

CHAPTER 8

Benthic Mineralization and Bacterial Production

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8.1 INTRODUCTION

In the past, more emphasis has been placed on the process of mineralization than on the production of bacterial biomass, during the degradation of organic detritus in marine sediments. There has also been more emphasis on indirect measurements of mineralization rates rather than on their direct determination (Blackburn, 1983; Klump and Martens, 1983; Nixon *et al.*, 1976). Because of this past bias, this review will concentrate on the more direct methods which have been used to measure nitrogen mineralization rates. In addition, methods for the direct and indirect measurement of bacterial biomass production in sediment are assessed. Due to the relative newness of these latter methodologies, there are very limited data available. It is therefore useful to employ more general methods (e.g. carbon dioxide production) to calculate biomass production.

The importance of benthic mineralization is that it makes nitrogen, often considered to be a limiting nutrient, available to the pelagic algae in the water overlying the sediments. Considerable emphasis has been placed on evaluating the extent to which nitrogen, mineralized in the sediment, can contribute to the nitrogen requirements of these primary producers (Billen, 1978, 1982; Blackburn and Henriksen, 1983). In many situations the major portion of detritus that falls to the sediment surface originates from the pelagic algal cells. It would thus seem that a simple method to determine the proportion of the pelagic algae that sediments in this way would be to catch the cells as they sediment through the water column. An additional measurement of the rate at which nitrogen accumulates in the sediment would give the proportion of the sedimenting cell nitrogen which does not get mineralized. There are, unfortunately, serious technical problems in measuring the rate at which detritus rains onto the sediment surface, mostly due to resuspension of particles from the sediment surface. There are equally daunting problems in determining the rate of organic nitrogen accumulation, particularly in inshore sediments subject to bioturbation.

The simplistic input/output measurements must usually be passed over in favour of more complex, but hopefully more reliable, methods.

Regarding the second theme of this review, the incorporation of nitrogen into bacterial cells, it might be asked if this is an important process in the sediment. There is no simple answer to this question. If the rate of bacterial biomass synthesis is sufficiently high, and occurs in a region in which there is contact with

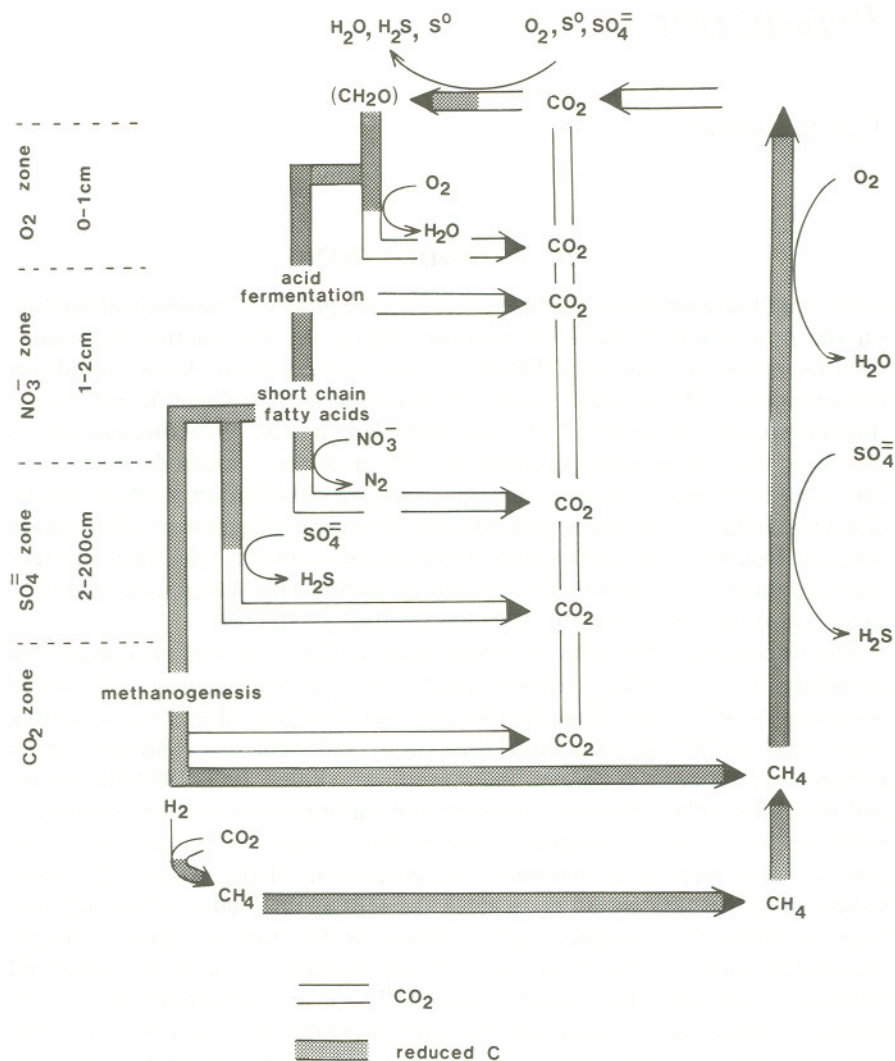


Figure 8.1. The zones of mineralization dominated first by oxygen, then by nitrate, sulphate and carbon dioxide. (Redrawn from Fenchel and Blackburn, 1979.)

organisms from higher trophic levels, then predation can occur and some higher purpose is possibly satisfied. If bacterial production occurs in isolation, then it must be assumed that bacteria die, and are mineralized by bacteria in a somewhat closed system. The rate of bacterial synthesis under these conditions would not seem to be of much importance.

Sediment mineralization and bacterial synthesis occur in zones dominated by different electron-acceptors in marine sediment (Figure 8.1). This figure illustrates the fate of organic carbon as it is oxidized, first by oxygen and subsequently by nitrate and sulphate. It is finally converted into methane after many years of burial (at approximately 2 m depth). As carbon is oxidized in these different zones, organic nitrogen is also mineralized to ammonium ion. Ammonium can only be oxidized by oxygen, and it therefore accumulates in lower, anoxic sediment layers, from where it diffuses up to the oxic surface. It is only at the sediment surface that nitrification can occur, unless the sediment surface is extended downward by the action of burrowing animals or by plant roots. It is probably only in these oxic regions that sufficient energy is available for the formation of enough bacterial biomass to be of significance in further food chains. I suggest that, in the anoxic sediment, most interest should lie not in the quantity of bacterial biomass produced, but rather in the types of bacteria involved in the mineralization processes, particularly in the mineralization of organic nitrogen. In marine sediments sulphate reduction can be a very important process in the oxidation of organic carbon (Jørgensen, 1977), yet sulphate-reducing bacteria have no capacity to hydrolyse particulate organic matter (Jørgensen, 1983). Other bacteria must therefore also be present, which are capable of performing these complex hydrolyses, obtaining energy from the fermentation of hydrolytic products. It is these bacteria that are important in organic nitrogen mineralization, while it is the whole consortium that is important in nitrogen assimilation (also referred to as incorporation or immobilization). These processes imply the building of a nutrient into biomass.

The review is mostly concerned with three types of method: those methods which describe the relatively well-defined procedures for the measurement of N-mineralization rates (Section 8.2) and cell biomass synthesis (Section 8.3), and the derivation of these rates from models, which involve C:N relationships (Section 8.4).

8.2 RATES OF NITROGEN MINERALIZATION

A distinction is made between the total rate of organic N-mineralization and the net rate (total rate minus rate of N-assimilation). It is usually the net rate that is measured, and that is of most interest to marine biologists, because this is the rate which determines the recycling of nitrogen to the overlying water. It should be stressed that most of these methods aim at measuring rates of mineralization in the absence of the benthic macrofauna. As other chapters in this book will

demonstrate, this will significantly underestimate the true mineralization rate. It is not intended that this be a comprehensive review of N-mineralization, it merely lists the procedures that have been used to generate the data so well reviewed by Klump and Martens (1983), and which indicate that benthic N-regeneration contributes significantly to primary productivity, particularly in shallow-water sediments. A value of 30% was frequently observed.

8.2.1 Sediment incubations

Samples of sediment may be incubated for varying time periods and the rate of ammonium accumulation is measured (Aller, 1980; Aller and Yingst, 1980; Billen, 1978; Rosenfeld, 1981). Usually these incubations are made anaerobically, to avoid nitrification and subsequent denitrification. This imposes a degree of artificiality, since oxygen would normally play a significant role in the decomposition process. There is the added danger that any obligate aerobes may die, and that their decomposing biomass may contribute to the measured rate of mineralization. Because ammonium is located in two pools, dissolved in porewater and adsorbed to sediment, it is necessary to ensure that the total ammonium pool is measured (Blackburn, 1979a,b; Rosenfeld, 1979a). The extraction of the adsorbed ammonium pool by different methods may give different results (Blackburn and Henriksen, 1983; Mackin and Aller, 1984). Short incubations of intact cores, which are then analysed for changes in ammonium concentration, will give a rate of net ammonium production minus the rate of nitrification (Blackburn and Henriksen, 1983); the addition of an inhibitor of nitrification will give the true net ammonium production rate (Henriksen, 1980).

8.2.2 Sediment ^{15}N -ammonium incubations

This is a variation on the method described in Section 8.2.1, with $^{15}\text{NH}_4^+$ being injected into intact cores or mixed into anoxic slurries (Blackburn, 1979a). In addition to obtaining information on the rates of net mineralization, the gross rate of mineralization is also measured. This can be of value in calculating the rate of ammonium uptake into bacterial cells. In addition, where there is interference in the bacterial sediment processes (for example, if there are many vascular plant roots present), a net rate of ammonium production can be calculated from the gross rate, by making some assumptions regarding the efficiency of bacterial ammonium assimilation (McRoy and Blackburn, unpublished manuscript). A comparison of the two methods of $^{15}\text{NH}_4^+$ application are seen in Figure 8.2. There is good agreement between the results obtained by injection of cores and by mixing the tracer into the sediment for anoxic incubation, except for sandy sediments which presumably contained aerobic species that died and decomposed. This might give the impression that the injection of cores always gives good results; this is not necessarily true. In order for the method to work there

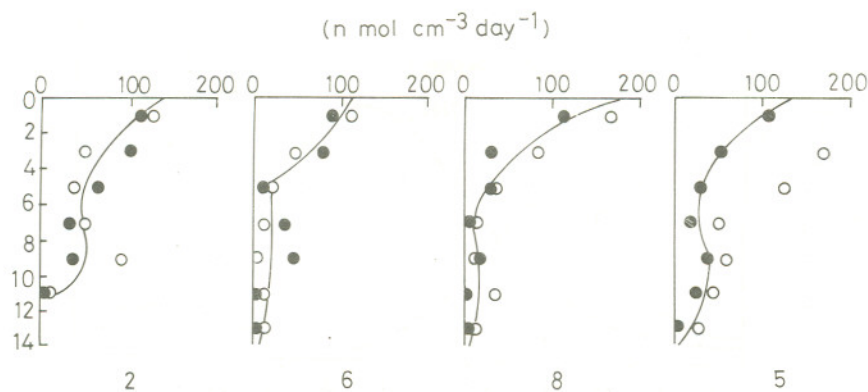


Figure 8.2. Rates of total ammonium production measured by $^{15}\text{NH}_4^+$ -dilution with a mixed (○) and an injected (●) procedure plotted against sediment depth. The results are for July at four stations. (Redrawn from Blackburn and Henriksen, 1983.)

must not be much variation between replicate cores. This is because the calculation is based on differences in ^{15}N -content between cores that have been incubated for different lengths of time, and it is assumed that all the cores were initially identical. This is seldom true. The data in Figure 8.2 demonstrate the decrease in the mineralization rate with sediment depth, as would be expected from the presence of less readily degradable organic detritus in the lower sediment strata.

The ^{15}N -ammonium dilution technique has been used to measure total N-mineralization in seagrass sediments (Iizumi *et al.*, 1982) and in freshwater marsh sediments (Bowden, 1984).

8.2.3 Exchange measurements

The exchange of ammonium, nitrate and nitrite across the sediment interface is measured, and the flux (usually out of the sediment) represents the net rate of organic-N mineralization minus the rate of denitrification. The measurements may be made on cores (Blackburn and Henriksen, 1983), which is often the only method available when working in deep water. A better alternative is to use chambers that are placed on the sediment surface (Smith *et al.*, 1972; Hartwig, 1976; Klump and Marten, 1981). Exchange measured within the chambers represents the whole community activity, whereas with cores, fluxes are measured in the absence of the macrofauna. Klump and Martens (1983) have reviewed the dependence of N-fluxes on season and temperature. This effect is also illustrated in Figure 8.3, where it is seen that the highest flux is in the summer and in the form of ammonium. During the spring the flux is smaller and in the form of nitrate.

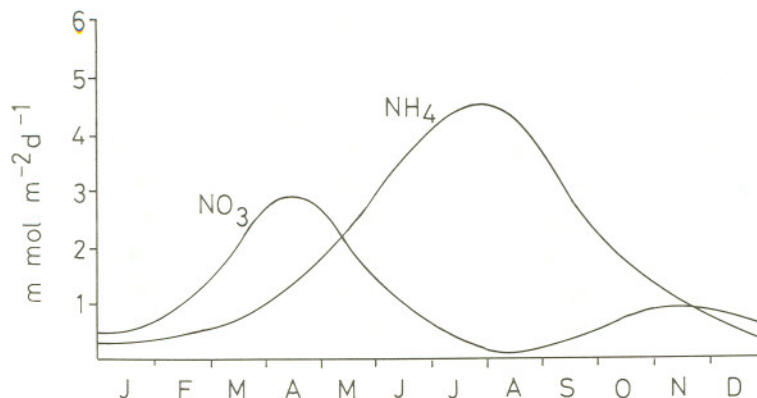


Figure 8.3. The yearly flux of inorganic nitrogen from sediments in the Limfjorden. The measurements are an average from five stations. (Redrawn from Blackburn and Henriksen, 1979.)

8.2.4 Gradient measurements

The concentration of ammonium in the pore water is measured, the gradient is determined, and the rate of organic-N mineralization is derived from a model (Berner, 1980). Klump and Martens (1983) have reviewed this procedure very thoroughly. It has proved to be a very valuable tool, particularly for non-bioturbated sediments. It has obvious drawbacks where clearly defined pore-water gradients are not observed.

8.2.5 Specific N-substrate mineralization

Specifically this concerns the mineralization of amino acids in sediments. These are not the only N-containing, dissolved organic molecules being degraded, but they may be the most important. There is a rapid turnover of amino acids in marine sediments, but there is evidence that much of the 'free' dissolved amino acid in porewater may not be biologically available (Christensen and Blackburn, 1980), just as 'free' dissolved acetate is not completely available (Christensen and Blackburn, 1982). The capacity of amino acid to bind to sediments is well established (Rosenfeld, 1979b), but the nature of their non-availability in porewater is not known. Care must be exercised to base calculations of rates on biologically available pools. Amino acid degradation occurs in the zone of sulphate reduction. Until recently it was thought that sulphate-reducing bacteria were incapable of utilizing amino acids, but their capacity to do this has now been demonstrated (Stams *et al.*, 1985).

8.3 RATES OF NITROGEN ASSIMILATION

As with Section 8.2, this is not a comprehensive review of all literature relating to the incorporation of nitrogen into bacterial cells. The aim is to give an indication of the methods that have been employed. Results of some experiments in which some of these methods have been used will be discussed later. Excellent reviews of pelagic bacterial productivity have recently been written by van Es and Meyer-Reil (1982), and Williams (1983). There are no comprehensive reviews of bacterial productivity in sediment. The methods are not exclusively for N or for C, they often are for biomass, but may be expressed in terms of C or N. Most methods thus involve a measurement of bacterial biomass. One of the simplest methods for biomass measurement is by counting bacteria, which must first be separated from sediment particles (McDaniel and Capone, 1985).

8.3.1 Thymidine assimilation

The incorporation of [^3H]thymidine into pelagic bacterial cells has been used to measure the rate of cell production, usually in terms of cell C, but the calculations are equally valid for cell N synthesis (Fuhrman and Azam, 1982). The method has also been applied to the measurement of bacterial productivity in seagrass sediments (Moriarty and Pollard, 1981, 1982; Moriarty *et al.*, 1985a), marine sediments (Fallon *et al.*, 1983; Moriarty *et al.*, 1985b,c) and freshwater sediments (Findlay *et al.*, 1984). The principle of the method is elegant and simple: the absolute quantity of thymidine that is incorporated into DNA is calculated from the amount of label in the DNA and the specific activity of the [^3H]thymidine (taking into account any dilution by extracellular and intracellular thymidine pools). An assumption is made that thymidine is one-quarter of the DNA, that each cell contains one DNA molecule and that all DNA molecules are of an equal (and defined) size. It is thus possible to calculate the rate of nuclear replication, and cell division. If the number of cells is also counted, or the biomass determined by other methods (White *et al.*, 1979b), the turnover time of the bacterial population is derived. It is usual to calibrate the method against the growth rates of natural populations of bacteria (Fuhrman and Azam, 1982).

8.3.2 Adenosine assimilation

This method, first used to estimate growth of pelagic bacteria (Karl, 1981), has now been applied to the estimation of growth in sediment bacterial populations (Craven and Karl, 1984; Novitsky and Karl, 1986). High specific activity [^3H]adenine is added to sediment samples, and after suitable incubation the radioactivity in ATP, RNA and DNA is counted, and the intracellular ATP

concentration is measured. The rate of DNA synthesis and of cell replication is calculated from the label in DNA, the ATP content of DNA and the quantity of DNA per cell. The cell biomass (C or N) is calculated from the intracellular ATP pool multiplied by a factor. The turnover time is derived from the biomass divided by the cell replication rate. The method should be calibrated against bacterial cultures (Winn and Karl, 1984).

8.3.3 Frequency of dividing cells (FDC)

This again is a method that was developed to measure the growth rate of pelagic bacteria (Hagström *et al.*, 1979). It has also been used to determine the growth rate of bacteria in sediments, where the method gave erroneously high values (Newell and Fallon, 1982), due possibly to adhesion of cells after division (Fallon *et al.*, 1983). Only dividing cells should be counted, not those whose division might have occurred generations before. The proportion of dividing cells is used to calculate the growth rate.

8.3.4 Incubation and direct counts

In principle, a bacterial count is made on a sample of sediment, the sediment is incubated and the increase in bacterial numbers is related to time of incubation, giving a rate of bacterial production. Implicit in this procedure is the removal of the bacterial predators, which would otherwise have consumed the newly synthesized bacteria. In water samples the predators may be removed by filtration (Fuhrman and Azam, 1982), although difficulties may be encountered with this procedure due to adhesion of bacteria to larger particles, and to the passage of microflagellates through the filter (Newell and Fallon, 1982). In sediments and microcosms the problems are even greater, since the predators cannot be removed without disturbing the integrity of the sediment structure (this problem exists for other methods also). In order to count the bacterial cells in the sediment they must be separated from the sediment particles, usually by blending or by ultrasonic treatment (McDaniel and Capone, 1985), neither of which is completely satisfactory (Ellery and Schleyer, 1984). It is usual to convert cell numbers to cellular C, from measurements of cell volume, making some assumptions concerning the cells' specific gravity, water content, and percentage C in the cells' dry biomass (Fallon *et al.*, 1983; Meyer-Reil *et al.*, 1980, 1983). Natural variations in bacterial numbers have been used to estimate bacterial growth rates in a saltmarsh (Kirchman *et al.*, 1984).

A variation on the counting procedure, as applied to natural communities, is to add specific substrates and to follow the change in substrate and bacterial biomass with time. Saltmarsh debris (Newell *et al.*, 1983), phytoplankton debris (Newell *et al.*, 1981), and macrophyte debris (Koop *et al.*, 1982; Newell *et al.*, 1982) have been used in this manner.

8.3.5 Phospholipid measurements

The quantity of phospholipid gives a very good indication of active biomass, and its turnover (^{32}P -labelled) can be used to measure growth (White *et al.*, 1979a,b). There is the additional advantage that different microorganisms have specific phospholipids (Gillam and Hogg, 1984). This has been used as evidence for the presence of a predominantly anaerobic flora in seagrass sediments (Moriarty *et al.*, 1985a). These authors also made a comparison of the phospholipid and thymidine incorporation methods; both methods gave similar values. Unfortunately this is one of the rare cases when a comparison of two methods was made directly. For this reason it is very difficult to distinguish between the merits of the adenosine and thymidine methods, but clearly the high sensitivity of the phospholipid method makes it very attractive. In general it is the high sensitivity of the thymidine, adenosine and phospholipid procedures that makes them useful for short incubations. They are therefore extremely valuable for making comparisons between sites or time series. They are possibly less useful for assessing absolute rates. These can be measured by one of the methods that can be related more directly to total sediment activity or to N-uptake.

8.3.6 Incorporation of ^{15}N -ammonium

The incorporation of $^{15}\text{NH}_4^+$ into bacterial cells in seagrass sediments gives an estimation of bacterial growth (Iizumi *et al.*, 1982). There is a danger in this type of experiment, that plant root tissue may also assimilate $^{15}\text{NH}_4^+$. The main difficulty with this experimental approach is that there is usually a high organic-N background, against which it is difficult to measure the small amount of label incorporated into the bacterial protein. Usually direct uptake can only be measured where there is high bacterial biomass, a fast growth rate, and a relatively low organic-N background. Ultimately the limit of detection is set by the sensitivity of the equipment used to measure the isotope ratio. Instead of measuring incorporation of $^{15}\text{NH}_4^+$ directly, the disappearance of label from the ammonium pool can be used to calculate the rate of uptake into bacterial cells (Blackburn, 1979a). The procedure is essentially that which is outlined in Section 8.2.2; mineralization of organic-N and bacterial growth can be measured from the same experimental data. Nitrification must not be allowed to occur, otherwise it must be measured independently.

For these methods to be useful it must be assumed that sediment bacteria utilize ammonium, rather than directly incorporate organic-N, e.g. amino acids. There is evidence that this assumption is correct, because amino acids are very rapidly oxidized (Christensen and Blackburn, 1980), and ammonium is the only dissolved N-species that is present in high concentration.

If net ammonium production is not measured at the same time as total ammonium production, ammonium incorporation cannot be determined, but a

calculation of probable ammonium incorporation may be made. This can only be done when the N:C ratio of the substrate is known, and this can be a problem, as will be discussed in relation to Figure 8.4.

8.4 CALCULATIONS FROM C:N MODELS

The mineralization of organic-N to ammonium, and the synthesis of microbial cells for two situations, is shown in Figure 8.4. In the first situation, cells are mineralized *in situ*. They may self-destruct, or they may be digested by other species; the mechanism of mineralization is not important. Detritus has a N:C ratio N_s and is degraded at the rate d_s . Cells (N:C= N_c) are synthesized at rate i and degraded at rate d_c . In steady-state conditions these two rates are equal and $d_s = d - i$. The rate d describes the degradation of the mixed detritus + cells, whose N:C ratio (N_{sc}) is unknown. If only d is measured, it is difficult to make further calculations from this total rate of ammonium production.

In the second situation the cells that are produced are not degraded in the system. This could occur where cells are produced at the sediment surface and are grazed and removed from this site. The rate d now describes the breakdown of the detritus, whose C:N ratio can be measured.

The relationship between the N-models outlined in Figure 8.4 and C-

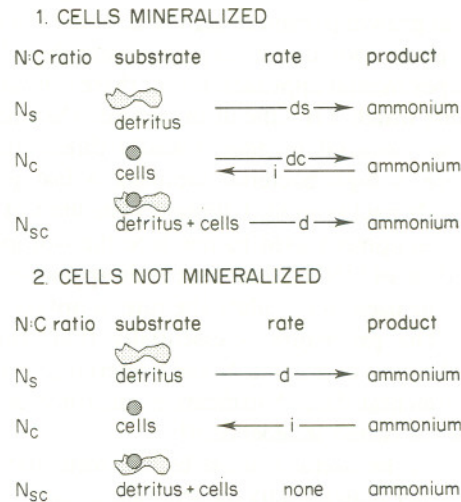


Figure 8.4. The mineralization of organic-N in sediment is shown for two situations: (1) where the rate of bacterial cell production is matched by the rate of cell mineralization; and (2) where there is a net production of cells, which are not mineralized

metabolism is shown in Figure 8.5. More specifically, the relationship to carbon oxidation (C_o) is shown. Carbon oxidation is a parameter that may be measured in a number of ways, and it is thus useful to be able to relate this measurement to cell nitrogen incorporation. The rate of ammonium incorporation into bacterial cells is described by the same equation, whether or not cells are mineralized *in situ*. The situation is different for the other two rates, d and $d - i$, which have different equations for the two different situations.

The rate of carbon oxidation may be measured, or calculated, in a number of ways, that will now be briefly reviewed.

Carbon dioxide exchange between sediment and the overlying water can be measured in enclosures placed over the sediment surface (Hargrave and Phillips, 1981). Exchange can also be measured in sediment cores in the laboratory.

The rate of carbon oxidation may be calculated from the various oxidants that have been consumed. The most important oxidant is oxygen. Oxygen is used to oxidize carbon, but it also reoxidizes the sulphide produced as a result of sulphate reduction (Jørgensen, 1977), and it can also oxidize reduced iron and manganese. Simultaneous measurements of carbon dioxide production and oxygen uptake show that carbon dioxide production is the greater of the two (Hargrave and Phillips, 1981; Andersen and Hargrave, 1984). This would indicate that the produced products of anoxic oxidations, e.g. sulphide, are not reoxidized immediately by oxygen during the experiment. Presumably oxidation is periodic, and occurs when the sediment is disturbed at a later time. It may be concluded that oxygen uptake is mostly due to carbon oxidation. In order to calculate all carbon oxidation it is necessary to calculate the carbon oxidized by sulphate, nitrate, and any other oxidant that may be present.

$$E = C_i / (C_i + C_o) \text{ where } C_i = \text{C-incorporation, } C_o = \text{C-oxidation}$$

1. CELLS MINERALIZED

$$\begin{aligned} i &= C_o N_c E / (1 - E) \\ d &= C_o N_{sc} / (1 - E) \\ (d - i) &= C_o N_s \end{aligned}$$

2. CELLS NOT MINERALIZED

$$\begin{aligned} i &= C_o N_c E / (1 - E) \\ d &= C_o N_s / (1 - E) \\ (d - i) &= C_o (N_s - N_c E) / (1 - E) \end{aligned}$$

Figure 8.5. The interconnections between C- and N-cycles in marine sediment are shown for the two situations described in Figure 8.4, where bacterial cells may or may not be mineralized in the sediment

8.5 CONCLUSIONS

There is an obligation to present a summary table showing comparative values, produced by different methods, for bacterial N-incorporation, in the sediments of the world. Table 8.1 does not meet these criteria, and the time is not ripe for such a synthesis to be made. The table is given in terms of nitrogen, but these values have largely been converted from carbon, assuming a N:C molar ratio of 0.16. Many liberties have been taken in attempting to convert data to a common base, $\text{mg N m}^{-2} \text{d}^{-1}$. Table 8.1 shows some interesting points, in spite of the difference in locations, temperatures and sediment characteristics. The net production values of Meyer-Reil (1983) would be expected to be minimal values, but they are quite high in comparison with most thymidine uptakes, indicating a possible lack of predation. Thymidine uptake appears to give reasonable values, in conformity with its proven usefulness in pelagic measurements. The ^{15}N -dilution procedure gives results that conform reasonably well with carbon oxidation calculations, but are somewhat higher than the latter (data not shown). There is such a degree of uncertainty about all these methods that it is necessary to check any measurement against as many other sediment rates as possible. It is only in this way that

Table 8.1. Rates of cell production

Sediment type	Method	$\text{mg N m}^{-2} \text{d}^{-1}$	References
Inshore	difference	24	Meyer-Reil (1983) ^a
Inshore	difference	42	
Beach	glucose	13	Meyer-Reil <i>et al.</i> (1980) ^b
Inshore	FDC	747	Newell and Fallon (1982) ^c
Inshore	THY	6	Fallon <i>et al.</i> (1983) ^d
Offshore	THY	1	
Beach	AD	560	Craven and Karl (1984) ^e
Offshore	AD	4	
Seagrass	THY	6	Moriarty <i>et al.</i> (1985a) ^f
Seagrass	LIP	5	
Inshore	^{15}N	2–13	Blackburn and Henriksen (1983) ^g

^a Sediments from Kiel Bight, where the *in situ* increase in cell biomass was measured; the first value was for the autumn and the second was for the spring.

^b Sediment from a German beach; the rate of cell synthesis was calculated from the rate of glucose uptake.

^c Sediments were from Sapelo Island, Georgia; rates were determined from the frequency of dividing cells.

^d Sediments were inshore and offshore Sapelo Island; rates were determined by thymidine uptake.

^e Sediments were from reef and offshore in Hawaii; rates were determined by adenosine uptake.

^f Sediments from shallow Australian seagrass beds; rates were by thymidine uptake and ^{32}P incorporation into lipid.

^g Sediments from inshore Danish sediments; rates were calculated from $^{15}\text{NH}_4^+$ dilution kinetics.

some confidence can be placed in the results. Unfortunately such cross-checks are seldom made, and uncertainty remains as to the extent of microbial synthesis in many marine sediments, and of the efficiency of C-assimilation. More confidence can be placed in the rates of organic mineralizations, because of the relative ease of measuring end product production.

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