Supporting Information

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SI Methods

Isolation of Respiratory CO_2. The Respiratory Carbon Recovery System (ReCReS) was designed to quantitatively recover the CO_2 derived from bacterial respiration after incubation of freshwater samples (1) where the background dissolved inorganic carbon (DIC) values have been reduced by >98%. The two-component ReCReS consists of an airtight incubation system (20 L) for short-term regrowth incubations (2–4 d) of filtered water samples reinoculated with ambient bacteria and a harvest system to recover the respiratory CO_2 produced during these incubations. Extensive preliminary tests confirmed both the quantitative recovery and the isotopic fidelity of the respiratory CO_2 recovered (1, 2). The recovered CO_2 was extracted from ReCReS traps, expanded on a vacuum extraction line, stripped of residual moisture, and quantified manometrically (0.5 mmol sensitivity; Baratron) before transfer to break seals (1/10 $\delta^{13}C$:9/10 $\Delta^{14}C$) (1).

Incubations to Correct for Macrophyte-Derived Organic Carbon to Respiratory CO_2 Signature in Des Monts. To assess the relative bioavailability of terrestrial vs. macrophyte-derived organic carbon (OC), bioassays of single-source leachates were conducted. Soil, litter, and macrophyte samples were collected and leached in triplicate in deionized water for 24 h in the dark at room temperature on a shaker table. The leachate was then passed through a combusted (525 °C for 4 h) Millipore AE glass fiber filter (1.0-mm nominal pore size) to remove particulates. Samples were inoculated with a 10% bacterial innoculum, which consisted of a whole water sample filtered through a combusted Millipore AE glass fiber filter to exclude particles and not the microbial consortium. The leachates were incubated in the dark for 14 d in nonnutrient limiting conditions, and the percent bioavailable dissolved organic C (DOC) was calculated from initial and final DOC concentrations. Approximately 18 ± 3% of the soil/litter-derived DOC was bioavailable compared with 60 ± 5% of macrophyte-derived DOC.

Natural Abundance ($\delta^{13}C$ and $\Delta^{14}C$) Isotopic Analysis. Subsamples of acidified bulk particulate C and lyophilized DOC 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 (3). Samples for $\Delta^{14}C$ DIC were acidified using 85% $H_3PO_4^-$. The sample was sparged using UHP N_2 gas, and the evolved CO_2 was collected cryogenically, purified on a vacuum extraction line at G. G. Hatch Laboratory, University of Ottawa, and collected in a break-seal tube. The CO_2 from break seals was subsequently reduced to graphite in an atmosphere of H_2 over cobalt catalyst (4). Graphite targets were analyzed at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. $\Delta^{14}C$ is defined as the (per million) deviation of a sample from the ^{14}C activity of a 1950 standard corrected for fractionation using the sample $\delta^{13}C$ (5). Total measurement uncertainties for $\Delta^{14}C$ analyses of these samples were typically ±5‰.

SI Results and Discussion

Ranges in Age of Aquatic Respiratory CO_2 Estimated by Indirect Approaches. Measurements of the age of C respired in aquatic systems are scarce compared with measurements from terrestrial systems. This scarcity is, in part, because of much lower respiration rates in aquatic systems relative to terrestrial rates. Furthermore, the bicarbonate buffering system provides the added analytical challenge of discerning small changes in CO_2 superimposed on a much greater background of DIC [$CO_2(aq)$ + HCO_3^- + CO_3^{2-}]. Resolution of the isotopic signature of respiratory CO_2 in aquatic systems is further complicated by internal [$CO_2(aq)$, HCO_3^-, and CO_3^{2-}] and external (aquatic and atmospheric) exchanges and isotopic fractionations (1).

Table S1 presents all of the estimates of the age of C respired in aquatic systems determined by indirect approaches and an overview of regional measurements of the age of C respired in terrestrial systems for comparison. The few studies that have explored the age of C cycling through freshwater systems have focused primarily on bulk inorganic C and OC pools. Using a combined ^{13}C and ^{14}C analysis of the DIC pool, the work by Mayorga et al. (6) suggested that the CO_2 efflux in the lower Amazon basin was supported primarily by OC fixed within the last 5 y. In contrast, mineralization of ancient OC (8,000–16,650 y B.P.) in the riparian zone of streams in the Pawcatuck River watershed (Rhode Island, United States) may have comprised 50–100% of the OC respired in experimental incubations (7).

More recently, studies have been conducted to measure the age of CO_2 evaded from streams and rivers, and they suggest that the C evaded may range from 400 to 2,000 y B.P. (8, 9). However, the age of C evaded is not solely a result of in situ bacterial respiration and may contain contributions from allochthonous inputs of soil CO_2, groundwater DIC, and mineral weathering, which obscure the ability to elucidate the bacterial respiratory component. Collectively, these studies suggest a wide range of potential ages of the OC processed in aquatic systems but have further highlighted major difficulties in determining the age of respired OC fueling the CO_2 flux using indirect approaches, which include additive errors inherent in isotopic mass balances (1, 2, 6, 10) and other confounding factors such as local chemistry (6, 7, 11).

Recent Evidence to Suggest That Old and Stable Organic Matter Is Not Chemically Recalcitrant and Biologically Unavailable. For over a decade, the terrestrial C cycling community has been evaluating the age of C respired in various biomes (12), whereas the direct measurement of the age of respiratory CO_2 in aquatic systems has lagged behind until this study. In part, the disparities of progress in terrestrival vs. aquatic systems are because of the difficulties discussed above. Logically, the age of OC would seem to provide a plausible assessment of its bioavailability given the supposition that OC persists as a result of its chemical recalcitrance. However, a reoccurring theme in terrestrial research (12–15) and a recently emerging theme in aquatic OC cycling (7, 16–19) is the decoupling between age and bioavailabilty as a result of (*i*) mineral association and protection from enzymatic attack (14, 15, 20, 21) and (*ii*) energy limitation with subsequent priming of preaged OC metabolism by inputs of fresh OC (15, 16, 21–25). For example, the work by Caraco et al. (18) recently showed that zooplankton in the Hudson River could be consuming a C source that is between 3,400 and 50,000 y B. P. The priming effect theory, which was initially hypothesized to operate in terrestrial systems (24), was later substantiated with dual isotope tracing experiments that increased deep soil mineralization and released carbon that had been stored for over 2,000 y B.P. (22). Our own direct measurement of 1,000–3,000 y B.P. OC respired in northern lakes provides unmistakable evidence that similar processes may be operating in aquatic systems (21, 23).

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Table S1. Summary of ages of C respired by bacteria in aquatic systems and comparison with soils

Region	System	Age of C metabolized	Method	Ref.
Aquatic				
Amazon River (Brazil)	River (floodplain)	Modern (<5 y)	^{14}C mass balance	1
Spring Creek (United States)	River	Modern (years-decades)	^{14}C-DIC	2*
York River (United States)	River (mainstem)	Modern	^{14}C-DOC from long-term incubations	3
Hudson River (United States)	River (mainstem)	1,000–5,000 y B.P.	Modeled from system metabolic balance	4
Hudson River (United States)	River (mainstem)	Preaged (hundreds-thousands)	^{14}C mass balance of C pools	5
Hudson River (United States)	River (mainstem)	~20,120 y B.P.	Isotopic mixing curves (^{14}C, ^{13}C)	6
Pawcatuck River (United States)	Riparian zone	4,000–16,663 y B.P.	^{14}C-DIC	7
Eastern Township (Canada)	Lakes (epilimnion)	1,000–3,000 y B.P.	In vitro measurement of respiratory CO$_2$	This study
Northern Peatland (United Kingdom)	Streams	850–1,455 y B.P.	^{14}C of evaded CO$_2$	8
Gulf of Alaska (United States and Canada)	Streams (glacial watersheds)	3,900 y B.P.	Regression of percent labile DOC and Δ^{14}C-DOC	9
Arctic (Siberia)	Thermokarst lakes	>35,000 y B.P.	^{14}C of evaded CH$_4$	10
Soils				
Manitoba (Canada)	Boreal soil	30 y	^{14}C of evaded CO$_2$	11
Massachusetts (United States)	Temperate soil	8 y	^{14}C of evaded CO$_2$	11
Eastern Amazonia (Brazil)	Tropical soil	3 y	^{14}C of evaded CO$_2$	11
Massif Central (France)	Deep grassland soil (0.6–0.8 m)	2,570 y B.P.	^{14}C of evolved CO$_2$ from laboratory incubations	12
Langlands Moss (United Kingdom)	Deep Peat (100–400 cm)	975–4,000 y B.P.	In situ passive sampling	13
Arctic	Tundra soil	1,990–4,020 y B.P.	^{14}C-evaded CO$_2$	14

*Buffalo Bayou was excluded as significant. CaCO$_3$ from pedogenic carbonate, crushed limestone/dolomite, and oyster shell contributed to the age of the DIC relative to respiration.

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Table S2. Lake characteristics

Water body	Total nitrogen (mg L^{-1})	pH	Alkalinity (μeq L^{-1})	Maximum depth (m)	Surface lake area (km^2)	Temperature at sampling (°C)
Bran de Scie	0.29	7.3	493	7.6	0.1	20.5
Des Monts	0.33	7.7	402	6.1	0.3	20.9
Stukely	0.16	7.5	320	30.0	4.0	20.9
Bowker	0.10	7.2	236	60.0	2.5	20.3
Fraser	0.25	7.1	303	18.9	1.6	19.8