

Respiration of allochthonous organic carbon in unproductive forest lakes determined by the Keeling plot method

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Abstract

We carried out short-term (2 d) experiments in nine unproductive lakes in northern Sweden in order to investigate organic carbon sources supporting lake water respiration. Surface water was incubated in gas-tight bottles in the dark, and the concentration and isotopic composition ($\delta^{13}\text{C}$) of dissolved inorganic carbon (DIC) were measured at the start and end of the incubations. Keeling plot analyses revealed that the $\delta^{13}\text{C}$ of the respired carbon was between -28.4‰ and -30.6‰ in the lakes and that the respired carbon was mainly of allochthonous organic carbon (AlloOC) origin. The respiration of AlloOC corresponded well with metabolic imbalances indicated by negative net ecosystem production (NEP) values in the lake waters. Keeling plot analysis of DIC accumulating in the hypolimnion of two lakes during summer stratification showed $\delta^{13}\text{C}$ values of around -26.6‰ for excess DIC, implying that the accumulation of DIC was mainly derived from respiration of AlloOC. Our data provide direct evidence that net heterotrophy of these lakes is caused by input and respiration of AlloOC. We conclude that the Keeling plot method is a powerful technique that enables characterization and quantification of the organic carbon sources contributing to respiration in aquatic systems.

The view of lake ecosystems as self-sustaining systems driven by autochthonous primary production (PP) has changed considerably in the last decades through the recognition that allochthonous organic carbon (AlloOC) supports lake food webs. It has been shown that bacteria are able to grow on AlloOC (Tranvik 1988) and, in that way, mobilize energy for secondary production in lakes. However, bacterial production (BP) on AlloOC is inefficient (Biddanda et al. 2001), and considerable amounts of assimilated organic carbon are respired directly by bacteria, in addition to respiration by consumers of bacteria. Input and subsequent respiration of AlloOC cause lakes to be net heterotrophic, in the sense that community respiration (R) exceeds gross PP (GPP < R) and net ecosystem production (NEP = GPP - R) is negative (del Giorgio and Peters 1994). Consequently, ecosystem net heterotrophy implies that the lakes act as a net source of CO_2 to the atmosphere (Cole et al. 1994).

Net heterotrophy can be sustained only if lake community respiration is subsidized by external carbon. Although several studies have indicated that unproductive lakes are

generally net heterotrophic, measures of metabolic balances are not always as straightforward as determinants of respiration of AlloOC within the system. Lakes are not homogeneous systems but may contain habitats that are coupled by allocation of energy and nutrients (Schindler and Scheuerell 2002). Thus, temporal and spatial separation between PP and respiration can, in theory, cause net heterotrophy of the sampled water without respiration of AlloOC. There also exist uncertainties in estimates of metabolic balances, e.g., to what extent measurements of PP and respiration reflect in situ values (del Giorgio and Peters 1994). Also, although low PP:BP ratios stress the importance of AlloOC for lake metabolism (Jansson et al. 2000; Karlsson et al. 2002), this criterion is uncertain due to variation in bacterial growth efficiency (Biddanda et al. 2001). Further, it is unclear to what degree primary production is respired, and, by definition, estimates of NEP represent the minimum respiration of AlloOC in the system.

The most commonly used indicator of net heterotrophy is supersaturation of CO_2 in surface waters of lakes (Cole et al. 1994). The CO_2 saturation value integrates the metabolic processes of the whole ecosystem, and supersaturation is a strong indication that AlloOC is respired in the lake (Cole et al. 1994). However, CO_2 saturation is also affected by processes other than the metabolism in the lake, and the degree of CO_2 saturation cannot be used alone as a measure of the respiration of AlloOC in a lake. Both photolytic degradation of AlloOC (Bertilsson and Tranvik

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2000) and input of CO₂ from the catchment (Riera et al. 1999) can contribute to significant portions of the CO₂ supersaturation. Also, losses of CO₂ via the outlet could cause underestimates of CO₂ production in the lake. The relative contribution of different CO₂ sources in different types of lakes remains unclear. In fact, there exists no direct evidence that respiration of AlloOC is a major source of CO₂ in lake ecosystems.

The Keeling plot method (Keeling 1958) is a powerful tool for determination of the isotopic signature of respired dissolved inorganic carbon (DIC) and characterization of its sources. In this method, the inverse of the increase of DIC over time due to respiration is plotted against the related change in $\delta^{13}\text{C}$ signature of DIC. The method is commonly used in terrestrial biogeochemistry to determine the isotopic composition and carbon sources for ecosystem respiration (Pataki et al. 2003), but few studies exist where the method has been used to investigate $\delta^{13}\text{C}$ of respired carbon in lakes (Miyajima et al. 1997). The aims of our study were to determine the isotopic signature of the organic carbon used for respiration in nine unproductive forest lakes, and to estimate the relative contribution of AlloOC and autochthonous organic carbon (AutoOC) sources to respiration in these lakes. For this purpose, we monitored changes in concentration and $\delta^{13}\text{C}$ of DIC over time in short-term experiments of epilimnion water and in hypolimnion water during summer stratification, and we used the Keeling plot method to estimate the $\delta^{13}\text{C}$ of respired carbon. We compared the results with estimates of metabolic balances and $\delta^{13}\text{C}$ values of organic carbon sources in the lakes.

Materials and methods

Eight lakes in temperate (64°N, 19°E) and one lake in subarctic northern Sweden (68°N, 18°E; Lake Diktar Erik) were studied. The lakes were small (area: 0.1–1.1 km²) with catchments dominated by birch forest (Diktar Erik) or coniferous forests and mires (all other lakes). No human settlements or agricultural activities were present in the catchments. We chose brown-water lakes for our study since respiration of AlloOC is expected to be a significant source of CO₂ in this type of lake (Cole et al. 1994).

Water samples were collected once in August from 1 m depth in the middle of the lakes using a Ruttner sampler. The samples were used for analysis of dissolved organic carbon (DOC), total nitrogen (Tot-N), and total phosphorus (Tot-P), for analysis of PP, and for experimental incubations. DOC, Tot-N, and Tot-P were analyzed by standard methods at the Department of Limnology, Uppsala University (see Karlsson et al. 2002). PP was measured during 4-h incubations using the ¹⁴C method. The incubation was carried out in a climate chamber at in situ temperature and with a photosynthetic active radiation of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Crustacean zooplankton were collected by vertical hauling of the upper 2 m of the water column using a plankton net with a mesh size of 100 μm . Zooplankton were separated into cladocerans and copepods when possible. The zooplankton were dried (65°C) and stored frozen prior to isotopic analyses.

In order to determine the $\delta^{13}\text{C}$ of respired carbon in the lake water, we carried out short-term experiments where changes in concentration and $\delta^{13}\text{C}$ of DIC were measured over time in lake water during dark incubations. Water collected from 1-m depth was transferred directly from the Ruttner sampler to 335-mL glass bottles (8–10 per lake) without creating air bubbles by overfilling the bottles to minimize contact with air. Half of the bottles were analyzed directly, and the remaining bottles were brought to the laboratory and were incubated in the dark at in situ temperature for 36–48 h, for which period respiration can be expected to be linear (M. Berggren, unpubl. data). The experimental design, with relatively long incubation time and distribution of the sampling points to the end points of the incubation period, should have minimized the variance when estimating $\delta^{13}\text{C}$ of respired CO₂ (Olsson et al. 2005). The bottles were analyzed for concentration and $\delta^{13}\text{C}$ of DIC before and after the incubation by using a headspace equilibration technique (Cole et al. 1994; Miyajima et al. 1995). The bottles were acidified (HCL, pH <2) and 50-mL He were added to create a headspace. The bottles were equilibrated by shaking for 1 min and left to stand for an additional minute. The headspace gas was transferred to evacuated 12-mL glass tubes and analyzed for CO₂ concentration and $\delta^{13}\text{C}$ signature. From the concentration of CO₂ in the headspace, the concentration of DIC in the water of each bottle was calculated according to Cole et al. (1994) using Henry's law and the fugacity-pressure relationship presented by Weiss (1974). The $\delta^{13}\text{C}$ of DIC in the water was obtained from the $\delta^{13}\text{C}$ of the headspace CO₂ after correction for the isotopic fractionation between water and gas phase (Mook et al. 1974).

In Lake Diktar Erik, we also performed monthly (June, July, August) sampling of the lake water over the greatest depth of the lake (16.5 m). Lake water was sampled from different depths in the epilimnion (0, 1, 2, 3, 4, 5 m) and in the hypolimnion (8, 15 m) and was analyzed for the concentration and $\delta^{13}\text{C}$ of DIC as above. We also included data on concentration and $\delta^{13}\text{C}$ of DIC from the hypolimnion of Lake Örträsket during the summer of 1994 obtained from Jonsson et al. (2001). Örträsket is a deep (64 m) lake in the same region as the temperate lakes of this study. From a detailed carbon budget, Jonsson et al. (2001) estimated that the respiration in hypolimnion was derived solely from respiration of AlloOC. The use of hypolimnion data enabled comparison of short-term experimental data with independent, long-term measures of carbon sources supporting respiration.

Analyses of CO₂ concentration and $\delta^{13}\text{C}$ in gas samples were carried out using an ANCA-NT gas purification module and a Mod. 20-20 stable isotope analyzer (Europa Scientific Ltd, Crewe, U.K.), and analyses of $\delta^{13}\text{C}$ in zooplankton were carried out using a Europa Scientific carbon and nitrogen analyzer connected to a Europa 20-20 stable isotope analyzer. Results are expressed by the δ notation in per mil (‰) as:

$$\delta^{13}\text{C} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000, \quad (1)$$

where R = ¹³C/¹²C. The analytical precision of isotopic analysis was <0.3‰.

Table 1. Characteristics of the nine forest lakes during the sampling in August, including experimental results of R and NEP. Zooplankton $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{Zoo}}$) represents cladocerans or copepods or the mean (± 1 SD) of these.

Lake	Area (km ²)	DOC (mg L ⁻¹)	Tot-P ($\mu\text{g L}^{-1}$)	Tot-N ($\mu\text{g L}^{-1}$)	R ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	NEP ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	$\delta^{13}\text{C}_{\text{Zoo}}$ (‰)
Diktar Erik	0.1	6.7	8.3	126	3.1	-2.3	-31.2 \pm 0.1
Granträsk	1.1	14.2	25.0	234	4.7	-2.3	-34.3
Grundsjön	0.2	14.1	19.1	259	4.5	-3.2	-35.2 \pm 0.1
Mellan-Betsarn	0.7	6.2	11.8	149	5.2		-33.5 \pm 0.1
Stor-Supanträsket	0.3	8.4	21.1	305	5.6	-3.7	-33.2
Hötjärn	0.2	16.5	21.1	241	6.6	-3.9	-35.8
Baksjön	0.3	17.5	14.1	267	3.7	-2.5	-33.2
Mörtsjön	0.6	10.2	20.7	284	9.8	-3.0	-35.5
Gäddtjärnen	0.3	13.2	15.3	219	4.1	-2.3	-33.7 \pm 0.4

The isotopic composition of the respired carbon source in the hypolimnion and in the experiments was estimated from the change in concentration and $\delta^{13}\text{C}$ of the DIC pool over time by using the Keeling plot method. During the course of the incubation, respiration of organic carbon adds CO_2 to the background DIC, increasing [DIC] and changing $\delta^{13}\text{C}$ of the DIC pool. When respired CO_2 is added to background DIC with constant concentration and isotopic composition, a linear relation exists between $1/[\text{DIC}]$ and $\delta^{13}\text{C}$, where the intercept corresponds to the isotopic composition of respired CO_2 (Keeling 1958; Pataki et al. 2003).

We estimated the fraction of AlloOC that contributed to respiration (R_{Allo}) in the experiments by assuming that the $\delta^{13}\text{C}$ of respired CO_2 ($\delta^{13}\text{C}_{\text{R}}$) is a mixture of CO_2 derived from respiration of autotrophic phytoplankton OC ($\delta^{13}\text{C}_{\text{Auto}}$) and AlloOC ($\delta^{13}\text{C}_{\text{Allo}}$), and by accounting for a fractionation during respiration of 0.5‰ (Hullar et al. 1996), such that

$$R_{\text{Allo}} = (\delta^{13}\text{C}_{\text{R}} - 0.5 - \delta^{13}\text{C}_{\text{Auto}}) / (\delta^{13}\text{C}_{\text{Allo}} - \delta^{13}\text{C}_{\text{Auto}}). \quad (2)$$

The $\delta^{13}\text{C}_{\text{Allo}}$ was set to -27.0‰ (Lajtha and Michener 1994). The difficulty in determining $\delta^{13}\text{C}_{\text{Auto}}$ is a well-known obstacle when using stable isotopes in aquatic food web analyses, and especially so in unproductive lake waters. Estimating $\delta^{13}\text{C}_{\text{Auto}}$ using, for example, sedimentation (Grey et al. 2001) or density fractionation in colloidal silica (Hamilton et al. 2005) is not an alternative when phytoplankton communities are dominated by small-sized mixotrophic species (e.g., chrysophytes), as in the lakes in this study. Other estimates have been based on the isotopic signature of POC, which is not very precise in unproductive lakes, or by modelling approaches (Meili et al. 1996; Karlsson et al. 2003). Recent methods include compound specific isotopic analysis (e.g., Sachs et al. 1999), but these methods are not practical alternatives at present in unproductive lakes with very low phytoplankton biomass. To obtain representative mean $\delta^{13}\text{C}_{\text{Auto}}$ values in each of the studied lakes, we used the $\delta^{13}\text{C}$ of metazoan zooplankton ($\delta^{13}\text{C}_{\text{Zoo}}$) sampled in connection with sampling for the Keeling incubations. Several studies have shown that metazoan zooplankton ingest organic carbon

from both allochthonous and autochthonous sources. Thus, we assumed that zooplankton contained 44% AlloOC ($Z_{\text{OoAllo}} = 0.44$), which is the mean of published summer values from unproductive lakes, i.e., eight Swedish forest lakes (range 6–77%; Meili et al. 1996, 2000; Karlsson et al. 2003), three U.S. lakes (range 30–62%; Carpenter et al. 2005), and one Scottish lake (20%; Grey et al. 2001). Consequently the phytoplankton organic carbon component of the zooplankton was 56%, and the $\delta^{13}\text{C}_{\text{Auto}}$ was calculated as:

$$\delta^{13}\text{C}_{\text{Auto}} = [\delta^{13}\text{C}_{\text{Zoo}} - F \times (\text{TP} - 1) - \delta^{13}\text{C}_{\text{Allo}} \times Z_{\text{OoAllo}}] / (1 - Z_{\text{OoAllo}}). \quad (3)$$

We assumed that cladocera and copepods were primary (trophic position [TP] = 2) and secondary (TP = 3) consumers in the lakes (Karlsson et al. 2004), respectively, and adopted a trophic fractionation factor (F) of 0.4‰ (Post 2002). When both cladocerans and copepods were present, we performed the calculations using both species, and we present herein the mean R_{Allo} of these calculations. The effect of uncertainties in the calculation of $\delta^{13}\text{C}_{\text{Auto}}$ was tested by assuming Z_{OoAllo} between 24% and 64% (± 1 standard deviation [SD] of the mean of reported values).

Results and discussion

The chemistry and metabolic balances of the surface water of the study lakes (Table 1) were typical for forest lakes in northern Sweden (Jansson et al. 2000; Karlsson et al. 2002). Tot-P varied between 8.3 and 25.0 $\mu\text{g L}^{-1}$, Tot-N varied between 126 and 305 $\mu\text{g L}^{-1}$, and DOC varied between 6.2 and 17.5 mg L^{-1} . PP was between 0.7 and 6.7 $\mu\text{g C L}^{-1} \text{ h}^{-1}$ during the 4-h incubations. Dark respiration was between 3.1 and 9.8 $\mu\text{g C L}^{-1} \text{ h}^{-1}$ during the 36–48-h incubations. The NEP in the surface water, calculated as the difference between volumetric rates of GPP and R, was between -2.3 and -3.9 $\mu\text{g C L}^{-1} \text{ h}^{-1}$ (Table 1). The metabolic imbalance, as indicated by negative NEP values, amounted to between 31% and 76% (mean ± 1 SD: 59 \pm 13%) of the dark respiration in the lake waters.

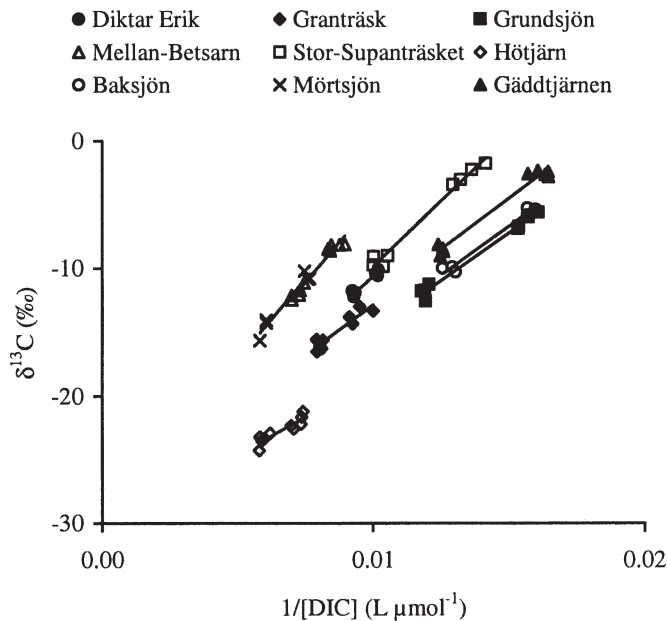


Fig. 1. Keeling plot showing the relationship between the stable isotopic composition ($\delta^{13}\text{C}$) of DIC and the inverse of the concentration of DIC ($1/[\text{DIC}]$) during dark incubation of lake water in nine forest lakes in northern Sweden.

The concentration of DIC increased and the $\delta^{13}\text{C}$ of DIC decreased during the dark incubations. Thus, respiration added isotopically light CO_2 to the DIC pool. The Keeling plots (Fig. 1; Table 2) show that the $\delta^{13}\text{C}$ of the respired carbon was between -28.4‰ and -30.6‰ in the incubations. The $\delta^{13}\text{C}$ values of respired carbon are similar to $\delta^{13}\text{C}$ of terrestrial vegetation and soil organic carbon, but they are clearly enriched in ^{13}C compared to estimates of phytoplankton organic carbon obtained in this study (-36.0‰ to -44.2‰) using the zooplankton $\delta^{13}\text{C}$ (Table 1) and the average carbon support of zooplankton from allochthonous and autochthonous sources. These $\delta^{13}\text{C}_{\text{Auto}}$ values are within the range (-35.7‰ to -47‰) reported from other unproductive lakes in Scandinavia (Meili et al. 1996, 2000; Jones et al. 1999; Karlsson et al. 2003) and North America (Rau et al. 1978; Hecky and Hesslein 1995). Low $\delta^{13}\text{C}$ of the inorganic carbon source and high

fractionation during carbon uptake explain the low $\delta^{13}\text{C}$ of phytoplankton in unproductive lakes (Hecky and Hesslein 1995).

The $\delta^{13}\text{C}$ values of respired carbon therefore indicate a clear dominance of AlloOC sources in dark respiration. AlloOC was estimated to cover $79.2 \pm 5.0\%$ (mean \pm SD) of the carbon used in dark respiration (Table 2). Uncertainties in assumed values of $\delta^{13}\text{C}_{\text{Auto}}$ cause relatively small variation in estimated respiration of AlloOC (Table 2). In fact, even if we assume that zooplankton relies solely on AutoOC, which gives $\delta^{13}\text{C}_{\text{Auto}}$ values between -31.8‰ and -36.3‰ , the respiration is still dominated by AlloOC sources ($62.6 \pm 8.9\%$). The results indicate that AlloOC dominates respiration and that this respiration of AlloOC is large enough to cover the metabolic imbalance as indicated by the negative NEP in the surface waters of the lakes (Table 1). No relationship ($p < 0.05$) was observed between respiration of AlloOC, expressed as either relative or absolute values (Table 2), and the DOC content of the lake water.

The measured respiration represents heterotrophic respiration and dark respiration by photoautotrophs. One important question is how this respiration during the incubation experiments relates to in situ respiration. It could be argued that the respiration of AutoOC is underestimated in our experimental study because of reduced input of new AutoOC during incubation in darkness. Unnaturally low contributions of AutoOC should not affect absolute respiration values of AlloOC but could indeed cause an overestimation of the relative contribution of AlloOC in respiration. However, parallel measurements of respiration in light and dark incubations in the epilimnion of Lake Diktar Erik showed that PP did not support a larger fraction of respiration in the light compared to in the dark (J. Åberg, unpubl. data). Hence, we likely did not underestimate the importance of AutoOC sources for community respiration.

The concentration and $\delta^{13}\text{C}$ of DIC was similar at different depths in the epilimnion of Diktar Erik, with minor differences between dates (Fig. 2), reflecting the exchange of CO_2 between lake water and the atmosphere. In the hypolimnion, the concentration of DIC was higher and the $\delta^{13}\text{C}$ of DIC lower compared to epilimnetic values. The DIC concentration increased and the $\delta^{13}\text{C}$ decreased in

Table 2. Results from Keeling plot analysis and modelling of the respiration of AlloOC (R_{Allo}) from experimental data. The standard error (SE) is given for the intercept. The range given for R_{Allo} shows the values assuming ± 1 SD of mean values of phytoplankton contribution to zooplankton carbon demand.

Lake	Keeling plot			R_{Allo}	
	r^2	Intercept	SE	(%)	($\mu\text{g C L}^{-1} \text{h}^{-1}$)
Diktar Erik	0.94	-28.9	2.1	74 (66–84)	2.5
Granträsk	0.89	-28.5	1.7	87 (83–92)	4.1
Grundsjön	0.99	-29.6	0.9	81 (75–88)	3.5
Mellan-Betsarn	0.96	-29.6	1.4	77 (70–86)	4.0
Stor-Supanträsket	0.98	-30.3	1.2	72 (62–82)	4.0
Hötjärn	0.84	-30.6	1.2	77 (70–86)	4.6
Baksjön	0.97	-29.6	1.7	76 (69–85)	2.9
Mörtsjön	0.96	-29.7	1.6	82 (75–88)	7.5
Gäddtjärnen	0.97	-28.4	1.4	86 (82–91)	3.5

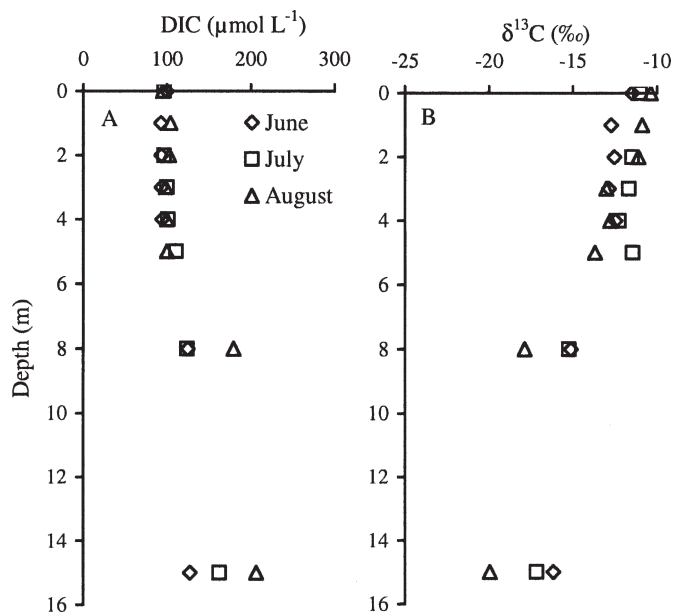


Fig. 2. (A) Concentration of DIC and (B) stable isotopic composition ($\delta^{13}\text{C}$) of DIC at different depths in Lake Diktar Erik during summer stratification in 2004.

the hypolimnion over time. The Keeling plot of hypolimnion data shows that the $\delta^{13}\text{C}$ of the added DIC source was -26.5‰ (Fig. 3). Figure 3 also includes data from Lake Örträsket where the $\delta^{13}\text{C}$ of the added DIC was estimated as -26.7‰ . Depleted $\delta^{13}\text{C}$ of DIC in the hypolimnion of both lakes Diktar Erik and Örträsket could theoretically be a result of oxidation of CH_4 produced in anaerobic sediments. However, other studies show no or very low CH_4 flux at the sediment-water interface and absence of CH_4 in the hypolimnion of these lakes (Algesten et al. 2005; J. Åberg, unpubl. data). Thus, we assume that oxidation of CH_4 is negligible and does not affect the isotopic results of this study. The Keeling plot values from Diktar Erik and Örträsket (Fig. 3) were very close to the assumed $\delta^{13}\text{C}$ of CO_2 from respiration of AlloOC (-26.5‰) used in the model (Eq. 2); this is in accordance with other studies in these lakes that have shown that pelagic photosynthesis is very low (Jansson et al. 2000; Karlsson et al. 2002) and that AlloOC is the predominant source for hypolimnion respiration (Jonsson et al. 2001; Åberg et al. 2005). The Keeling plots also show that the results from our short-term experiments were reflected in long-term in situ data. The slightly lower $\delta^{13}\text{C}$ values of respired carbon in the epilimnion experiments compared to the in situ hypolimnion values are logical since respiration of AutoOC sources, both through dark respiration by phytoplankton and heterotrophic respiration of AutoOC, should be highest in the epilimnion.

We conclude that the Keeling plot method has large potential for investigations of carbon sources supporting respiration in aquatic systems. The results show that surface-water respiration in the study lakes was dominated by AlloOC sources and that this respiration was high enough to cause high community respiration compared to primary production and hence cause a high degree of net

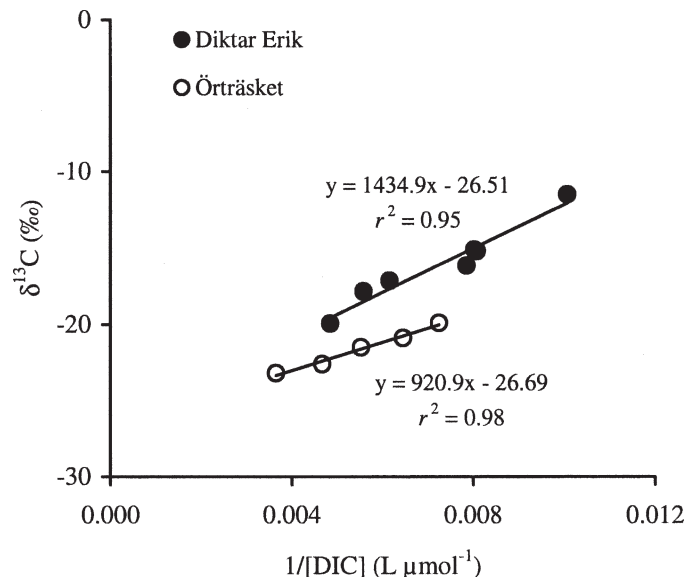


Fig. 3. Keeling plot for data on stable isotopic composition ($\delta^{13}\text{C}$) and inverse concentration of DIC ($1/[\text{DIC}]$) in the hypolimnion of Lake Diktar Erik in 2004 and lake Örträsket in 1994 (Jonsson et al. 2001). In both lakes, one value (i.e., the point with highest $\delta^{13}\text{C}$ and $1/[\text{DIC}]$ in the figure) from the surface water at the start of stratification is included. This value is assumed to represent the concentration and $\delta^{13}\text{C}$ of DIC in the hypolimnion at the start of the stratification period. The standard error for the intercept is 1.1 for Diktar Erik and 0.4 for Örträsket.

heterotrophy. This study, thus, provides direct evidence that respiration of AlloOC causes net heterotrophic conditions in unproductive lakes.

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