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Rates of Microbial Processes in Sediments [and Discussion]

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Rates of microbial processes in sediments

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The following methods of measurement of sediment parameters are discussed: (1) rate of diffusional exchange of reactants and products across the sediment–water interface; (2) the concentration profiles of these reactants and profiles in the sediment, and (3) profiles and integrated rates of reactions in the sediment. The interaction of the processes of reaction and diffusion are illustrated using a simulation model. The effect of increasing organic loading (6.2, 37.2 and 62.0 mmol $C\ m^{-2}\ d^{-1}$), with organic matter distributed in three ways: close to the sediment surface, a linear gradient downwards or evenly mixed throughout the sediment. Predictable increases in anoxic processes occurred with increasing organic loading. There were higher diffusional losses of dissolved organic matter when organic degradation occurred close to the sediment–water interface. The model predicted that degradation of organic matter at depth could have the following effects: an increase in the depth of oxygen penetration, an increase in the ratio of CO_2/O_2, and an increase in nitrification and denitrification.

1. Introduction

One very important interest is in marine sediment processing of detrital organic matter which has entered the sediment from the overlying water. What is of most concern is the extent to which organic matter is mineralized and the state of the products of mineralization. The extent of mineralization determines the amount of organic matter that is buried, not to reappear in thousands of years and potentially may be the source of future fossil fuels. The products of organic matter mineralization are primarily C-, N-, and P-compounds. The latter do not participate in oxidation–reduction reactions and are always in the form PO_4^{3-}. Carbon is oxidized completely to CO_2, but nitrogen can exist in several oxidation states, of which the most important are NO_3^-, NH_4^+ and N_2. Dinitrogen cannot be used as a nitrogen source by most primary producers. Processes which lead to the production of N_2 are thus important as they determine the availability of mineralized nitrogen. Similarly, processes which lead to the burial of organic nitrogen determine the extent to which this essential nutrient is recycled from the sediment to the pelagic primary producers. Dinitrogen is produced mostly from NO_3^- which may be present in the overlying water or may be produced in the sediment. The extent of denitrification depends on the availability of NO_3^- and on its entry into anoxic environments. In this paper, emphasis will be placed on the processes of nitrification and denitrification and the manner in which denitrification is affected by other sediment processes.

The processing of organic matter within sediments is studied by three main methods: (1) measurement of fluxes of reactants and products across the sediment–water interface; (2) measurement of concentrations of reactants and

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products in the sediment pore water or sediment solid phase; and (3) measurement of rates of reactions in the sediment layers. Some of these methods used to measure process rates are described and evaluated. The results of a simulation analysis are then presented to illustrate the effect of the quantity and distribution of POC on the relative importance of these processes in sediments. This type of simulation makes possible the analysis of interactions at a resolution impossible by other methods. It is only feasible to measure certain parameters in real systems and never to separate out specific influences.

2. Methods

Measurement of fluxes across the sediment–water interface usually involves sediment cores with some overlying water, usually stirred and capable of being closed to prevent loss or gain of gasses (Blackburn 1987*a*). Typically, fluxes are measured of O_2, of components of the nitrogen cycle (NO_3^-, NH_4^+, N_2 and dissolved organic nitrogen) and of the carbon cycle (CO_2 and dissolved organic carbon) in batch or with continuous perfusion (Blackburn 1987*b*). There are variants on this methodology in which areas of the sea floor are enclosed and fluxes measured (Rutgers van der Loeff *et al*. 1984).

Recent measurements of dissolved nutrients in pore water have emphasized the importance of obtaining high resolution profiles. This can be readily done for O_2 (Revsbech *et al*. 1980), HS^- (Blackburn *et al*. 1975), but there are problems with the measurement of NH_4^+ and NO_3^- in small volumes (Blackburn & Blackburn 1993*b*). A recent method for NH_4^+ and CO_2, which requires less than 100 µl samples should be suitable for high resolution pore water analysis (Hall & Aller 1992).

Sulphate reduction rate measurements must account for the ^{35}S-label which is trapped in pyrite and elemental sulphur (Howarth & Jørgensen 1984). Rates of organic nitrogen mineralization can be measured by the dilution of added $^{15}NH_4^+$ (Blackburn 1979), by the turnover of amino acids (Christensen & Blackburn 1980), and of urea (Lund & Blackburn 1989). Intermediate carbon metabolites may be measured by the turnover of acetate (Christensen & Blackburn 1982). Nitrification rates are generally measured by the use of inhibitors leading to the accumulation of NH_4^+ (Sloth *et al*. 1992). Rates of denitrification have been measured by the inhibition of N_2O reduction by acetylene (Sørensen 1978), a method which is not satisfactory in all situations (Seitzinger 1988). Better methods involve the measurement of N_2 production in sediment cores incubated under a 20% atmosphere of O_2 in an inert gas (Seitzinger 1988) and by N_2 flux from sediment enclosures (Devol 1991). A more versatile method uses the addition of $^{15}NO_3^-$ to water overlying sediments and measuring the rate of production of $^{15}N^{14}N$ and $^{15}N^{15}N$ (Nielsen 1992). Exclusive production of $^{15}N^{14}N$ indicates denitrification from sediment-produced NO_3^-, whereas $^{15}N^{15}N$ production is dependent on sources of NO_3^- external to the sediment. These rates and those of other sediment processes can be incorporated into models, in which interrelationships and interdependencies may be investigated.

Modelling of diagenetic processes was stimulated by the pioneering work of Berner (1975). Many of the models are constrained by the number of components that can be used; this is often restricted to two. The initial models and modifications of these (Jahnke *et al*. 1989) partitioned the sediment into a maximum of three zones. Nitrification occurred in the upper zone, sometimes with the consumption of O_2, while denitrification consumed NO_3^- in the lower anoxic zone. Organic matter degradation was coupled to NO_3^- production or NO_3^- production to O_2 consumption

Table 1. *Distribution of particulate organic carbon (POC) in the three sediment types*

		concentration of POC/(mmol cm^{-3})		
layer	thickness/cm	TOP	LINEAR	MIXED
water	2000.00	0	0	0
sediment	0.05	116.01	11.35	1.62
sediment	0.05	0.08	11.27	1.62
sediment	0.05	0.08	11.19	1.62
sediment	0.05	0.08	11.10	1.62
sediment	0.10	0.08	5.47	1.62
sediment	0.10	0.08	5.39	1.62
sediment	0.10	0.08	5.30	1.62
sediment	0.20	0.08	2.57	1.62
sediment	0.40	0.08	2.49	1.62
sediment	0.80	0.08	1.16	1.62
sediment	1.60	0.08	0.50	1.62
sediment	1.60	0.08	0.17	1.62

by zero-order reactions. None of these models included consumption of O_2 by carbon or the diffusion of NH_4^+ from lower sediment layers. A system dynamic model has been described which accounted for all the major sediment processes and which linked reactants and products by diffusion equations (Blackburn 1990). This model had restricted spatial resolution. A new modelling environment was designed and the processes in sediment were simulated by a system dynamic reaction–diffusion model (Blackburn & Blackburn 1993*a*), which had been shown to reproduce sediment O_2, NH_4^+, and NO_3^- profiles (Blackburn & Blackburn 1993*b*). The model had 20 m overlying water, which served as a source for O_2 and as a sink for products diffusing from the sediment. There were 12 sediment layers ranging in thickness from 0.05 cm at the surface to 1.6 cm at 5.3 cm depth (table 1). The present model was run under steady-state conditions, to facilitate comparison of the results of changes in the initial conditions. Steady-state was achieved by having a very large reservoir of particulate organic carbon (POC), 62 mol m^{-2}. POC was hydrolysed to dissolved organic carbon (DOC) at rates which did not significantly deplete the reservoir. Three rates of hydrolysis were used: 6.2, 37.2 and 62.0 mmol C m^{-2} d^{-1}, determined by the first-order rate constants of 1×10^{-4}, 6×10^{-4} and 10×10^{-4} d^{-1}. The POC was distributed in three different ways: (1) mostly in the top sediment layer (TOP), (2) in a linear gradient from the sediment surface downward (LINEAR), and (3) evenly distributed throughout the sediment (MIXED). There was no POC in the water and no reactions were allowed to occur in the water. PON was $\frac{1}{6}$ of POC and had the same rate constant of hydrolysis. The rate constants (d^{-1}) for nitrification, denitrification, DOC oxidation by O_2, HS^- oxidation by O_2 and sulfate reduction were 30, 30, 30, 30 and 5 respectively. Oxygen completely inhibited denitrification and sulphate reduction at 30 nmol cm^{-3}. Oxygen-dependent processes were at V_{max}, when O_2 was greater than 0 mmol cm^{-3}. Diffusion coefficients were in the range 1.64 to 1.81 cm^2 d^{-1}, except for DON and DOC which were 0.5 cm^2 d^{-1} and HS^- which was zero, on the assumption that all sulfide reacted with iron and did not diffuse (Blackburn & Blackburn 1992).

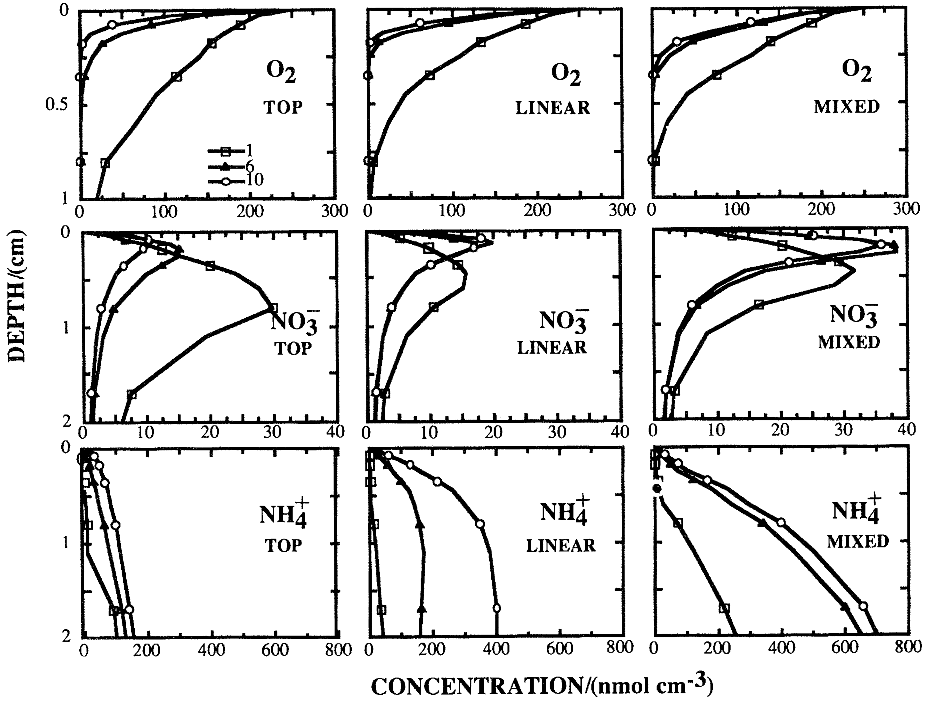

Figure 1. The distribution with depth of oxygen, nitrate and ammonium. POC was distributed as follows: mainly in the upper sediment (TOP), in a linear gradient from the surface (LINEAR) or was evenly distributed (MIXED). Three levels of organic loading were examined: 6.2, 36.2 and 62.0 mmol C m^{-2} d^{-1}, indicated by 1, 6 and 10 respectively.

3. Simulations

The output from the simulations is in the form in which data is usually collected in real experiments: pore water profiles, rate profiles, integrated rates and rates of diffusional flux of dissolved species across the sediment–water interface.

The depth profiles of O_2, NO_3^-, and NH_4^+ resulting from the nine treatments (three distributions and three different POC loadings) are seen in figure 1. Increased POC hydrolysis resulted in decreased O_2 penetration for each of the three POC distributions. At the lowest POC, the order for O_2 penetration was TOP > LINEAR > MIXED. There was a different order for the higher POC loadings, where MIXED > TOP > LINEAR. These profiles reflected the availability of POC close to the sediment surface; LINEAR was probably higher in subsurface DOC than TOP from which much DOC was lost by diffusion. The NO_3^- concentration profiles are similar to those of O_2 as increased POC hydrolysis resulted in decreased depth at which NO_3^- was maximum, for each of the three POC distributions. In all cases there was evidence for denitrification in the convex downward gradients. There was a complex relationship between POC distribution and NO_3^- distribution. This was probably because of the dependence of nitrification on the concentrations of both O_2 and NH_4^+. The highest NO_3^- concentrations coincided with the highest NH_4^+ concentrations (MIXED), but the relatively high NH_4^+ in LINEAR did not result in high NO_3^- due to the low availability of O_2. The very low NH_4^+ concentrations in TOP-distribution was mostly due to low NH_4^+ production below the surface, but also to active nitrification.

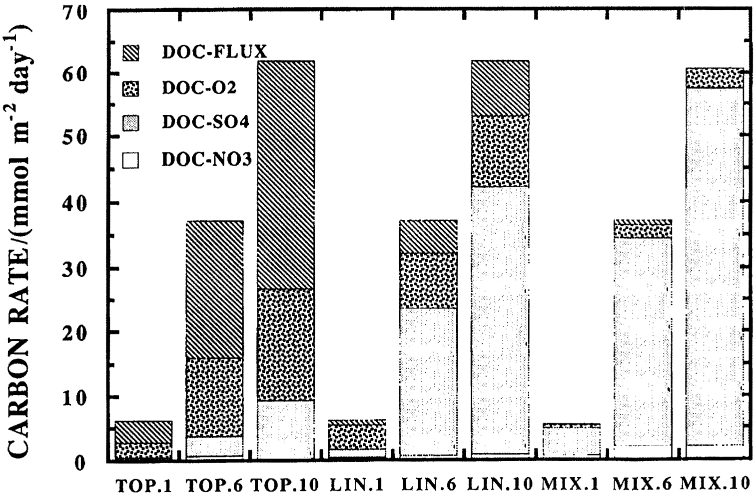

Figure 2. The integrated rates of carbon metabolism. POC was distributed as follows: mainly in the upper sediment (TOP), in a linear gradient from the surface (LIN) or was evenly distributed (MIX). Three levels of organic loading were examined: 6.2, 36.2 and 62.0 mmol C m^{-2} d^{-1}, indicated by 1, 6 and 10 respectively. The rates were: DOC flux from the sediment (DOC–FLUX), and the oxidation of DOC by O_2 (DOC–O_2), by SO_4^{2-} (DOC–SO_4) and by NO_3^- (DOC–NO_3).

There was evidence of nitrification in most of the NH_4^+ profiles which showed concavities between 0 and 1 cm depth.

The POC hydrolysis budget is summarized by the integrated rates of carbon metabolism: DOC-flux and the oxidation of DOC by O_2, NO_3^- and SO_4^{2-} (figure 2). The POC hydrolysis rates were 6.2, 37.2 and 62.0 mmol C m^{-2} d^{-1}. The TOP-distribution of POC resulted in large losses of DOC (3.39, 21.13 and 35.22 mmol C m^{-2} d^{-1} for the three loading). There were also large diffusional losses of DOC from the LIN-distribution, but virtually no losses from MIX. These DOC losses were related to the closeness of POC hydrolysis to the sediment–water interface. Rates of sulphate reduction were negatively related to DOC losses, reflecting the anoxic mineralization of DOC at depth in the sediment. Carbon oxidation by NO_3^- was insignificant in all treatments, but were highest in the MIX-distribution (0.75, 2.24 and 2.10 mmol m^{-2} d^{-1} for the three loadings).

The ratios of the four carbon rates give a better overview of their relative importance (figure 3). The very high losses of DOC (55, 57 and 57% for the three loadings in TOP-distribution contrast with the much lower losses in LIN-distribution (12, 13 and 14%) and the tiny losses from MIX (2, 2 and 0%). The predominant oxidant of DOC in TOP was O_2 (37, 33 and 28% of the three POC loadings). Oxidation by NO_3^- decreased, and by SO_4^{2-} increasing with loading. This pattern was repeated for LIN and MIX; O_2 and NO_3^- contribution decreased and the influence of SO_4^{2-} as oxidant increased, reflecting the increased anoxia of the sediments. The maximum contribution of NO_3^- to carbon oxidation (14%) was in MIX 1. In the most anoxic situation, MIX 10, SO_4^{2-} reduction contributed to 91% of DOC oxidation.

DON-flux paralleled the DOC-flux and was $\frac{1}{6}$ of the latter, as was the PON-hydrolysis rate $\frac{1}{6}$ that of POC (figure 4). There was an increase in NH_4^+ flux with increasing organic loading and going from TOP to MIX to LIN. The pattern of nitrification (NO3-flux + N2-flux) was more complex. The rates were (in mmol m^{-2} d^{-1}) 0.36, 1.48 and 1.14 for TOP distribution, i.e. a nitrification optimum

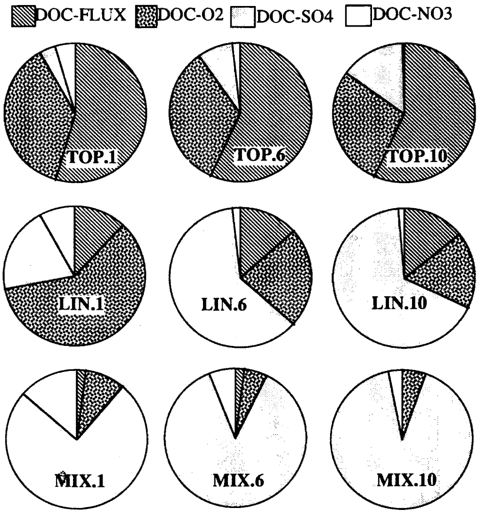

Figure 3. The ratios of the integrated rates of carbon metabolism. See figure 2.

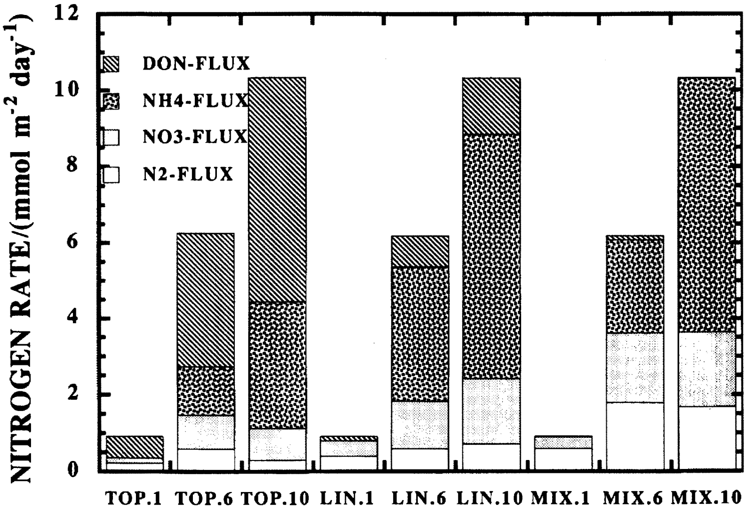

Figure 4. The integrated rates of nitrogen metabolism. PON was distributed as follows: mainly in the upper sediment (TOP), in a linear gradient from the surface (LIN) or was evenly distributed (MIX). Three levels of organic loading were examined: 1.0, 6.0 and 10.3 mmol N m^{-2} d^{-1}, indicated by 1, 6 and 10 respectively.

at TOP.6, where PON mineralization was 6.0 mmol m^{-2} d^{-1}. There was a general increase in nitrification with loading in the LIN-distribution, but in MIX there was almost no difference in nitrification rates between the two highest loadings (3.61 and 3.64 mmol m^{-2} d^{-1}). The proportion of the nitrogen budget going to nitrification decreased in the TOP-distribution, with organic loading (figure 5). The decrease was greatest for denitrification, N2-flux, which changed from 25 to 10 and 3%. This trend was observed for all the PON distributions, but nitrification and denitrification were still important processes in MIX 10, 19 and 16% of PON hydrolysis, respectively. The proportion going to NH_4^+ flux increased with increased loading and from TOP (0, 20 and 32%) to LIN (0, 57 and 62%), but not so regularly to MIX (0, 40 and 65%).

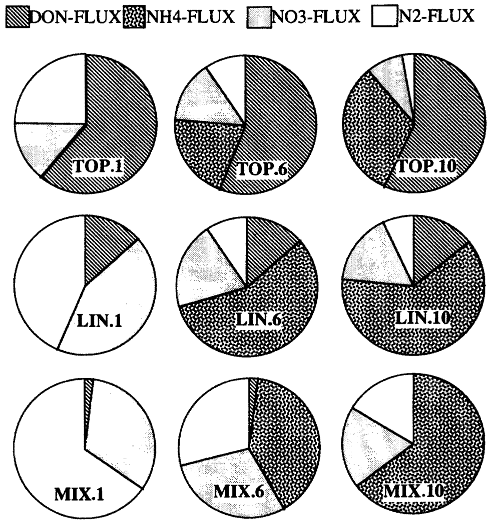

Figure 5. The ratios of the integrated rates of nitrogen metabolism. See figure 4.

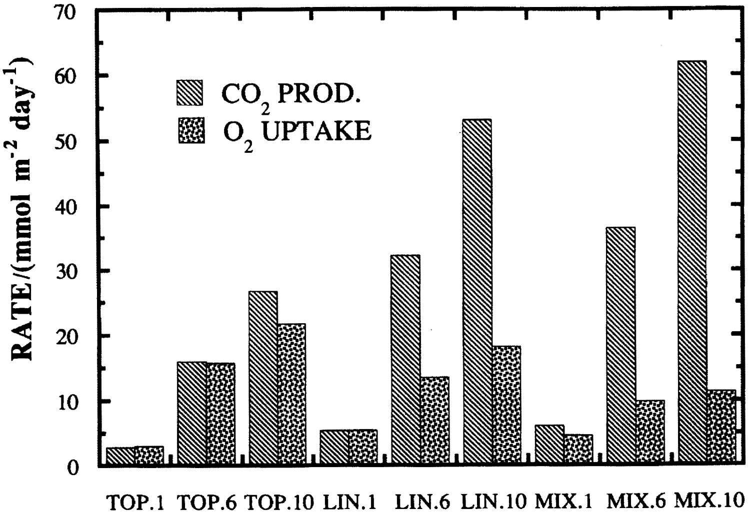

Figure 6. The integrated rates of CO_2 production and of O_2 uptake. POC was distributed as follows: mainly in the upper sediment (TOP), in a linear gradient from the surface (LIN) or was evenly distributed (MIX). Three levels of organic loading were examined: 6.2, 36.2 and 62.0 mmol C m^{-2} d^{-1}, indicated by 1, 6 and 10 respectively.

It is generally accepted that O_2 uptake should be approximately equivalent in magnitude to CO_2 production, but this is not always found (Blackburn 1987*b*; Hargrave & Phillips 1981). These rates were not equal in this series of simulations (figure 6). They were almost equal for the TOP-distribution and for the lowest rate of POC hydrolysis in LIN and MIX. At the highest rates of POC hydrolysis, there was a marked discrepancy. It is clear that this difference was due to anoxic respiration of DOC at the higher loading. This led to CO_2 production, but little HS^- was reoxidized by O_2. The proportion of O_2 going to HS^- oxidation increased with organic loading and was highest for the LIN-distribution (figure 7). In LIN 10, HS^- oxidation

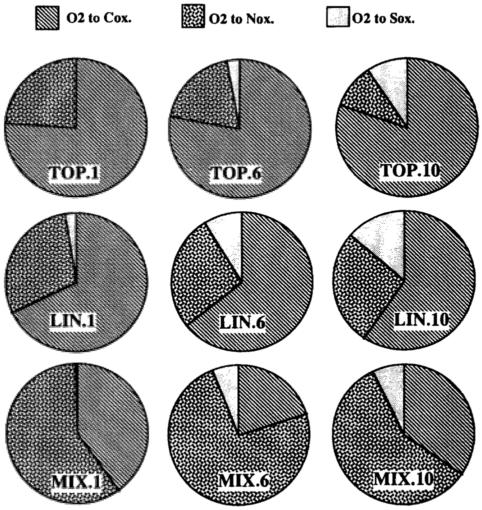

Figure 7. The ratios of the integrated rates of O_2 consumption.

accounted for 14% of the O_2 flux. The proportion of O_2 going to carbon DOC oxidation increased with POC loading, while the proportion going to NH_4^+ oxidation decreased in the TOP-distribution. The same pattern held, to a lesser extent, in the LIN-distribution. The situation was quite different in the MIX-distribution. The proportion of O_2 going to DOC oxidation was 40, 20 and 35%, and to NH_4^+ oxidation was 59, 74 and 58%, with increasing POC loading.

The higher rate of NH_4^+ oxidation in relation to DOC oxidation by O_2 may be explained as follows: In situations where POC was hydrolysed at some depth in the sediment, e.g. in the MIX- and to a lesser extent in the LIN-distributions, DOC was oxidized by SO_4^{2-} to CO_2, but the NH_4^+ could not be oxidized. This NH_4^+ diffused up to the oxic interface where it was oxidized to NO_3^-. As this was a region of relatively low DOC concentration, the NH_4^+ did not have to compete with DOC for O_2. This situation of high NH_4^+ oxidation was most apparent at MIX 6 (figure 5). At the higher organic loading, MIX 10, the proportion of O_2 going to DOC oxidation increased, presumably due to the increased DOC concentrations in the upper sediment layers.

All the predictions of the model make sense and substantiate each other. It should be possible to deduce these predictions by reasoning, but some of the conclusions might be missed. The value of this type of modelling is its ability to illuminate what should be obvious, but may not be so. Modelling also makes possible an examination of interrelated processes at high resolution. These relationships, e.g. the processes involving O_2 consumption, are difficult to evaluate by real analyses. A very exciting future development is the creation of a data bank of simulations of sediment processes under conditions of different quantities and distributions and degradable organic matter. A neural network will match measured O_2 and NH_4^+ gradients with the simulation profiles, thus predicting all other parameters associated with the measured gradients.

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Discussion

R. J. G. Mortimer. Dr Blackburn described sediments exposed to three different levels of organic loading and three distributions, but do such sediment conditions exist naturally?

T. H. Blackburn. They probably do. The TOP distribution is equivalent to freshly sedimented organic material. Bioturbation, etc., gives partial/complete mixing. Loading 1 is equivalent to sediment under 1000 m water, loading 6 is comparable with nearshore sediment and loading 10 would be near a sewage outfall.

M. L. Coleman. Is there evidence of NO_3^- reduction or nitrification in ancient sediments?

T. H. Blackburn. Probably not. Denitrification might be observed in N_2/Ar ratios in pore water. N-isotopic fractionation which occurs with denitrification might leave a useful signal.

M. L. Coleman. I have invoked nitrate reduction as the cause of carbonate hardgrounds, safe to invoke but not rigorous.

D. R. Lovley. Does the model include dissimilatory reduction of nitrate to ammonia?

T. H. Blackburn. The model did not include this process which is only important if the nitrate levels are high in overlying water. In this case most is in the sediment.