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Biochemical Composition of Dissolved Organic Carbon Derived from Phytoplankton and Used by Heterotrophic Bacteria

INGVAR SUNDH†

Uppsala University, Institute of Limnology, Box 557, S-751 22 Uppsala, Sweden

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The molecular size distribution and biochemical composition of the dissolved organic carbon released from natural communities of lake phytoplankton (photosynthetically produced dissolved organic carbon [PDOC]) and subsequently used by heterotrophic bacteria were determined in three lakes differing in trophic status and concentration of humic substances. After incubation of epilimnetic lake water samples with $H^{14}CO_3^-$ over one diel cycle, the phytoplankton were removed by size-selective filtration. The filtrates, still containing most of the heterotrophic bacteria, were reincubated in darkness (heterotrophic incubation). Differences in the amount and composition of PDO^{14}C between samples collected before the heterotrophic incubation and samples collected afterwards were considered to be a result of bacterial utilization. The PDO^{14}C collected at the start of the heterotrophic incubations always contained both high (>10,000)- and low (<1,000)-molecular-weight (MW) components and sometimes contained intermediate-MW components as well. In general, bacterial turnover rates of the low-MW components were fairly rapid, whereas the high-MW components were utilized slowly or not at all. In the humic lake, the intermediate-MW components accounted for a large proportion of the net PDO^{14}C and were subject to rapid bacterial utilization. This fraction probably consisted almost entirely of polysaccharides of ca. 6,000 MW. Amino acids and peptides, other organic acids, and carbohydrates could all be quantitatively important parts of the low-MW PDO^{14}C that was utilized by the heterotrophic bacteria, but the relative contributions of these fractions differed widely. It was concluded that, generally, low-MW components of PDOC are quantitatively much more important to the bacteria than are high-MW components, that PDOC released from phytoplankton does not contain substances of quantitative importance as bacterial substrates in all situations, and that high-MW components of PDOC probably contribute to the buildup of refractory, high-MW dissolved organic carbon in pelagic environments.

The dissolved organic carbon (DOC) released from phytoplankton (photosynthetically produced DOC [PDOC]) is an important carbon source for natural assemblages of pelagic heterotrophic bacteria. Sometimes, more than 50% of the total bacterial carbon demand is met by PDOC (2, 44, 49). Exceptions to this general pattern are systems with large inputs of allochthonous DOC, such as marine estuaries (10) and humic lakes (49, 51). To some extent, the biochemical composition of released PDOC is related to the phytoplankton species present (19, 36) and dependent on abiotic environmental conditions, such as light and nutrients. Work with unialgal laboratory cultures of phytoplankton has led to the identification of numerous compounds in PDOC, and the biochemistry of PDOC released from phytoplankton was reviewed by Hellebust (20) and Jensen (26). Characterization of in situ PDOC (performed mainly on PDO^{14}C from ^{14}C tracer experiments) has mostly been limited to molecular size fractionation. Such experiments have demonstrated that either high- or low-molecular-weight (MW) substances can dominate and that the composition of PDOC released from natural phytoplankton communities exhibits both within- and between-system variations (reviewed by Sundh [47]). However, our knowledge is still very limited concerning the overall biochemical composition of in situ PDOC.

Findings of several studies suggest that as substrates for heterotrophic bacteria, low-MW components of PDOC are quantitatively most important (27, 36, 37). Nevertheless, evidence that high-MW components are utilized as well, but often at lower rates, has accumulated (9, 25, 31, 33, 47). An evaluation of the relative importance of high- and low-MW PDOC as bacterial substrates, however, is problematic, since the methods used for size fractionation of PDOC have often differed between studies. Activities of extracellular hydrolytic enzymes can be significant in lake water (8, 22, 42), suggesting that high-MW substrates, including those recently released as PDOC, are potentially important to the bacteria. Amino acids have been suggested to be an important fraction of the total bacterial production in both marine and freshwater environments (15, 29). However, comparative studies which take all of the various biochemical groups in PDOC into consideration are lacking.

The main objective of this study was to determine which components of the PDOC released from phytoplankton are quantitatively most important as a substrate for the pelagic heterotrophic bacteria in lakes. The ^{14}C tracer method was used, and the bacterial utilization of different fractions in labelled PDOC was monitored. Experiments were done at various times during the phytoplankton growing season in three lakes differing in trophic status and concentration of humic substances.

MATERIALS AND METHODS

The experiments were carried out in three temperate lakes in eastern central Sweden: Lake Vallentunasjön (59°29′N, 18°2′E), Lake Erken (59°51′N, 18°35′E), and Lake Siggeforasjön (59°58′N, 17°9′E). All three lakes are normally cov-

† Present address: Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, S-750 07 Uppsala, Sweden.

ered with ice for several months in winter. Lake Vallentunasjön is eutrophic and has a surface area of 6.2 km^2 and a maximum depth of 5.0 m. Owing to its shallowness, it only occasionally stratifies in summer. The summer mean concentration of total phosphorus is ca. 100 $\mu g\ liter^{-1}$, and the Secchi depth, which is largely determined by dense phytoplankton populations, is ca. 0.5 m. After a diatom biomass peak in spring, the phytoplankton community is normally dominated by colonial cyanobacteria (mainly *Microcystis* spp.). Mesotrophic Lake Erken has a surface area of 24 km^2 and a maximum depth of 20 m. Thermal stratification normally develops by the end of May and persists until September. The mean Secchi depth and epilimnion total phosphorus concentration during summer are ca. 3.5 m and ca. 20 $\mu g\ liter^{-1}$, respectively. In most growing seasons, the phytoplankton community of Lake Erken is characterized by a spring bloom of diatoms, followed by more diverse summer and autumn communities. Oligo- to mesotrophic Lake Siggeforasjön has an area of 0.76 km^2 and a maximum depth of 11 m. Like Lake Erken, it is normally stratified during summer. Lake Siggeforasjön is slightly humic (water color, 60 to 80 mg Pt $liter^{-1}$), and in most situations the dissolved humic substances determine the Secchi depth, which is ca. 2.5 m. The epilimnetic mean total phosphorus concentration in summer is ca. 15 $\mu g\ liter^{-1}$. The phytoplankton community of Lake Siggeforasjön is diverse in most situations. Further details of the abiotic and biotic characteristics of the lakes were described by Boström et al. in 1989 (Lake Vallentunasjön) (5), Pettersson in 1990 (Lake Erken) (40), and Heyman and Blomqvist in 1984 (Lake Siggeforasjön) (21).

Methods of sampling, incubation, filtrations, and treatment of filters and filtrates are described only briefly here. For a detailed description, see reference 49. Mixed water samples (from the epilimnion during stratification and from the euphotic zone during circulation periods) were gently sieved through a 100-μm-pore-size net to remove macrozooplankton and subsequently incubated with $H^{14}CO_3^-$ in duplicate. The incubations were started in the morning and carried out in situ at a depth of 0.5 to 1.0 m or at in situ temperature in the laboratory at 110 to 250 microeinsteins $m^{-2}\ s^{-1}$ during daytime and in darkness at night. After ca. 24 h (1 day followed by night and a shorter light period on the following day), one 5- to 15-ml aliquot was filtered sequentially through 2-μm (Nuclepore polycarbonate; gravity filtration)- and 0.2-μm (Schleicher and Schüll membrane; vacuum pressure never lower than 100 mm Hg)-pore-size filters, and the filtrate was prepared for $PDO^{14}C$ characterization after removal of $^{14}CO_2$ by acidification and air bubbling. Another 5- to 15-ml aliquot was passed only through a 2-μm-pore-size filter (Nuclepore polycarbonate; gravity filtration) to remove the phytoplankton but not the bacteria. The filtrates containing the heterotrophic bacteria and the $PDO^{14}C$ were subsequently reincubated in darkness at in situ temperature to allow continued bacterial utilization of $PDO^{14}C$. In the July 1988 experiment at Lake Erken, heterotrophic incubations were started both after ≈14 h (at the end of day 1) and 24 h preincubation with $H^{14}CO_3^-$. In all experiments in 1988, only one incubation time (4.4 to 7.6 h) was used for the heterotrophic incubations, whereas in 1989, one comparably short incubation time (6 to 22 h) and one long incubation time (51 to 93 h) were used. The heterotrophic incubations were stopped by filtration through a 0.2-μm-pore-size Schleicher & Schüll membrane filter. The filtrates were acidified and bubbled with air to remove radioactive inorganic carbon. Size fractionation and biochemical characterization were subsequently performed on $PDO^{14}C$ collected both before and after the heterotrophic incubations.

The first step in the characterization of the $PDO^{14}C$ was Sephadex G-50 molecular size fractionation. Material from the ^{14}C peaks of the Sephadex chromatograms was separately pooled and stored frozen at −20°C until biochemical characterization. High-MW peaks were further fractionated, by routine biochemical methods, into lipids (chloroform-methanol extraction), proteins (phenol extraction), nucleic acids (trichloroacetic acid precipitation), and polysaccharides (still dissolved in the remaining water phase). Low-MW peaks were further fractionated, by ion-exchange chromatography, into amino acids (retained by an acidic cation exchanger at acidic pH), other organic acids (retained by an anion exchanger at basic pH), and carbohydrates (not retained by any of the ion exchangers). Biochemical characterizations of the peaks were always preceded by filtration of the thawed peak material through a 0.2-μm-pore-size Schleicher & Schüll membrane filter. More details on the fractionation methods are given by Sundh (48).

RESULTS

The Sephadex G-50 size distribution chromatograms obtained for samples taken before and after the heterotrophic incubations are shown in Fig. 1. In general, the net $PDO^{14}C$ collected before the heterotrophic incubations always contained both high-MW (eluted at the size exclusion limit of the gel) and low-MW (eluted close to the total volume of the gel) substances. Sometimes (in all experiments at Lake Siggeforasjön), an intermediate-MW peak also appeared. The relative contributions of high-, intermediate-, and low-MW substances, however, differed substantially between experiments at each of the lakes. The total radioactivity of each individual peak was assessed by integration, and the rates of change of ^{14}C in the individual peaks during the heterotrophic incubations are presented in Table 1. In both experiments at eutrophic Lake Vallentunasjön, the high-MW substances were used very slowly or not at all while the low-MW substances were subject to fairly rapid bacterial utilization (Fig. 1 and Table 1). At Lake Erken, an additional small peak of intermediate MW appeared in all experiments (Fig. 1). The ^{14}C of the low- and intermediate-MW peaks in experiments at this lake always decreased during heterotrophic incubation, while that of the high-MW peak decreased significantly in two of the four experiments (Table 1). In experiments at humic Lake Siggeforasjön, the intermediate-MW peak was not only a quantitatively very important component of the net $PDO^{14}C$ but was also subject to the highest rates of bacterial utilization (Fig. 1 and Table 1). In the 1989 experiment, the intermediate-MW peak had vanished completely after 51 h of heterotrophic incubation. It is important to bear in mind that an equal relative decrease of ^{14}C in two peaks may represent very different amounts of carbon, since the amount of ^{14}C in the peaks (and the specific activity) may differ from the start.

The radioactivity of the high-MW peak decreased significantly in only two of the heterotrophic incubation experiments (Lake Erken on 5 May 1988 and one of the experiments on 20 July 1988); in these cases, the decrease appeared in the polysaccharide fraction (Fig. 2). Radioactivity in the intermediate-MW peaks in experiments at Lakes Vallentunasjön and Erken after the heterotrophic incubations was too low for reliable biochemical characterization. However, in experiments at Lake Vallentunasjön on 27 September 1989 and Lake Erken on 5 May 1988, the inter-

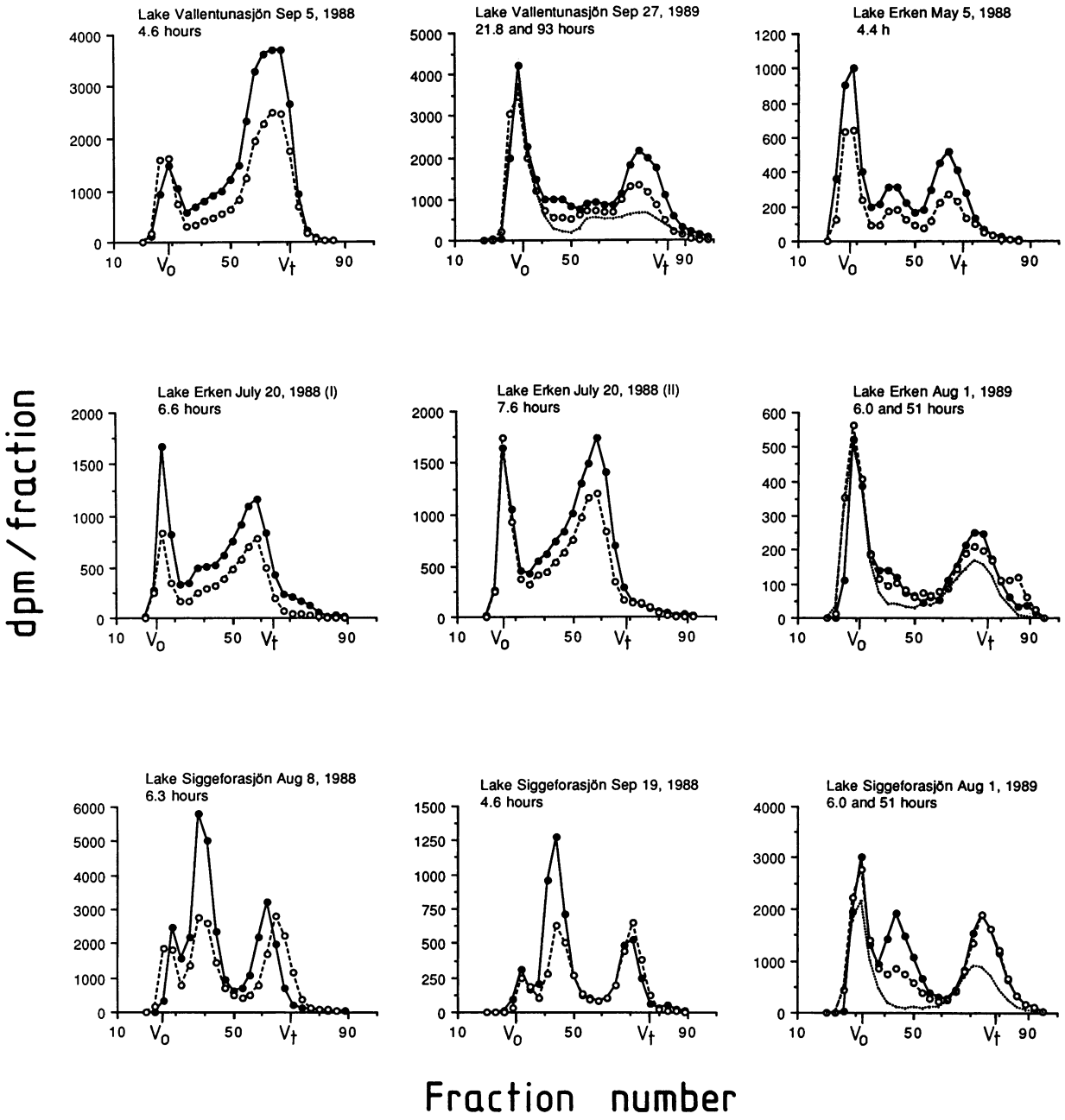

FIG. 1. Sephadex G-50 size distribution chromatograms of PDO^{14}C before (●) and after short (○) and long (·····) heterotrophic incubations. V_0, upper size exclusion limit; V_t, total volume of the gel (as determined with blue dextran and NaCl, respectively).

mediate-MW peak of the net PDO^{14}C collected before the heterotrophic incubation was strongly dominated by polysaccharides (48). Thus, the decrease in intermediate-MW substances during the heterotrophic incubations was at least partly a result of polysaccharide utilization. At Lake Siggeforasjön, where the intermediate-MW peak was quantitatively dominant, it was almost entirely composed of polysaccharides; hence, the rapid decrease in this peak represented polysaccharide utilization (Fig. 3). At one time or another, the amino acid, organic acid, and carbohydrate fractions were all quantitatively important components of the low-MW peak in the net PDO^{14}C. In Lake Vallentunasjön, carbohydrates dominated the low-MW peak and were also subject to the highest utilization rates in the 1988 experiments, while in the 1989 experiment, the same was true for the organic acid fraction (Fig. 4). In the May and both of the July experiments at Lake Erken, the amino acid and other organic acid fractions were quickly utilized, whereas the carbohydrates were utilized slowly or not at all. The fairly slow utilization of the low-MW peak in the 1989 experiment (Fig. 1) was confined mainly to the carbohydrate fraction (Fig. 4). In the only experiment at Lake Siggeforasjön in which the low-MW peak decreased significantly during heterotrophic incubation (the long incubation on 1

TABLE 1. Change in $DO^{14}C$ levels over time in individual Sephadex G-50 size fractionation peaks during heterotrophic incubations

Site and date	Heterotrophic incubation time (h)	Recovery (%)[a]	$DO^{14}C$ change[b]		
			High MW	Intermediate MW	Low MW
Lake Vallentunasjön					
5 Sept. 1988	4.6	93	+4.1		−8.8
27 Sept. 1989	21.8,	90,	+0.01,	−1.6,	−1.7,
	93	106	−0.06	−0.7	−0.6
Lake Erken					
5 May 1988	4.4	85	−7.8	−10	−12
20 July 1988 (I)	6.6	87	−7.3		−5.2
20 July 1988 (II)	7.6	85	−1.4		−5.6
1 Aug. 1989	6.0	81	+4.1		−0.8
	51	81	+0.3		−0.7
Lake Siggeforasjön					
8 Aug. 1988	6.3	84	+0.9	−6.9	−0.9
19 Sept. 1988	4.6	81	−3.6	−10	+5.5
1 Aug. 1989	6.0	76	+1.2	−8.3	−0.7
	51	99	−0.3	−2.0	−0.9

[a] Total radioactivity eluted from the Sephadex column as a percentage of the radioactivity applied to the column.
[b] Expressed as a percentage of the initial activity per hour.

August 1989), the decrease was a result of utilization of organic acids and carbohydrates (Fig. 4).

DISCUSSION

Methodological considerations. The heterotrophic incubation technique has some inherent limitations. One is that the $PDO^{14}C$ utilized during the incubation are the net products of the preincubation; thus, their composition has already been altered by bacterial utilization. It is possible that readily utilizable components of the PDOC are released in large amounts but rapid bacterial uptake during the preincubation keeps the dissolved pool small. Theoretically, however, if the heterotrophic incubation period is short enough, this method should give valid information on the relative bacterial turnover rates of the various fractions in the net $PDO^{14}C$.

The other important limitation of the heterotrophic incubation technique is that the released substances may already be present in unlabelled form in the water. If they are present in different concentrations, uniform labelling of the dissolved pools cannot be assumed. This, of course, makes the comparison of the utilized proportions of carbon in the various fractions highly uncertain and means that the ^{14}C tracer method may seriously underestimate the total bacterial utilization of released PDOC. In particular, the lower bacterial turnover rates of the high-MW PDOC components seen in this study imply that their ^{14}C specific activity may be markedly lower, which in turn would result in underestimation of the utilization of the high-MW components relative to that of the low-MW components. On the other hand, by using the ^{14}C tracer method it has been established that in most systems and situations (including the lakes sampled in this investigation), PDOC accounts for more than half of the carbon substrate for heterotrophic bacteria (2, 44, 49). Thus, although in a very indirect way, this general relationship between PDOC uptake measured with the ^{14}C tracer method and total carbon uptake suggests that the ^{14}C method does not seriously underestimate the bacterial utilization of PDOC owing to dilution of the labelled compounds. Hence, the overall utilization of the high-MW components relative to that of the low-MW components (in absolute carbon units) is not likely to have been seriously underestimated. So, with the drawback that the relative contribution of high-MW components to the total bacterial PDOC utilization could have been underestimated in some experiments, the heterotrophic incubations may nevertheless be interpreted as showing which PDOC fractions are, in general, quantitatively most important to the bacteria.

In the experiments presented here, the bacterial turnover rates of the total $PDO^{14}C$ pool were estimated by two different methods: the fractionated filtration technique (turnover rates calculated as total ^{14}C release after the first light period of the preincubation divided by the ^{14}C of the bacterial size fraction, with correction for 50% respiration), the results of which have been given by Sundh and Bell (49), and the heterotrophic incubations presented here. Assuming that careful use of the fractionated filtration technique (involving, as a minimum of precautions, gravity filtration when possible and correction for photosynthetic CO_2 uptake in the bacterial size fraction) yields good estimates of the total bacterial utilization of PDOC, the bacterial turnover rate estimates of the heterotrophic incubation experiments should be similar to those estimated with the fractionated filtration technique. Table 2 shows that this was not always the case. Thus, in the 1989 experiment at Lake Erken the hypothetical turnover time of $PDO^{14}C$ estimated with the heterotrophic incubation technique was several times longer than that estimated on the basis of the fractionated filtration technique. Even after 51 h of heterotrophic incubation, only a small portion of the net $PDO^{14}C$ had been removed from solution (Fig. 1 and Table 1). Interestingly, the relative rate of $PDO^{14}C$ uptake in this experiment, measured with the fractionated filtration method, was higher than in any of the other experiments (57% of the released $PDO^{14}C$ was utilized during the preincubation with $H^{14}CO_3^-$). Thus, a probable reason for the slow $PDO^{14}C$ utilization in the heterotrophic incubation of this experiment is that a large proportion of the labile $PDO^{14}C$ had already been utilized, resulting in enrichment of the refractory components in the net $PDO^{14}C$. Likewise, the heterotrophic incubations yielded longer turnover times at Lake Vallentunasjön in September 1989. In this

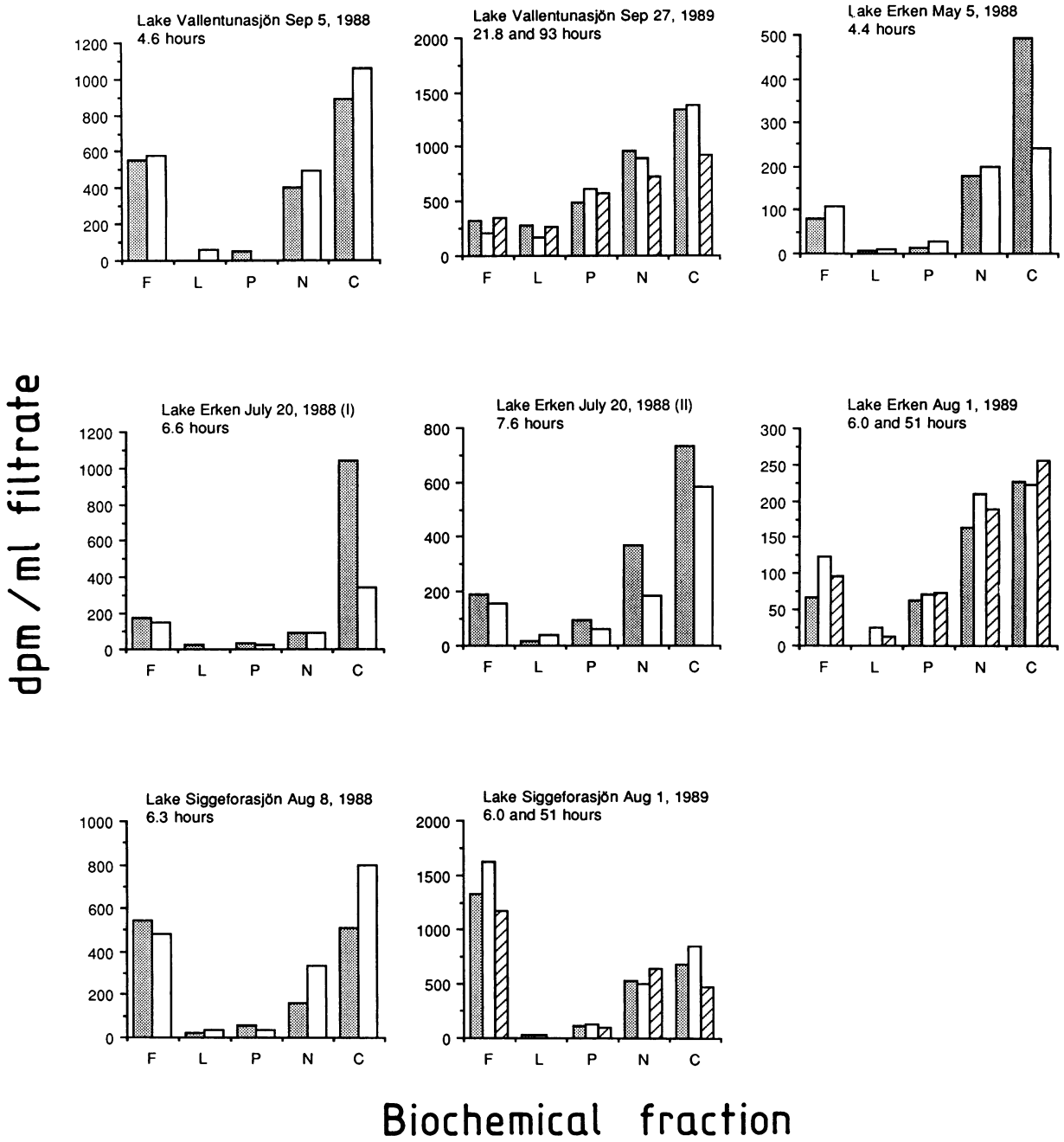

FIG. 2. Biochemical composition of the high-MW peaks from Sephadex G-50 before (▨) and after short (□) and long (▨) heterotrophic incubations. Abbreviations: F, precipitated during freezing and thawing; L, lipids; P, proteins; N, nucleic acids; C, carbohydrates.

latter case, however, the longer turnover times can probably be attributed to the longer heterotrophic incubation times, since the longer the incubation period, the greater the degree of enrichment of refractory components of the PDOC. Considering the uncertainties in the estimates (for example, the same respiration quota, i.e., 50% of the bacterial PDO^{14}C uptake, was used in all experiments), however, the fairly good overall agreement between values obtained with the two methods lends some credibility to the heterotrophic incubation experiments.

A more accurate method for assessing the magnitude of bacterial PDOC uptake and determining the composition of the utilized components would be to compare the net PDOC with the gross PDOC. Gross PDO^{14}C can be prepared only if the processes of phytoplankton PDOC release and bacterial PDOC utilization in natural samples can be uncoupled. One way of doing this is to inhibit bacterial activity with antibiotics. Although this approach has sometimes been successfully applied to the study of natural plankton communities (7, 27, 47), inhibition of the bacteria is often incomplete and there can be a pronounced simultaneous inhibition of the photosynthetic organisms as well (28). In the 1989 experi-

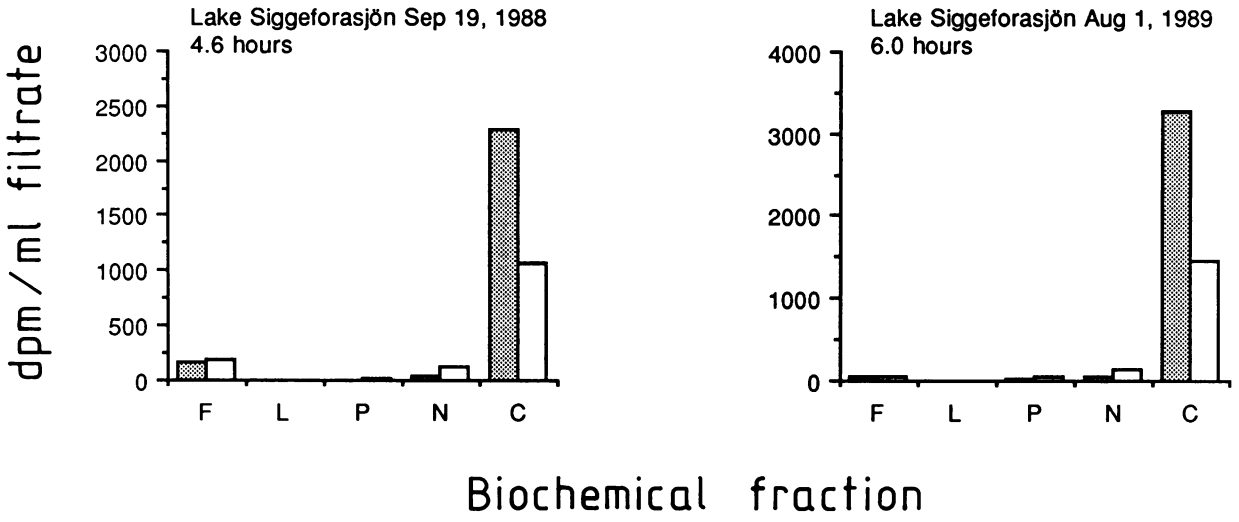

FIG. 3. Biochemical composition of the intermediate-MW peaks from Sephadex G-50 before and after heterotrophic incubations. Symbols and abbreviations are identical to those in Fig. 2.

ments of this study, streptomycin (added to 30 mg liter^{-1} [final concentration]) was added to samples in parallel with untreated samples. Unfortunately, however, addition of the antibiotic always reduced the total primary production by ca. 50% (data not shown), rendering the results of the antibiotic additions unreliable.

The total radioactivity of the high-MW peak in the second heterotrophic incubation at Lake Erken on 20 July 1988 and at Lake Siggeforasjön on 8 August 1988 did not change significantly during the incubation period (Table 1). The total radioactivity in the peaks, calculated as the sum of the individual biochemical fractions, however, decreased in the Lake Erken experiment and increased in the Lake Siggeforasjön experiment (Fig. 2). This difference can be explained by the fact that the ^{14}C recoveries obtained for the biochemical characterization of the high-MW peaks in PDO^{14}C collected before the heterotrophic incubation differed from those obtained for samples collected afterwards. Thus, the recoveries differed by 18% for the Lake Erken peak and by 20% for the Lake Siggeforasjön peak. Otherwise, differences in recovery were smaller than 15% and thus did not influence the results markedly.

Interpretation of the Sephadex G-50 chromatograms is not always a simple and straightforward process. For example, in the September 1988 experiment at Lake Siggeforasjön, the level of radioactivity in the low-MW peak was slightly higher after the heterotrophic incubation. Although it has been shown that most of the activity of extracellular hydrolytic enzymes is present in the particulate fraction (8, 22) and that hydrolytic cleavage and subsequent uptake can therefore be assumed to be tightly coupled processes, it is possible that some ^{14}C-labelled low-MW cleavage products re-enter the dissolved phase after enzymatic hydrolysis (23). Thus, even if there is some bacterial utilization of low-MW substances of true phytoplankton origin, this utilization may be hidden by their simultaneous production, and as a consequence radioactivity in the low-MW peak may increase. Another problem in the interpretation of the size fractionation chromatograms is that some of the low-MW substances taken up by bacteria may be transformed to macromolecules which are subsequently rereleased to the dissolved phase as high-MW polymers (14, 24). Although the extent to which this process influences the size distribution of the net PDO^{14}C cannot be evaluated, the presence of high-MW DO^{14}C after periods of incubation with $H^{14}CO_3^-$ much shorter than 24 h (48) suggests that most of the high-MW DO^{14}C was of direct phytoplankton origin.

Ecological implications. In contrast to the newly released PDOC of phytoplankton origin, of which a large part is readily available for bacterial utilization and often turns over rapidly (12, 27, 49), the total pool of DOC is characterized by fairly stable concentrations and low turnover rates. Accordingly, it has been demonstrated that most of the total DOC pool in the pelagic zone of lakes is resistant to bacterial utilization (45, 50). In most situations, the total DOC pool contains both high (in the range of thousands)- and low (in the range of hundreds)-MW components (1, 18, 52). It has been suggested that it is some of the low-MW components that are available to the bacteria, whereas the high-MW components are more or less refractory to utilization (17, 46). The work of Meyer et al. (35) and Tranvik (52), however, suggests that at least in humic systems, the difference in availability between the high- and low-MW DOC components is not large. The suggestion that high-MW substances can be important bacterial substrates is also supported by the fact that pelagic heterotrophic bacteria possess extracellular hydrolytic enzymes. Some attention has recently been focused on activity measurements of such enzymes (8, 33, 43). Although the present study does not reveal the continuous seasonal patterns of variation in the biochemical composition of PDOC or in the bacterial turnover rates of its different fractions, the experiments cover a wide range of trophic states and phytoplankton taxonomic composition (48) and therefore permit some general conclusions in this respect. Thus, the results of the heterotrophic incubations show that the low-MW PDOC components turn over much more rapidly than those of high MW. This finding indicates that PDOC contributes to the accumulation of high-MW refractory DOC in the pelagic zone of lakes, as suggested by Cole et al. (11). Moreover, assuming that variations in specific activity between the PDO^{14}C size fractions (see discussion above) do not seriously distort the major patterns of utilization, and keeping in mind that bacterial utilization of the low-MW PDOC components in

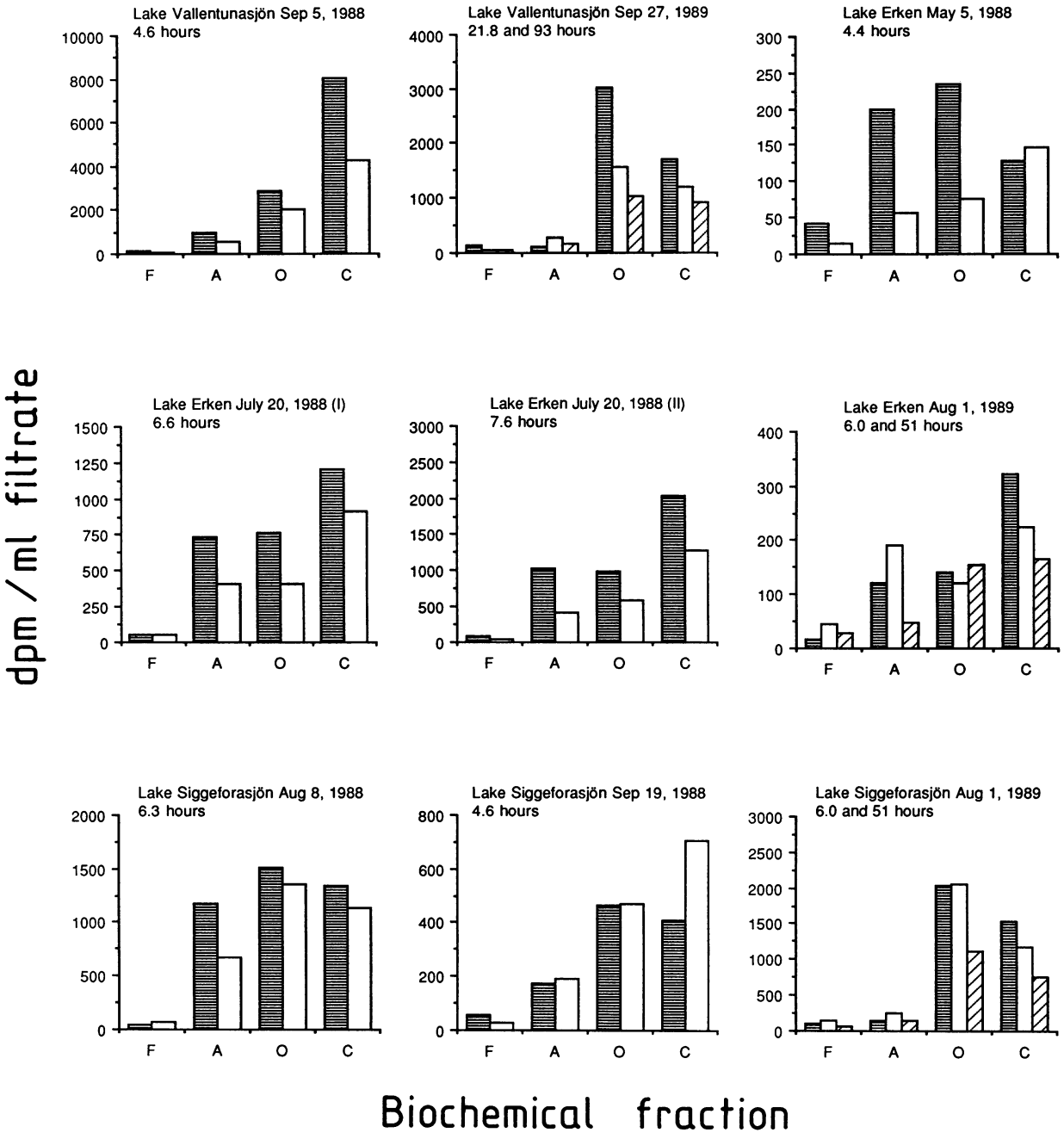

FIG. 4. Biochemical composition of the low-MW peaks from Sephadex G-50 before (▤) and after short (□) and long (▨) heterotrophic incubations. Abbreviations: F, precipitated during freezing and thawing; A, amino acids; O, organic acids; C, carbohydrates.

Lake Siggeforasjön could have been underestimated (see above), it is clear from Fig. 1 and Table 1 that on a quantitative basis, the results imply that the low-MW PDO^{14}C components are those generally of greatest importance to heterotrophic bacteria.

In view of the probable polysaccharide nature of the intermediate-MW substances that accounted for a very large fraction of the bacterial utilization of PDO^{14}C in Lake Siggeforasjön (and that sometimes, but to a lesser degree, were quantitatively important in Lake Erken), their MW is probably ca. 6,000. Polysaccharides of ca. 6,000 MW were also found to constitute the main part of the carbon flow from autotrophic phytoplankton to heterotrophic bacteria in a similar study in another moderately humic Swedish lake (47). It is not clear from the results of these studies, however, why intermediate-MW polysaccharides were so important in the humic lakes but not in the clearwater lakes. In fact, it is possible that compounds interpreted as high- and intermediate-MW PDO^{14}C resulted from binding of low-MW components of PDO^{14}C to higher-MW, unlabelled carbon skeletons (the concentrations of which are likely to be higher in humic than in nonhumic lakes) already present in the water (6, 35). Furthermore, Meyer et al. (35) found that the bacterial growth on high-MW components of DOC from a

TABLE 2. Theoretical bacterial PDOC turnover times estimated with the fractionated filtration and heterotrophic incubation techniques

Site and date	Heterotrophic incubation time (h)	Bacterial PDOC turnover time (h)	
		Heterotrophic incubation method	Fractionated filtration method
Lake Vallentunasjön			
5 Sept. 1988	4.6	14.6	36.1
27 Sept. 1989	21.8	81.8	33.5
27 Sept. 1989	93	141	33.5
Lake Erken			
5 May 1988	4.4	9.2	13.1
20 July 1988 (I)	6.6	17.6	45.8
20 July 1988 (II)	7.6	33.3	45.8
1 Aug. 1989	6.0	99.4	15.1
1 Aug. 1989	51	48.4	15.1
Lake Siggeforasjön			
8 Aug. 1988	6.3	31.9	19.0
19 Sept. 1988	4.6	16.5	42.0
1 Aug. 1989	6.0	45.8	37.3
1 Aug. 1989	51	68.9	37.3

humic river occurred at the expense of low-MW substances bound to refractory carbon skeletons. Two findings, however, provide some evidence against the hypothesis that the intermediate-MW peak in experiments at Lake Siggeforasjön resulted from abiotic binding reactions. (i) The Sephadex G-50 size distribution of the total DOC pool in Lake Siggeforasjön in August 1989 (DOC was measured by A_{254}) was characterized by a broad peak of fairly low MW and a smaller peak at the size exclusion limit of the G-50 gel, while absorbance was at a pronounced minimum at the position of the intermediate-MW PDO^{14}C peak (48a). Thus, if this peak were a result of abiotic binding, this would require binding to carbon skeletons which are present in comparatively low concentrations in the water. (ii) In September 1988, material from the low-MW PDO^{14}C peak from Lake Siggeforasjön was mixed with lake water filtered through a 0.2-μm-pore-size filter. After 3 days at in situ temperature in darkness, however, intermediate-MW PDO^{14}C was still not present. Although low-MW substances from gross PDO^{14}C should ideally have been used for this test, this finding nevertheless suggests that the intermediate-MW peak was of phytoplankton origin and not a result of abiotic adsorption processes. In any case, as long as the origin of the intermediate-MW peak in the humic lakes is somewhat uncertain, this peak should be interpreted with caution.

Although it has been established that a dominant part of the dissolved amino acids in marine pelagic environments occurs as combined amino acids (13, 34, 41), data from lakes are few and somewhat conflicting. Thus, Hama and Handa (18) found that the contributions of combined (MW, greater than a few thousand) and free dissolved amino acids (or very short peptides) were roughly equal, while Jørgensen and Søndergaard (30) found that a very large proportion of the dissolved amino acids was actually in free form. Oligopeptides (up to ca. six amino acids long) may be taken up directly, without prior hydrolysis, by heterotrophic bacteria (38), and Kirchman and Hodson (32) found that marine bacteria actually preferred peptides over amino acids. Thus, although the relative importance of combined amino acids in lakes cannot be established, it is likely that part of the decrease in the amino acid fraction during heterotrophic incubations results from bacterial uptake of short peptides.

It must be stressed that the heterotrophic incubation technique does not consider PDO^{14}C utilization by large bacteria or by bacteria attached to detrital aggregates or large phytoplankton. In the two September experiments at Lake Vallentunasjön, more than half of the [^{3}H]thymidine incorporation was confined to large or attached bacteria (>2-μm fraction [49]). The phytoplankton communities were dominated by colonial cyanobacteria (mainly *Microcystis* species). Bacteria in the gelatinous mucilage of the cyanobacteria probably accounted for a large part of the [^{3}H]thymidine incorporated in this size fraction (4, 39). It is likely that the mucilage bacteria meet a large part of their carbon demand by taking up organic compounds released by the host cyanobacteria (organic compounds which thus do not enter the truly dissolved phase), but it is possible that they utilize organic compounds from the truly dissolved phase as well. Since the heterotrophic incubation technique does not consider the activity of large or attached bacteria, the patterns of PDO^{14}C utilization in the September experiments at Lake Vallentunasjön were due to the activity of only the free and suspended bacteria. Omission of large or attached bacteria could have influenced the outcome of the rest of the heterotrophic incubations as well. This is not probable, however, since direct counts of acridine orange-stained cells (49) revealed that large or attached bacteria made only a small contribution to the total bacterial biomass in these cases.

In conclusion, the present study suggests that the size distribution and biochemical composition of PDOC released from phytoplankton vary greatly both between lakes and over time within lakes. Moreover, the particular blend of the PDOC components utilized by heterotrophic bacteria can vary considerably, and it appears that PDOC released from phytoplankton does not contain any component that is of quantitative importance to the heterotrophic bacteria in all situations. Because heterotrophic bacteria often strongly rely on PDOC as a carbon source, these results imply that the bacterial assemblage is subject to large seasonal variations in the amount and composition of utilizable substrates. Therefore, to sustain growth rates, the bacterial assemblage must continually alter its efficiency in utilizing various types of substrates. The possibility that pelagic bacterial assemblages can adapt to specific substrates was suggested by Bell and Sakshaug (3) and Fukami et al. (16). Such adaptation could occur in the form of taxonomic succession or as a change in the uptake kinetics of established taxonomic units. The results of the present study also suggest that despite the large variations in the biochemical nature of the low-MW PDOC utilized by the bacteria, the low-MW components of PDOC turn over much more rapidly and are, in general, quantitatively more important as bacterial substrates than are the high-MW components. In most situations, the high-MW components are processed at very low rates and probably contribute to the buildup of refractory high-MW DOC in pelagic environments. Studies in this area should focus on the patterns of seasonal variation in PDOC composition and the relationships between these patterns and the phytoplankton species composition. Furthermore, we need to learn more about how the uptake capacities of the bacterial assemblage respond to such variations in PDOC composition.

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