δ^{13}C (−40‰ to −26‰). POC displayed the widest range and most Δ^{14}C enriched values with a mean of +117 ± 38‰ and a range from +74‰ to +179‰ (Fig. 3). The more Δ^{14}C enriched POC values reflect the input of bomb carbon most likely in the form of modern photosynthetically fixed vascular plant OC, which was stored in soils and marshes for decades prior to its export to the aquatic environment. In contrast to POC, DIC was significantly ($p < 0.0005$) Δ^{14}C depleted with a mean of +2 ± 50‰ (Fig. 3), suggesting an older, recycled component in the lake inorganic C pool. Zooplankton were also significantly ($p < 0.001$) Δ^{14}C depleted relative to POC but overlapped completely with the DIC signature (range from +18 ± 16‰, Fig. 3). Δ^{14}C notation includes a correction for δ^{13}C, thereby equating the Δ^{14}C of $CO_{2(aq)}$

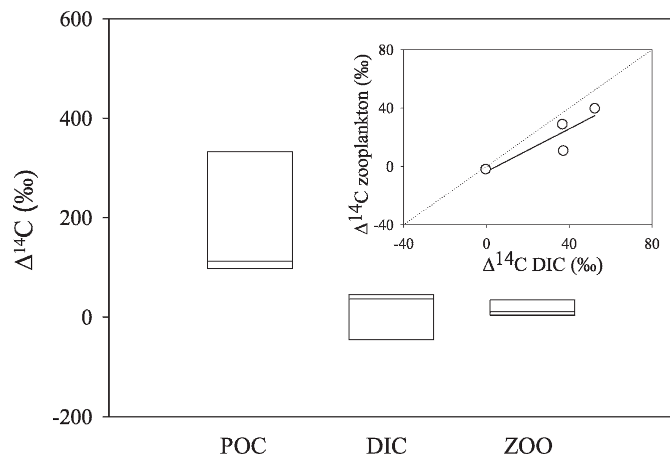

Fig. 3. Box and whisker plot showing the range, upper and lower quartiles, and median (n = 4 to 7) of Δ^{14}C signatures of potential algal proxies. Stream values are excluded. Inset shows Δ^{14}C of zooplankton as function of Δ^{14}C − DIC; the regression line is the least squares fit to the data. Dashed line is the 1 : 1 line.

and DIC (Stuiver and Polach 1977). If we assume an algal signature equivalent to Δ^{14}C-DIC (McCallister et al. 2004), then the similarity of Δ^{14}C in both DIC and zooplankton supports the contention that zooplankton preferentially assimilate algal over detrital (terrigenous) C within the bulk POC (Fig. 3 inset). The isotopic data thus reveal (1) a consistency in the δ^{13}C signature between bulk lipids and zooplankton (Fig. 1); (2) a similar Δ^{14}C isotopic signature in zooplankton and DIC (an algal proxy, Fig. 3); and (3) a significant difference between the signature of both DIC and zooplankton with that of the bulk POC pool ($p <$ 0.001 Fig. 3). The Δ^{14}C data provide strong supplementary evidence for the hypothesis that zooplankton are preferentially removing and assimilating algal-derived carbon over other sources (e.g., terrigenous) in these lakes. Consequently, we adopt zooplankton (δ^{13}C) as an end member for the autochthonous signature, acknowledging that this is a conservative estimate since zooplankton most likely incorporate some portion of terrigenous OC (*see* below for further discussion).

Estimates of algal and terrigenous OC sources fueling BR and linkages to lake trophy—We used the algal endpoint discussed in the previous section, with a terrigenous OC end member of −27‰ (Boschker and Middelburg 2002), to determine the relative contributions of autochthonous and terrigenously derived OC sources to BR employing a δ^{13}C isotopic, two-source mixing model. The series of two equations and two unknowns is

$$\delta^{13}C_{CO_2} = f_1\delta^{13}C_{Terr} + f_2\delta^{13}C_{Auto} \quad (2)$$

$$f_1 + f_2 = 1 \quad (3)$$

where CO_2 δ^{13}C values are measured from the ReCReS, and f_1 and f_2 are the relative contributions of terrigenous (C_{Terr}) and autochthonous (C_{Auto}) sources to the CO_2 signature, respectively.

The percentage of terrigenous OC respired in lakes ranged from ~3% to 73% with a corresponding autochthonous algal contribution of ~27–97%. Along a relatively narrow gradient in Chl *a* (1.5–6.9 μg L^{-1}) there was a strong negative relationship ($r^2 = 0.72$; $p < 0.0005$) with the percentage of terrigenous OC fueling BR (Fig. 4A), suggesting that in the more eutrophic systems terrigenous OC contributes proportionately less to BR and that these external respiration substrates are quickly diluted by autochthonous sources as lake productivity increases. There was no significant relationship between the relative proportion of BR based on terrigenous OC and the total DOC in lakes. The absence of a link between DOC concentration and the percentage of terrigenous OC respired may result in part because the total DOC is a composite of autochthonous and terrigenous sources, and consequently increases in the total DOC may not reflect increases solely in the terrigenous component. We normalized the DOC concentration to Chl *a* (DOC : Chl *a*) as a means to account for changes in DOC related to primary production, and there was a positive relationship between DOC : Chl *a* and the fraction of terrigenous OC sustaining BR ($r^2 = 0.47$, $p < 0.01$; Fig. 4B), suggesting that in lakes with large external DOC loads terrigenous OC is a greater fraction of total BR. Thus we conclude that foremost the trophic status of lakes, and secondarily the loading of terrigenous DOC, are key factors that determine the

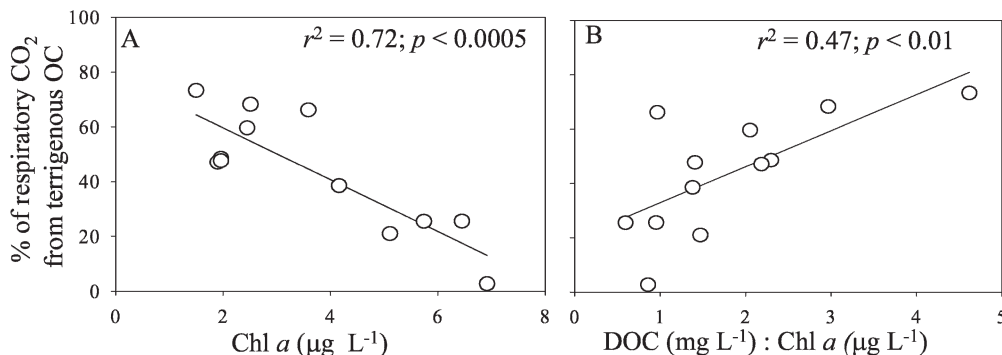

Fig. 4. Percentage of respiratory CO_2 from terrigenous OC as a function of (A) Chl *a* μg L^{-1} and (B) DOC (mg L^{-1})/Chl *a* (μg L^{-1}) in eight lakes from southeastern Québec. Lines and regression parameters are the least squares fits to the data.

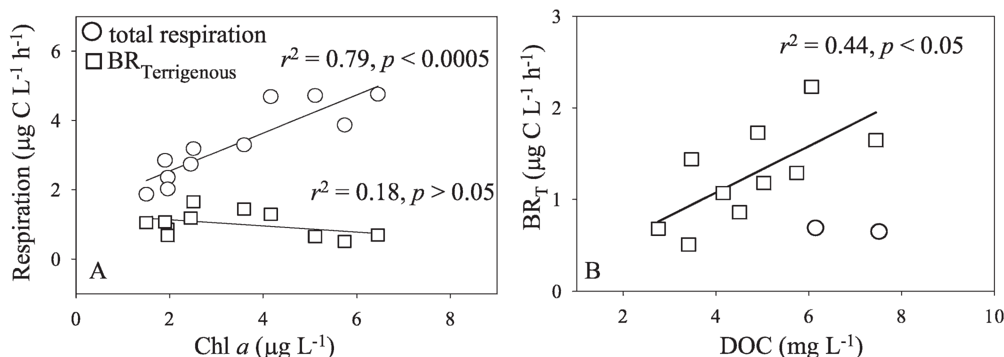

Fig. 5. Total planktonic respiration (μg C L^{-1} h^{-1}) and bacterioplankton respiration (BR) (μg C L^{-1} h^{-1}) derived from metabolism of terrigenous OC sources (BR_T) as a function of (A) Chl *a* (μg L^{-1}) and BR_T as a function (B) DOC (mg L^{-1}). Total BR was derived from our measured total plankton respiration (*see* text for details). Lines and regression parameters are the least squares fits to the data. Open circles in panel B denote samples from eutrophic Bran-de-Scie (*see* text for further discussion) and are not included in the regression.

relative proportion of terrigenous vs. autochthonous OC respired by bacteria, and consequently the metabolic status of lakes.

Partitioning of bacterial respiration into algally and terrigenously derived components—The total plankton *R* measured in parallel to the ReCReS experiments ranged from 1.5 to around 5.0 μg C L^{-1} h^{-1} and increased with Chl *a* concentration (Fig. 5A), a pattern that has previously been shown for these lakes (del Giorgio et al. 1999). The estimated total BR ranged from 1.3 to 3.4 μg C L^{-1} h^{-1} and its contribution to total *R* declined, from oligotrophic lakes, where it accounted for greater than 80% of the total *R*, to the more eutrophic lakes, where it comprised less than 50% of the total *R* (Fig. 5A); this pattern of declining contribution of bacteria to total plankton metabolism along trophic gradients has been described before (Biddanda et al. 2001; Roberts and Howarth 2006). Total BR increased with Chl *a* concentration, although most of this change was due to increases in the BR_A component, which was strongly positively correlated to Chl *a* ($r^2 = 0.88$; $p < 0.0001$; data not shown). BR_T, on the other hand, ranged from 0.5 to 1.7 μg C L^{-1} h^{-1} and had no significant relationship with Chl *a* (Fig. 5A). Rather, BR_T had a weak but significant ($r^2 = 0.44$; $p < 0.05$) relationship with DOC (Fig. 5B). Although DOC concentration in these lakes is largely a function of DOC loading from the catchment, it is also mediated by local processes, such as algal and macrophyte production. In our data set, for example, the only lake that does not fit the BR_T vs. DOC relationship is eutrophic Lake Bran-de-Scie, presumably because a larger portion of the DOC in that lake is of algal (Table 1) rather than terrigenous origin (Fig. 5B, open circles).

Ecosystem implications for the respiration of terrigenous DOC—Lake pCO_2 is strongly influenced, among other things, by primary production, so any potential influence of BR_T on pCO_2 could be completely masked by variations in CO_2 imposed by local production. To account for these local trophic effects we divided the average pCO_2 by the average lake Chl *a*. Figure 6A shows a positive, curvilinear relationship between the percentage of BR that is supported by terrigenous OC and the average pCO_2 per unit Chl *a* for our study lakes. This relationship suggests

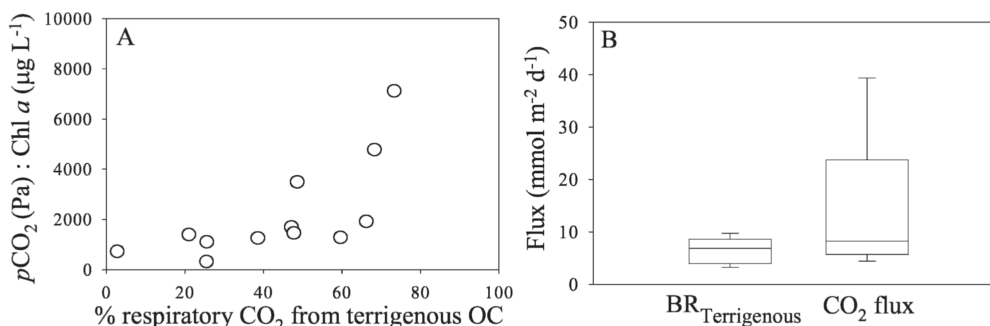

Fig. 6. (A) Ambient surface water pCO_2 (Pa) normalized to Chl *a* (μg L^{-1}) as a function of the percentage of respiratory CO_2 derived from terrigenous OC. (B) Box and whisker plot for estimates of the total carbon flux across the air–water interface calculated from CO_2 concentrations and the portion of CO_2 flux that can be accounted for by bacterial respiration of terrigenous OC sources (*see* Methods for details).

that for any given level of Chl *a*, and thus of local primary production, lakes with a higher proportion of BR based on terrigenous OC tend to have higher pCO_2 levels, suggesting that BR_T does contribute to CO_2 supersaturation in these temperate lakes. Figure 6B shows the average and range for both the CO_2 flux generated by the integrated BR_T and the estimated in situ CO_2 flux for all our study lakes and streams. The two overlap in magnitude, and the flux generated by BR_T accounts from 30% to over 80% (average of 60%) of the estimated in situ flux.

Discussion

Patterns in the relative importance of terrigenous OC to BR—Using the ReCReS we have empirically determined the $\delta^{13}C$ of respiratory CO_2 and have subsequently quantified the relative contribution of terrigenously and autochthonously derived OC to BR in lakes, using a mass balance approach. Further, there are systematic cross-lake patterns in the relative importance of these different sources of OC to lake metabolism. The observed patterns suggest that lake trophy plays a key role in this partition, with terrigenously derived OC as the main substrate for BR in oligotrophic and colored lakes and autochthonously derived OC overwhelmingly dominating BR as lakes become eutrophic (Fig. 4). Our results suggest that terrestrial OC, mostly in the form of DOC, plays a major role in lake metabolism, and yet in our subset of lakes, we found that factors related to primary production (Chl *a*, Fig. 4; TP, data not shown) were the primary drivers of the relative importance of the source (percentage authochthonous vs. terrignenous) of OC respired by bacteria. This presumed discrepancy can be reconciled in part by a consideration of the spatial scale over which this study was conducted. Since most of the lakes are located within the same catchment, they share such common regional and chemical characteristics as land cover, pH, alkalinity, and DOC characteristics. Conceivably, had we sampled lake regions with other catchment properties, such as the lakes in the Canadian Shield north of Montréal, we might have seen a stronger influence of DOC loading, concentration, and basin properties. Thus, it is not too surprising that the main driver determining the relative proportion of autochthonous vs. terrigenous OC respired is the production of algal OC (as indicated by Chl *a*, TP).

Further, total bacterioplankton respiration in lakes may be partitioned into components fueled by distinct OC sources whose magnitude and variability are regulated by very different ecosystem processes. Not surprisingly, the component of BR that is linked to authochthonous production, BR_A, strongly covaries with TP, Chl *a*, and other indices linked to algal primary production. BR_T, on the other hand, tends to covary with DOC concentration (Fig 5B), which itself is a function of drainage basin to lake size, percentage of wetlands in the drainage basin, and other factors linking the lakes to the catchment (Algesten et al. 2004; Pace and Prairie 2005).

More surprising, perhaps, is the almost complete independence of BR_A from BR_T. BR_T is large in relative terms in oligotrophic lakes, accounting for over 60% of total BR, which itself is a substantial portion of total plankton *R*. Consequently, over 50% of total water column respiration may be supported by terrigenously derived OC in unproductive and colored lakes. Although the actual rates of BR_T are of roughly the same magnitude between oligotrophic and eutrophic lakes (Fig 5A), the contribution of terrigenously derived OC to bacterial and total respiration becomes very small as lakes become more productive.

The fact that BR_T is both nontrivial in terms of its magnitude and independent of Chl *a* (Fig. 5B) points to the existence of a significant baseline bacterial metabolism in these temperate lakes, which although linked to the watershed, is largely uncoupled from lake primary production. Results from a long-term incubation (14 d) that we carried out in parallel to the regular ReCReS incubation (<3 d) for Lake Fraser suggest a shift away from the respiration of algal material in the short term ($\delta^{13}C = -28.4‰$; 25% algal) to an isotopic convergence with a terrigenous source in the long term ($\delta^{13}C = -27.3‰$; 5% algal) confirming that a baseline component of bacterial metabolism is supported by catchment-derived sources (McCallister and del Giorgio unpubl.). The concept of aquatic ecosystem respiration as the sum of components that may vary independently from each other, as well as the existence of a baseline ecosystem metabolism that is either weakly connected or completely unconnected to contemporary lake primary production, has been postulated before (Jansson et al. 2003; del Giorgio and Williams 2005).

Links between respiration of terrigenous DOC and lake CO_2 dynamics—There is now overwhelming evidence that most northern temperate and boreal lakes are consistently supersaturated in CO_2 and as a consequence are a significant source of CO_2 to the atmosphere. Substantial evidence has accrued suggesting that the pCO_2 is linked to allochthonous DOC loading and processing within lakes (Prairie et al. 2002; Sobek et al. 2003; Sobek et al. 2005). Since there is usually a tight coupling between the production of OC and its respiration in lakes, biologically induced CO_2 supersaturation can only be maintained through the respiration of significant amounts of terrigenous OC. It follows that if the respiration of terrigenous OC is a source of CO_2 supersaturation, the lakes with the highest relative contribution of terrigenous OC to BR should have the highest CO_2 concentrations. We have used our data to test this hypothesis by comparing the CO_2 fluxes that could be potentially generated by BR_T with the actual air–water CO_2 fluxes that have been estimated for these same lakes and streams (*see* Methods for details). It is important to note that the flux generated by BR_T in streams is most likely dwarfed by the input of CO_2 via groundwater sources, so that comparisons may not apply for these systems. The much greater variability in the estimated in situ flux relative to BR_T would suggest that BR_T may represent a baseline component of aquatic CO_2 supersaturation, which is relatively constant among lakes and upon which are superimposed other processes that also contribute to CO_2 flux, such as photochemical degradation, groundwater DIC injections, littoral and benthic metabo-

lism, and carbonate dissolution. Collectively these additional factors are much more variable than is BR_T among lakes. At the ecosystem scale, these results imply that BR of watershed-derived carbon contributes significantly to CO_2 supersaturation and thus to CO_2 efflux from lakes.

Our results confirm pervious reports that have highlighted the importance of terrestrial OC in shaping lake metabolism (i.e., del Giorgio et al. 1999; Kritzberg et al. 2005; Cole et al. 2007), most notably that of Karlsson et al. (2007), who also used changes in the isotopic signature of bulk DIC to assess the relative importance of terrestrial vs. algal sources in boreal Swedish lakes. These authors used Keeling plots for both in vitro incubations of lake water and lake hypolimnia over summer, as opposed to our approach based on the recovery of respiratory CO_2, and they also showed unequivocal evidence of respiration of significant amounts of terrestrial carbon in these northern Swedish lakes.

Assessment of potential errors—Although the direct approach of isolating and recovering respiratory CO_2 from short-term incubations is an improvement over previous indirect methods, there are still potential problems and biases associated with the approach that must be considered. While we have made an effort to minimize the impact of these issues, our results still need to be placed in the context of potential errors. There are two primary sources of error in our approach. The first arises from the inherent characteristics of the ReCReS incubation and its associated methodological manipulations. The second is more general and results from potential error in the isotopic approximation of the source end members, particularly the algal, for the OC source mass balance. This latter problem is common to all isotopic studies, whether experimental or empirical, and not solely to our approach, but still needs to be considered.

Potential biases from the incubation approach occur initially from filtration and isolation of a parcel of water from its in situ environment (e.g., lack of DOC and nutrient replenishment, removal of particulates, regrowth of different bacterial community) and subsequently from the methodological manipulations required to isolate a pure (bacterial) respiratory CO_2 signature (e.g., pH adjustments). Arguably, of the aforementioned, the greatest potential source of error is likely the decoupling of bacteria in incubations from fresh algal OC, thereby systematically underestimating the algal contribution to BR. We should point out that most experimental determinations of either plankton or bacterial respiration and of long-term carbon consumption require isolation and filtration that results in a decoupling of OC sources. In this regard our experimental approach is not substantially different from most other current approaches. Further, these types of approaches do not typically consider photochemical effects that may enhance the use of terrigenous DOC (McCallister et al. 2005). Incubation times in our experiments typically exceeded 3 d, longer than most conventional respiration measurements (Pace and Prairie 2005), but are required to secure enough respiratory CO_2 to enable both ^{13}C and ^{14}C determinations of the carbon. McCallister et al. (2006*b*) demonstrated that the ReCReS incubations are characterized by a long initial lag phase, but that once the lag phase is overcome, BR and production rates in the ReCReS are similar to those generated by conventional short-term incubations.

The second potential sources of error in our approach are related to the estimation of the $\delta^{13}C$ values for the OC end members necessary for the mass balance. While the ReCReS has enabled a direct measurement of the $\delta^{13}C$ signature of bacterial respiratory CO_2, thereby eliminating potential error related to mass balance determinations, the ability to accurately assess the proportions of terrigenous and algal OC respired remain limited by uncertainties in the isotopic estimates of these end members. In this paper we focused on the direct determination of the respiratory $\delta^{13}C$ signature. Translation of our $\delta^{13}C$ values to percentages of terrigenous and algal C respired through the use of estimates or proxies for these end members, rather than direct determination, leads to potentially large uncertainties in their absolute value (Table 2). Thus, while we have eliminated errors in the determination of the respiratory $\delta^{13}C$ signature, our resolution at the ecosystem level remains constrained by uncertainties in the isotopic signatures of the end members, particularly the algal component.

The $\delta^{13}C$ assignment of $-27‰$ as a terrigenous OC end member is relatively robust with minor (1–2‰) deviations (Lajtha and Michener 1994; Boschker and Middelburg 2002); isotopic shifts that occur with diagenesis will only slightly alter this average signature depending on the origin of the terrigenous OC (i.e., soils vs. surface runoff) (Benner et al. 1987). Variation of the terrigenous isotopic end member from $-26.5‰$ to $-27.5‰$ results in deviations in the mass balance solutions (3–67% and 3–81%, respectively) of the contribution of terrigenous DOC to respiratory CO_2 (Table 2). Thus a 1‰ change in the selection of the terrigenous end member results in solution estimates of the mass balance that range from insignificant to up to 17%, but with no change in the actual patterns described.

The isotopic signature of freshwater phytoplankton, on the other hand, has been traditionally difficult to estimate because algal biomass is typically a small fraction of the total POC (del Giorgio and France 1996) and the isotopic signature may vary more widely. Physically separating and isolating the algal carbon from this complex mix is extremely difficult, especially in oligotrophic lakes, where the ratio of algal OC to miscellaneous detritus is very small. A substantial number of studies have previously demonstrated that freshwater zooplankton selectively assimilate algal carbon from this mixed POC pool (del Giorgio and France 1996; Pel et al. 2003; Feuchtmayr et al. 2004), thereby suggesting that the zooplankton isotopic signature may be a more robust estimate of the algal signature than POC. In this regard, a recent study of sources of organic matter respired in northern Swedish lakes (Karlsson et al. 2007) also used the isotopic signature of zooplankton as a proxy of the authochthonous algal signature.

Our results tend to support this contention. $\Delta^{14}C$ nomenclature is corrected for $\delta^{13}C$ fractionations, and as a consequence the $\Delta^{14}C$ of DIC is equivalent to that of

Table 2. End member uncertainty analysis.

Date	Algal end member uncertainty analysis*				Terrigenous end member uncertainty analysis†			
	Zooplankton-based estimate		Algal fractionation-based estimate		Positive terrigenous (0.5‰ step)		Negative terrigenous (0.5‰ step)	
	Algal end member (‰)	Mass balance (% terrigenous)	Algal end member (‰)	Mass balance (% terrigenous)	Terrigenous end member (‰)	Mass balance (% terrigenous)	Terrigenous end member (‰)	Mass balance (% terrigenous)
20 May 04	−29.6	25	—	—	−26.5	21	−27.5	32
06 Jun 04	−33.3	66	—	—	−26.5	61	−27.5	72
13 Jun 04	−31.8	39	−32.2	44	−26.5	35	−27.5	43
04 Jul 04	−31.2	49	—	—	−26.5	43	−27.5	55
20 Jul 04	−30.6	47	−34.9	76	−26.5	41	−27.5	55
03 Aug 04	−34.0	26	−40.0	60	−26.5	24	−27.5	28
10 Aug 04	−32.1	3	−38.4	57	−26.5	3	−27.5	3
31 Aug 04	−34.0	21	−38.4	51	−26.5	20	−27.5	23
07 Sep 04	−33.0	68	−37.2	81	−26.5	63	−27.5	74
14 Sep 04	−30.6	60	−34.8	81	−26.5	52	−27.5	69
14 Sep 04	−31.2	48	−35.1	73	−26.5	43	−27.5	54
21 Sep 04	—	—	−40.1	83	−26.5	—	−27.5	—
21 Sep 04	—	—	−43.3	87	−26.5	—	−27.5	—
27 Sep 04	−32.1	73	−37.3	87	−26.5	67	−27.5	81

* Terrigenous end member fixed at −27.0‰.
† Zooplankton-based estimate used for algal end member.

algal biomass (Stuiver and Polach 1977; Druffel et al. 1996; Repeta and Aluwihare 2006). Supporting $\Delta^{14}C$ isotopic evidence (Fig. 3) suggests zooplankton are removing carbon that has a similar $\Delta^{14}C$ signature as the bulk DIC pool (e.g., an algal proxy) but that has a very different $\Delta^{14}C$ value (74–179‰) from the bulk POC pool (e.g., large terrigenous component) implying a preferential selectivity of algal OC (Fig. 3). Further, the $\delta^{13}C$ signature of the algae is constrained by both the $\Delta^{14}C$ and $\delta^{13}C$ of the POC and zooplankton. For example, the algae cannot be much more $\delta^{13}C$ depleted than predicted by the zooplankton-based estimate, since this would be inconsistent with the $\Delta^{14}C$ and $\delta^{13}C$ signatures of the POC and zooplankton. In this regard, our data support recent papers that have suggested that algal fractionation is generally less than the historically assumed 20‰. The average fractionation derived from our data is 14‰, which is consistent with these recent reports (Cole et al. 2002; Bade et al. 2006).

The consequence of selecting zooplankton as the algal end member is a potential underestimation of the contribution of terrigenous OC to bacteria. Alternatively, if we instead use the algal estimate based on the isotopic signature of $CO_{2(aq)}$ and an assumed 20‰ fractionation (Fig. 2B), arguably the most negative of the possible algal values, and solve the mixing model, the range of terrigenous OC respired in lakes increases from 3–74% to 19–85% (Table 2). Thus, while on one hand we are most likely underestimating the contribution of terrigenous OC due to the use of zooplankton as a proxy for the algal end member, on the other hand the ReCReS itself imparts a slight bias toward a more terrigenous signal as a result of the incubation (i.e., lack of replenishment). As a result we have biases in both directions, which though difficult to determine quantitatively, would tend to balance each other out and prevent extreme excursions in our estimates of the percentage of OC contribution to BR in either direction (Table 2). We thus acknowledge that there is still some degree of uncertainty in the absolute amount of terrigenous OC respired due to differences in the algal end member selection, but we also emphasize that the patterns across lakes and with lake trophy and DOC that we present here (i.e., Fig. 4) are robust.

Although ample circumstantial and indirect evidence has accumulated in recent years that terrigenous OC is respired by bacteria in most temperate northern lakes, direct evidence of this process has been lacking. Here we have provided direct experimental evidence not only that terrigenous OC is respired in temperate lakes in southern Québec, but also that it represents a major fraction of the OC respired by bacteria in many lakes. Terrigenous OC appears to support a relatively constant level of baseline metabolism in these northern temperate lakes. Further, this baseline activity explains a significant fraction of the CO_2 efflux that has been recorded in these lakes. It is evident that nutrients, particularly phosphorous, strongly influence total ecosystem respiration (Smith and Prairie 2004). What is less apparent is to what extent nutrients, and other factors such as ultraviolet radiation, interact with DOC loading to modulate this baseline metabolism supported by terrigenous OC.

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