

Degradation of dissolved organic matter in oxic and anoxic lake water

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Abstract

Decades of conflicting results have fueled a debate about how O₂ affects organic matter (OM) degradation and carbon cycling. In a laboratory study, using both OM taken directly from a humic lake and chemically isolated fulvic acid, we monitored the mineralization of dissolved OM in freshwater under purely oxic and anoxic conditions, under oxic then anoxic conditions, and under anoxic then oxic conditions, for 426 d. Between 5% and 24% of the initial OM was mineralized, with most extensive mineralization occurring under purely oxic and anoxic–oxic conditions. A sequential change in the O₂ regime did not result in greater overall degradation, but initially anoxic conditions favored subsequent oxic mineralization. A substantially greater fraction of the OM was degraded than in previous shorter studies, with as much as 50% of the total OM degradation occurring after 147 d into the experiment. Three fractions of the degradable OM were identified: OM degraded only under oxic conditions (68–78%), OM degraded more rapidly under anoxic conditions than under oxic conditions (16–18%), and OM degraded at equal rates under both oxic and anoxic conditions (6–14%). The degradation patterns of natural dissolved OM from a humic lake and chemically isolated fulvic acid were very similar, which indicates a similar level of bioavailability. The difference between anoxic and oxic degradation was greater in our long-term studies than in previous short-term experiments, which indicates that the oxic and anoxic degradation potentials vary with increasing overall OM recalcitrance and that similar oxic and anoxic degradation rates can be expected in short-term experiments in which <30% of the long-term degradable OM is allowed to decompose.

The issue of how O₂ concentration affects organic matter (OM) degradation has received considerable attention in marine environments (Hedges and Keil 1995; Kristensen 2000). Under anoxic conditions, heterotrophic bacteria have a reduced energy yield per substrate unit consumed (Zehnder and Stumm 1988; Fenchel and Finlay 1995). In addition, the primary substrate attack by oxygenases or reactive oxygen species (e.g., H₂O₂) is restricted to oxic conditions (Zehnder and Svensson 1986; Schink 1988). Hence, OM degradation should be slower and less extensive in the absence of O₂ than under oxic conditions. Accordingly, many studies have indicated restricted degradation under anoxic conditions, which may explain the carbon preservation in marine sediments (e.g., Emerson and Hedges 1988; Harvey et al. 1995; Hedges and Keil 1995; Hedges et al. 1999). However, similar OM degradation rates under oxic and anoxic conditions are frequently found in short-term incubation studies (hours to weeks) with relatively fresh OM (e.g., Foree and McCarty 1970; Kristensen and Blackburn 1987; Lee 1992).

In addition, there is an emerging debate about how oscil-

lating redox conditions affect OM degradation. Aquatic OM is likely to be exposed to both oxic and anoxic conditions in water columns or in sediments, and sequentially oxic and anoxic conditions may stimulate the overall mineralization of OM (Aller et al. 1996; Hulthe et al. 1998). On the other hand, some authors have suggested that the bioavailability of OM is always greatest under oxic conditions and that the maximum mineralization of OM occurs under oxic conditions alone (Hedges and Keil 1995; Kristensen 2000; Sun et al. 2002).

Two primary explanations of the inconsistent results regarding the difference between oxic and anoxic decomposition have been proposed. One explanation refers to the degree of adsorption of OM to mineral particles. According to this hypothesis, most dissolved OM is equally available to both aerobic and anaerobic bacteria and will be degraded at similar rates regardless of the O₂ regime, whereas oxic conditions are necessary for the degradation of OM being tightly adsorbed to mineral particles (Mayer 1994*a,b*; Hedges and Keil 1995). The other explanation concerns the time of previous degradation—that is, the time during which the OM has been subjected to degradation (Henrichs and Reeburgh 1987; Hartnett et al. 1998; Hulthe et al. 1998; Hedges et al. 1999). Selective degradation will cause the remaining OM to be increasingly resistant to degradation. Thus, the labile OM, which is presumably equally accessible to enzymes regardless of the O₂ regime, will be depleted with time. Cor-

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respondingly, the proportion of more recalcitrant OM, which presumably can be accessed only by oxygenases or reactive oxygen species, will increase. Hence, a diagenetic change toward increased OM recalcitrance may restrict anoxic degradation more than oxic degradation.

Typically, previous studies focused on processes in marine sediments, acting over very long timescales, and/or relied on short-term incubation experiments (hours to weeks). We take a different perspective, focusing on the mineralization of lake water OM over intermediate timescales (months to years) under oxic, anoxic, and sequentially oxic–anoxic and anoxic–oxic conditions. We also simultaneously measured mineralization in two independent ways (the formation of CO₂ and loss of OM). Concentrations of electron acceptors were also measured, to provide additional insight about the pathways of carbon mineralization. Experiments were conducted under similar conditions as previous short-term studies (Bastviken et al. 2001), to allow the comparison of timescales. The questions addressed are (1) how does the time of degradation affect overall mineralization and differences between oxic and anoxic mineralization of dissolved OM, and (2) how do sequential oxic–anoxic or anoxic–oxic conditions affect OM mineralization? In addition, the change in O₂ regime during the experiment allowed the identification of subpools of OM as being differently susceptible to degradation under varying redox conditions.

Materials and methods

Experimental water—Water for one experiment (LILL) was retrieved from Lillsjön, a small oligotrophic, polyhumic lake in southern Sweden. The concentrations of total phosphorous, total nitrogen, and dissolved inorganic carbon were 0.5, 59, and 175 μmol L⁻¹, respectively, and the absorbance at 420 nm in water from this lake was 0.086 cm⁻¹. Equal parts of oxic surface water and anoxic bottom water were collected in plastic carboys using a submersible pump. The water was stored at 2°C until the following day, when the experiment was started. To ensure carbon limiting conditions, the water from Lillsjön was amended with PO₄³⁻ and NH₄⁺ to final concentrations of 3.7 μmol P L⁻¹ and 70 μmol N L⁻¹, respectively, immediately before the start of the experiment. For the second experiment (FA), we used artificial lake water, according to the method of Lehman (1980), with some modifications (Table 1). The only carbon source in this water consisted of Nordic reference fulvic acid isolated from a Norwegian lake using the method described by Thurman and Malcom (1981), obtained from the International Humic Substances Society (IHSS sample 1R105F; for chemical characterization, see <http://www.ihss.gatech.edu/chemistry.html>). Finally, a control for carbon contamination was based completely on Milli-Q water.

Experimental setup—Sixty liters of experimental water were filtered (using a 142-mm prerinsed Gelman A/E glass fiber filter) to remove particles and mixed in a plastic carboy. Some bacteria passed the filter and served as inoculum in the LILL experiment. The artificial lake water was inoculated with 0.6 liter of filtered water from LILL, to add a microbial community. No bacteria were added to the controls.

Table 1. Constituents and concentrations in the artificial lake water used in experiment FA (modified from Lehman 1980). NOR-FA denotes the fulvic acid, previously isolated from a Norwegian lake, that was added to the water as a source of organic carbon (see text for details).

Constituents	Final concentration (mg L ⁻¹)
Macro constituents	
CaCl ₂	4
MgSO ₄ × 7H ₂ O	2
NaHCO ₃	10
KHCO ₃	10
Na ₂ HPO ₄ × 2H ₂ O	0.6
NH ₄ Cl	3.8
Organic carbon (NORFA)	20 (equal to 10 mg C L ⁻¹)
Micro constituents	
FeCl ₃ × 6H ₂ O	0.7
CuSO ₄ × 5H ₂ O	0.01
ZnSO ₄ × 7H ₂ O	0.022
CoCl ₂	0.01
MnCl ₂ × 4H ₂ O	0.18
(NH ₄) ₆ Mo ₇ O ₂₄ × 4H ₂ O	0.006

For experiments LILL and FA, 900 ml of water was transferred from the carboy into each of 60 glass infusion bottles (1,000 ml each; Apodan). Half of the bottles in each experiment were made anoxic according to a two-step procedure. First, the water was purged with N₂ (N52, Air Liquide, <1 ppm O₂) for 1 h. Thereafter, the bottles were capped with Teflon-coated rubber stoppers (Apodan) under continuous N₂ flushing. The stoppers were secured with aluminum crimp-seal caps. In a second oxygen removal step, the capped bottles were connected to a vacuum pump and a N₂ supply via a valve, which allowed repeated evacuation of the headspace followed by the addition of N₂. The bottles were evacuated until boiling occurred on tapping the bottles with a plastic rod (<0.2 atm pressure in the bottles). Evacuation was sustained for 60 s and then followed by N₂ addition to ~2 atm pressure. The evacuation–N₂–addition cycle was repeated 9–14 times. The purging, together with the gas-exchange cycles, removed oxygen to below detection levels of both Winkler titration and an oxygen electrode (Orion model 835; detection limit of 0.005 mg L⁻¹). This procedure allowed minimal disturbance of the OM and bacteria compared with other oxygen removal methods such as boiling at 100°C or the addition of reducing agents such as sulfide. After the second removal step, excess N₂ was released from the anoxic bottles through a syringe needle until ambient air pressure was reached. To ensure that bottles for oxic treatments were oxygenated, they were subjected to gas-exchange cycles with artificial air (80% N₂ and 20% O₂; <1 ppm CO₂) instead of N₂.

Each experiment consisted of four treatments, as illustrated in Fig. 1. Treatments *anan* and *oxox* were anoxic and oxic, respectively, throughout the experiment (426 d). Treatment *anox* had anoxic conditions during the initial experiment period (period 1, lasting 147 d) and oxic conditions during the second period (period 2, lasting 279 d), whereas

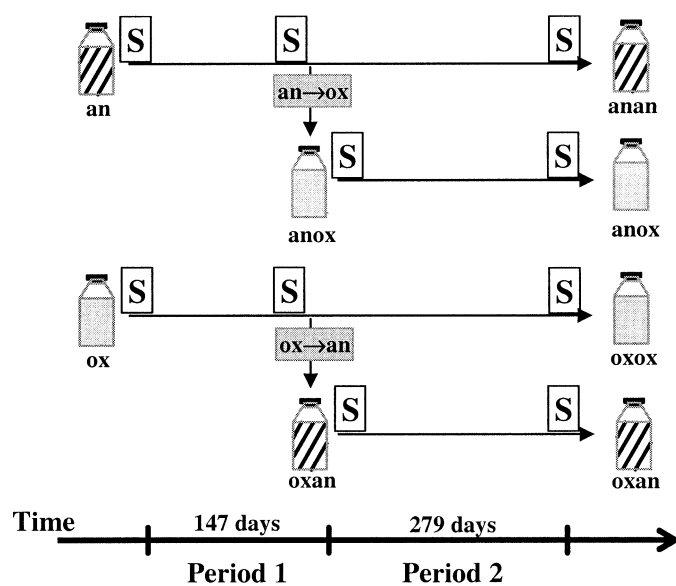


Fig. 1. Illustration of the experimental setup. For each experiment 30 anoxic (striped) and 30 oxic (gray) bottles were prepared. At each sampling, denoted by [S] in the figure, six bottles per treatment were sampled and thereby removed from the incubation. After 147 d, the oxygen regime was changed in half of the remaining bottles. This created four treatments. In two of those, the bottles were either anoxic or oxic throughout the whole 426-d experiment (denoted *anan* and *oxox*). In the other two treatments, the oxygen regimes had been changed from anoxic to oxic or from oxic to anoxic conditions (denoted *anox* and *oxan*, respectively). The experiment period from the initial to the middle sampling is referred to as period 1 in the text, and the second part of the experiment is denoted period 2. “an” and “ox” denote anoxic and oxic conditions, respectively, during period 1.

treatment *oxan* had oxic conditions during period 1 and anoxic conditions during period 2. Gas exchange with artificial air or N_2 , as described above, was used to change the O_2 regime in the *anox* and *oxan* bottles after period 1. Water from the oxic and anoxic bottles sampled 147 d into the experiment was used to inoculate the *anox* and *oxan* bottles, respectively, after the change in O_2 regime (8 ml of water was added per bottle). This was done to ensure that bacteria adapted to the new O_2 regime were present in the bottles. On each sampling occasion (Fig. 1), six replicate bottles per treatment were sampled and thereby withdrawn from the experiment. Bottles were sampled immediately prior to and immediately after the change in oxygen regime after period 1, to allow correction for effects induced by the gas exchange procedure. All bottles were incubated in the dark at 15°C, and the anoxic bottles were incubated submerged in water.

O_2 , nitrate, sulfate, and pH— O_2 concentrations were measured in subsamples from the bottles using an oxygen electrode (Orion model 835, detection limit of 0.005 mg L^{-1}). The opening of the anoxic bottles, as well as the O_2 measurements of their water, were carried out under N_2 atmosphere in a glove bag (I²R Instruments for Research and Industry), to avoid O_2 contamination.

Filtered water samples (Whatman GF/F) for nitrate

(NO_3^-) and sulfate (SO_4^{2-}), representing alternative electron acceptors under anoxic conditions, were transferred to pre-washed 20-mL scintillation vials and preserved by freezing before analysis. Concentrations were measured spectrophotometrically (Wetzel and Likens 1991) using an autoanalyzer system (Autoanalyzer 3; Bran-Luebbe). Nitrate and sulfate concentrations were analyzed in water from the LILL and FA experiments only.

Oxic and anoxic microbial processes affect pH differently. Under oxic conditions, CO_2 is a major pH-affecting product of the microbial metabolism, which leads to lowered pH values. In addition, nitrification (i.e., ammonia oxidation) can contribute to a lowered pH. Under anoxic conditions, microbial processes such as reduction of NO_3^- , Mn(IV), Fe(III), and sulfur compounds may increase the pH (i.e., counteract the effect of CO_2 formation). Therefore, we measured pH in a subsample from each bottle using a Radiometer PHM 93 pH meter.

OM mineralization—The total OM mineralization was estimated from the accumulation of CO_2 and CH_4 in the bottles. Plastic syringes (20 and 60 ml, Plastipak; Becton Dickinson) were used to withdraw samples. To retrieve headspace samples, 20 ml of the headspace gas in each bottle was withdrawn and transferred into an evacuated glass vial with a rubber septum. These vials were used in the autosampler for the GC (Varian 3800 with FID and methanizer) that simultaneously measured CO_2 and CH_4 (Klemetsson et al. 1997). For sampling dissolved gases, 60 ml of water was transferred to evacuated 120-ml infusion bottles holding 240 μl 2 mol L^{-1} H_2SO_4 and 20 g NaCl. This made the sample acidic and saturated with NaCl, which preserved the sample and facilitated the extraction of CO_2 into the headspace. Fifty milliliters of N_2 was added to each 120-ml bottle, to normalize the pressure. After equilibrating the gases between the headspace and the water, the headspace was sampled, transferred to evacuated glass vials, and analyzed on the GC (see above). Initial, middle, and final concentrations were recalculated to $\mu moles$ CO_2 and CH_4 formed per liter of water during each experiment period. The gas partitioning between the headspace and the water in the 120-ml infusion bottles was corrected for in the calculations. The mineralization corresponds to the accumulation of mineralization products during each time period (Fig. 1). To enable comparison of time periods, the mineralization was normalized to the length of each period and expressed as μmol C L^{-1} d^{-1} .

Dissolved organic carbon (DOC), particulate organic carbon (POC), bacterial biomass, and absorbance—Concentrations of DOC were measured initially and after the entire 426-d experiment period. Duplicate samples from each bottle were filtered (Whatman GF/F glass-fiber filter) and analyzed on a Shimadzu TOC-5000 system (Sugimura and Suzuki 1988; Benner and Strom 1993). POC was analyzed in the final samples only: 100–400 ml of water was filtered through precombusted Whatman GF/F filters, and the carbon content on the filters was analyzed using a LECO CHNS-932 analyzer.

For the determination of bacterial abundance and biomass, 5-ml samples were withdrawn and preserved by the addition

of borax-buffered formaldehyde to a 2% final concentration. Bacterial abundance was determined using flow cytometry (Becton-Dickinson FACSCalibur and CellQuest 3.1 software) after staining cells with Syto 13 (Molecular Probes) as described by del Giorgio et al. (1996). Bacterial cell volumes were measured using image analysis of 4',6'-diamino-2-phenylindole-stained cells using an epifluorescence microscope. Subsequently, cell volumes were converted to bacterial dry weight (m_b) using the equation $m_b = 435 \times V^{0.86}$ (Loferer-Krößbacher et al. 1998). To estimate bacterial carbon biomass, we assumed that carbon made up 50% of the bacterial dry weight.

Statistics—When testing for statistical differences between groups, we used common parametric tests (e.g., Student's *t*-test or analysis of variance [ANOVA]), using SPSS 10.0 for Windows. When ANOVA was used, it was combined with the Tukey HSD (honestly significantly different) method for post hoc tests of specific differences (Sokal and Rohlf 1995). Significant differences were also tested with corresponding nonparametric tests (e.g., the Mann-Whitney or Kruskal-Wallis tests), which always yielded similar results, indicating that the assumption of normally distributed data did not affect the comparisons. The significance level was 5% for all tests. Unless otherwise specified, results are reported as average \pm 1 SD or, alternatively, as ranges that include all measurements.

Results

O_2 , nitrate, sulfate, and pH—The O_2 concentrations were always below the detection limit in the anoxic treatments. In the oxic bottles, O_2 levels ranged 8.07–9.99 mg L⁻¹ initially. The O_2 levels decreased to 50–65% of the initial values in the oxic treatments of experiments LILL and FA but remained unchanged during the control experiment.

Initial nitrate concentrations were below or close to the detection limit and remained there in the LILL_{anan} and FA_{anan} treatments (Fig. 2, upper panels). In the LILL_{oxox} and FA_{oxox} treatments, nitrate levels increased throughout the whole experiment period. During period 2, nitrate concentrations decreased to the detection limit (LILL_{oxan}), remained unchanged (FA_{oxan}), or increased to the levels of the oxic treatments (LILL_{anox} and FA_{anox}; Fig. 2). Sulfate levels were more stable, and the most striking change was a small but consistent decrease in the LILL_{anan} treatment (Fig. 2, lower panels).

The variability in pH within groups was low, with a coefficient of variation <1%. The initial pH was 6.50 in the LILL experiment and 7.50 in the FA experiment. In the oxic treatments, pH decreased to 4.55 (LILL_{oxox}) and 5.25 (FA_{oxox}), whereas pH values remained similar in the anoxic treatments (6.88 in LILL_{anan} and 7.16 in FA_{anan}; final sampling). In treatments LILL_{oxan} and FA_{oxan}, the pH decreased during period 1 to 4.90 and 5.68, respectively, and then increased during the anoxic period 2 to 5.88 and 6.30. In the LILL_{anox} and FA_{anox} treatments, the pH remained at initial levels during period 1 and decreased to the final levels of the oxic treatments during period 2.

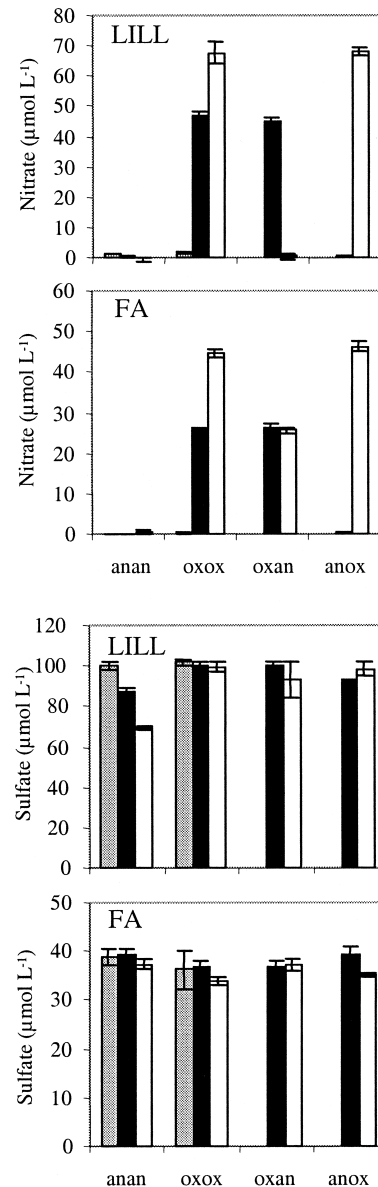


Fig. 2. Nitrate (NO_3^-) and sulfate (SO_4^{2-}) concentrations in the LILL and FA experiments. Average \pm 1 SD is shown. Gray, black, and white bars represent initial, middle, and final samples, respectively. *anan* and *oxox* denote treatments being anoxic or oxic throughout the entire experiment, and *oxan* and *anox* refer to treatments in which the O_2 regime was changed from oxic to anoxic or from anoxic to oxic conditions, respectively, after sampling at 7 months (see Fig. 1). Note the different y-axis scales.

OM mineralization—There was no accumulation of mineralization products in the control experiment. Methane was absent in all of the experiments, which indicates that the organic carbon mineralization primarily resulted in the formation of CO_2 . The oxic mineralization was 3–4 times greater than the anoxic mineralization, and overall mineralization was about twice as high in the LILL experiment as in the FA experiment (Fig. 3). In treatments with an oxic final step (*oxox* and *anox*), similar amounts of OM were mineralized during the 426-d experiment (one-way ANOVA for each ex-

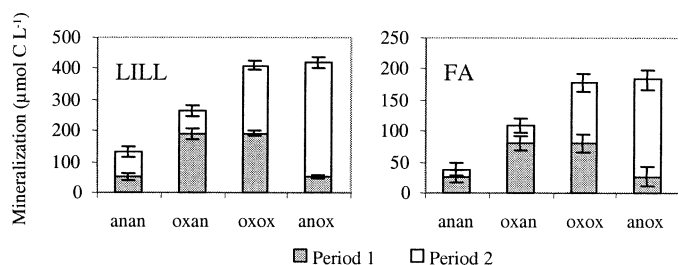


Fig. 3. Total organic matter mineralization (i.e., formation of CO₂ and CH₄) in the LILL and FA experiments. Average ± 1 SD is shown. Gray and white bars represent the mineralization during the first and the second experiment period (see Fig. 1). The treatments are explained in Fig. 1. Note the different y-axis scales.

periment comparing total mineralization in all four treatments, $P = 0.795$ for LILL and 0.873 for FA in the post hoc tests; Fig. 3). The other treatments were significantly different from each other and from the *oxox* and *anox* treatments, as seen in Fig. 3 ($P < 0.001$ in the post hoc tests). The order of the different oxygen regimes affected mineralization, as illustrated by a two-way ANOVA with total mineralization as the response variable and the oxygen regime during periods 1 and 2 as independent factors ($P < 0.001$ for the interaction).

In the LILL experiment, the *anan* mineralization rate during period 1 and the rates in the *anan* and *oxan* treatments during period 2 were similar (one-way ANOVA, $P = 0.1$ – 0.99 in the post hoc tests; Fig. 4). The mineralization rate was intermediate in the *oxox* treatment during period 2, and the highest rates were found in the *oxox* treatment during period 1 and in the *anox* treatment during period 2, which both had similar rates. The same pattern was found in the FA experiment, with the only exception being that mineralization rates during period 2 in the *anan* treatment were significantly lower than during period 1 ($P < 0.001$; Fig. 4). However, as in the LILL experiment, there was no significant difference between *anan* and *oxan* rates during period 2 ($P = 0.25$ in the post hoc test).

DOC, POC, bacterial biomass, and carbon budget—There was no accumulation of DOC in the control bottles with Milli-Q water, and average DOC concentrations were always $<45 \mu\text{mol L}^{-1}$ (0.54 mg L^{-1}). Initial DOC concentrations were $1,987 \pm 43$ and $806 \pm 12 \mu\text{mol L}^{-1}$, respectively, in the LILL and FA experiments. The concentration of DOC decreased significantly in all treatments during both experiments (t -tests, $P < 0.001$), and this decrease was smallest in the *anan* treatment, followed by the *oxan* treatment, and then by the *oxox* and *anox* treatments, which had the greatest loss of DOC (Fig. 5). The final POC concentration corresponded to a minor part of the DOC loss (6–17%; Fig. 5). Because we did not measure POC initially, particles that would be recovered on filters immediately after the initial filtration and experimental setup were not considered. Hence, our final POC concentration possibly overestimates the formation of POC. However, a microscopic examination of bacterial samples showed that nonbacterial particles were rare initially and much more abundant in the final samples. Therefore, we assume that final POC concentrations primar-

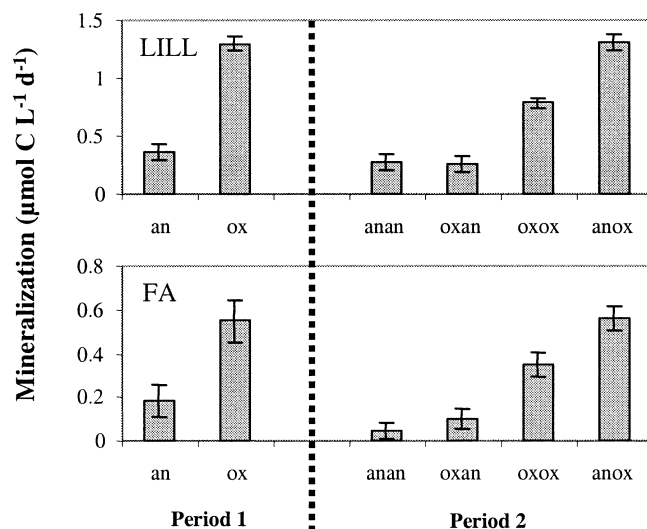


Fig. 4. Time-normalized organic matter mineralization during the two experiment periods in the LILL and FA experiments. Average ± 1 SD is shown. See text for details. The treatments are explained in Fig. 1. Note the different y-axis scales.

ily reflect the POC formation during the experiment. This assumption is conservative, given that POC was measured to make sure that it did not constitute a major carbon pool compared with DOC, the DOC loss, or the mineralization. In absolute terms, the POC formation was greatest in the

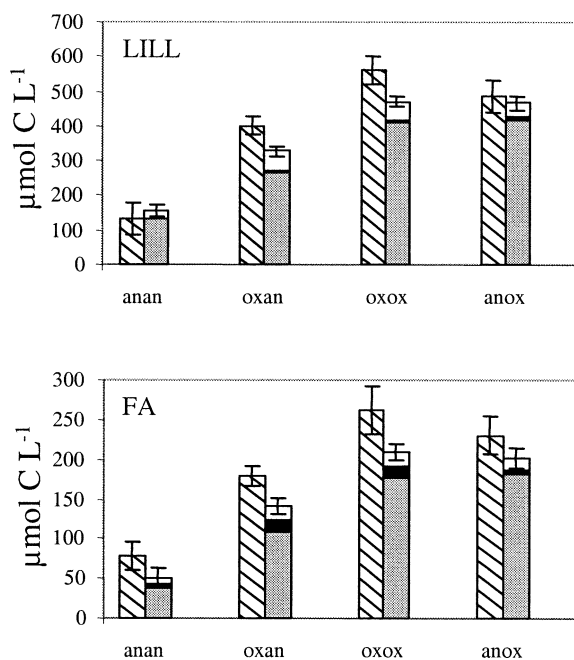


Fig. 5. Carbon budgets in which the loss of DOC (striped bars) is compared with the formation of mineralization products (gray bars), bacterial biomass (black bars), and POC (white bars) in the LILL and FA experiments. Average ± 1 SD is shown (for the combined bars; error bars represent the combined SD for all carbon pools included). The treatments are explained in Fig. 1. Note the different y-axis scales.

treatments having oxic conditions during at least one time period (Fig. 5).

The net formation of bacterial biomass was small compared with the DOC loss (1–8%; Fig. 5) and corresponded to 2.9–8.3 $\mu\text{mol L}^{-1}$ of C in the LILL experiment and 4.5–14.2 $\mu\text{mol L}^{-1}$ of C in the FA experiment. Most of the increase in bacterial biomass occurred during period 1 of the experiments.

Including the mineralization, the accumulation of bacterial biomass, and the POC formation, we recovered 66–119% of the DOC loss (Fig. 5). The mineralization represented the largest DOC loss, constituting 76–90% of the recovery. The proportion of the initial DOC being degraded during the experiments (i.e., being mineralized or transformed to bacterial biomass) varied between the treatments, corresponding to 5–7% and 13–15%, respectively, in the *anan* and *oxan* treatments. In the *oxox* and *anox* treatments, 21–24% of the initial DOC was degraded in both experiments.

Discussion

O₂, other electron acceptors, and pH—Both O_2 and pH measurements indicated that O_2 regimes clearly differed among treatments. Initial nitrate concentrations were low or below the detection limit. Under oxic conditions, nitrate was always formed in both experiments, presumably because of nitrification (ammonium oxidation). Nitrate was always consumed under anoxic conditions in the LILL experiment, which indicates that denitrification occurred during the second period of the LILL_{oxan} treatment, and a small amount of sulfate reduction occurred in the LILL_{anan} treatment (Fig. 2). In the FA experiment, nitrate concentrations remained constant in the FA_{oxan} treatment during period 2 (Fig. 2, upper panels). A potential explanation for this pattern is that denitrifying bacteria never established populations large enough to affect nitrate concentrations in the FA experiment. Sulfate reduction was probably of minor importance in the FA experiment (Fig. 2, lower panels). However, the high pH after anoxic periods in both experiments indicates that Mn(IV) and Fe(III) reduction may have been important, along with fermentation, in the anoxic treatments. Unfortunately, because of a lack of data, it is not possible to evaluate the importance of electron acceptors other than nitrate and sulfate.

OM transformations—The degradation pattern was very similar for the natural OM in both the LILL and the FA experiments. Similar fractions were degradable, and the differences between oxic and anoxic degradation were similar (Figs. 3, 4). This probably reflects that fulvic acids constitute a major OM pool in lake water (Thurman 1985), especially in humic lakes such as Lillsjön. These results also suggest that chemically isolated fulvic acids are not necessarily more refractory than untreated OM in natural waters. In addition, the isolation procedure is rather crude, and fulvic acids may include several groups of compounds, such as carbohydrates and amino acids (<http://www.ihss.gatech.edu/chemistry.html>).

The OM mineralization, together with the accumulation of bacterial biomass and POC, accounted for 66–119% of

the DOC loss (Fig. 5). Some of the DOC loss not accounted for may have been due to biofilm formation in the bottles. Overall OM bioavailability (i.e., the mineralization plus the accumulation of bacterial biomass) was higher than the bioavailability reported in previous short-term studies of oligotrophic lake water with varying humic content. Previous studies were generally done under oxic conditions, and the OM bioavailability was between 6% and 14% of the total DOC concentrations (Tranvik 1998). This is in the same range as in our oxic treatments during the period 1 (9–10% of the initial DOC was mineralized during this period), but considering both experiment periods, the OM availability was 21–24%, which reflects that more DOC can be degraded given a longer time of degradation. The substantial OM degradation in the *oxox* treatments during period 2 (Fig. 3) demonstrates that as much as 50% of the bioavailable material is still left to be degraded after 147 d of previous degradation.

The anoxic OM availability was 5–7% of the initial DOC. In a previous short-term anoxic batch-culture experiment with water from Lillsjön, the average accumulation of bacterial biomass corresponded to 5 $\mu\text{mol L}^{-1}$ (60 $\mu\text{g C L}^{-1}$; Bastviken et al. 2001). Under the assumption of a bacterial growth efficiency of 0.1–0.5 (del Giorgio and Cole 1998), this corresponds to a degradation of 10–50 $\mu\text{mol C L}^{-1}$ and to a short-term bioavailability of 0.5–2.6% of the initial DOC. Thus, the long-term OM availability under both oxic and anoxic conditions was greater than short-term estimates. Hence, the bioavailability, or the degradable fraction of the OM, clearly seems related to the time of degradation and should not be regarded as an absolute measure that is independent of the experimental conditions (*see also* Berner 1980). Experimental assessments of the labile fraction of dissolved OM are typically run for ≤ 2 weeks (Søndergaard and Middelboe 1995) and are set up as batch cultures. OM that is degraded within this time span, which includes minimal turnover of bacterial biomass, is defined as “bioavailable.” Such short-term degradation experiments may be highly relevant for studies concerning certain issues, such as food-web dynamics and nutrient remineralization. Our experiments were run on a timescale that is more relevant to the residence time of allochthonous OM in lakes (months to years) and highlight that the OM that is degraded within the first few weeks constitutes only a minor fraction of the OM that can be degraded over ecologically relevant timescales.

Most of the DOC loss could be attributed to mineralization, and the pattern was very similar in the LILL and the FA experiments (Figs. 4, 5). The oxic mineralization rates were 3–4 times greater than the anoxic rates. In combination with the results of previous experiments that have shown similar short-term oxic and anoxic OM degradation rates in water from Lillsjön and other lakes (Bastviken et al. 2001), this indicates that the time of degradation increases the differences between oxic and anoxic degradation rates. In other words, OM availability under oxic conditions seems greater not only for OM adsorbed to mineral particles (Hedges and Keil 1995) but also for partially degraded dissolved OM. It is widely recognized that partial degradation increases the overall recalcitrance of the OM, and it has been shown that the difference between oxic and anoxic degradation increas-

es with the age of the OM in marine sediments (Hulthe et al. 1998). However, it is not known whether this was due to the time of previous degradation per se or to the time of previous degradation in combination with adsorption to mineral particles so that the fraction of tightly adsorbed OM increased with time because of degradation processes. In our experiments with filtered water, on the other hand, partial degradation alone was likely the most important factor affecting OM recalcitrance and differences between oxic and anoxic degradation. The diagenetic change occurring during prolonged exposure and partial degradation in the environment includes the transformation of OM into compounds that are not recognizable by specific enzymes (McKnight 1998). Therefore, the ability to use oxygenases or reactive oxygen species in the primary attack may increase the oxic substrate availability not only for adsorbed OM but also for partially degraded dissolved OM.

These results indicate that the degradable OM fraction belonged to three different pools of OM. First, more OM was mineralized under oxic conditions (Fig. 3), which indicates that one pool of the degradable OM was only accessible under oxic conditions during the time course of the experiment (pool size = total oxic mineralization – total anoxic mineralization). Second, the time-normalized OM mineralization during period 2 in the *oxan* and *anan* treatments was similar in spite of a previous oxic mineralization in the *oxan* treatment during period 1 that was greater than the total mineralization in the *anan* treatments when both experiment periods are considered (Figs. 3, 4). This strongly indicates that OM mineralized during period 2 in the *oxan* treatments belonged to a pool of OM that was more susceptible to anoxic than to oxic degradation. Finally, the total OM mineralization was similar in the *oxox* and *anox* treatments, which suggests that all the OM that was bioavailable under anoxic conditions was also accessible under oxic conditions (i.e., 100% of the degradable OM was bioavailable under oxic conditions). Hence, the anoxic degradation not accounted for by the second pool above indicates the existence of OM being equally accessible under oxic and anoxic conditions.

Therefore, we suggest that the degradable OM can be divided into (1) OM that is only degraded under oxic conditions, (2) OM that is degraded more rapidly under anoxic than oxic conditions, and (3) OM that is degraded at similar rates under both oxic and anoxic conditions. The first pool probably contains highly refractory partially degraded OM that can only be accessed using oxygenases or reactive oxygen species (see above). The second pool has to consist of OM for which anoxic degradation is superior. Such organic compounds could have been present in the original OM or may have been formed under oxic conditions during period 1 of the experiment. Regardless of whether the OM was formed in the cultures or present originally, the demonstration of an OM pool that is more degradable in the absence of oxygen is controversial. Halogenated OM represents a well-studied group of compounds that may be preferentially degraded under anoxia (e.g., Armenante et al. 1999). However, the abundance of halogenated OM is generally low and can only account for a minor fraction of the OM that we found to be preferentially degraded in the absence of oxygen. Hence, further studies are needed to confirm and provide

biochemical support for the preferential anoxic degradation of OM. The third pool presumably contains simple low-molecular-weight compounds that are easily accessible for bacteria regardless of the O₂ regime. By simple subtraction procedures using the data in Fig. 3, we estimate that these pools constituted 68%, 18%, and 14% of the degradable OM in the LILL experiment and 78%, 16%, and 6% in the FA experiment, respectively. Thus, under the assumption that the third pool is most rapidly degraded, similar oxic and anoxic degradation rates can be expected in short-term experiments in which ~30% or less of the long-term degradable OM is allowed to decompose.

To our knowledge, different degradable pools of OM in freshwater environments have not been described and quantified previously. Hence, we rely on comparisons with OM from marine environments, although we are aware that the characteristics of the OM, as well as the timescales of degradation, differ between marine and freshwater environments. It has previously been suggested that OM in marine sediments can belong to one of three fractions from a degradation perspective: OM that is completely mineralized regardless of the redox condition, OM that is associated with particles that degrades slowly in presence of O₂ but not at all under anoxic conditions, and OM that is completely refractory (Hedges and Keil 1995; Kristensen 2000). Potentially, this is consistent with our results, but our results also suggest the presence of an OM pool being degraded most rapidly under anoxic conditions and show that oxic degradation can be superior also for OM that is not associated with particles.

The rapid degradation during period 2 in the *anox* treatments shows that previous anoxic degradation enhanced subsequent oxic degradation (Figs. 3, 4). Accordingly, Hulthe et al. (1998) found that the reexposure to O₂ of previously anoxic sediments promoted OM mineralization. This was possibly due to formation of easily degraded fermentation products (e.g., low-molecular-weight fatty acids and alcohols) during the anoxic period. Generally, the concentrations of fermentation products are very low in the environment, which suggests that terminal degradation steps rapidly consume all fermentation products formed (Kristensen et al. 1995; Postma and Jakobsen 1996). If fermentation products accumulated in the anoxic bottles, our measurements of mineralization underestimate the overall anoxic OM degradation.

In contrast to previous short-term studies, we show that the oxic mineralization of dissolved OM was clearly more extensive than the anoxic mineralization. This supports the idea that the extent of previous diagenetic change (i.e., the time of previous decay) affects the difference between oxic and anoxic degradation rates. Together with the identification of three subpools of degradable OM (see above), this provides a potential explanation for some inconsistencies between short-term studies and studies of long-term OM degradation in stable environments. Sequential oxic–anoxic or anoxic–oxic degradation did not increase the overall mineralization, but previous anoxic degradation enhanced subsequent oxic degradation, and anoxic–oxic degradation yielded the same mineralization as purely oxic conditions. Finally, our results indicate similar extent and patterns of the

microbial degradation of isolated fulvic acids and natural bulk lake water DOM.

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