
Nitrogen Cycling in Eutrophic Freshwaters [and Discussion]

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Nitrogen cycling in eutrophic freshwaters

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Agriculture and urbanization are important sources of N input to aquatic ecosystems, and this input is tending to increase as agricultural practice is intensified and as urban populations increase. Although a great deal of information is readily available on the concentrations of nitrate entering lakes, lochs and rivers, less is known about the precise fate of the added N, how it is cycled within ecosystems, and the ways in which it is lost from ecosystems. This paper presents information obtained from selected habitats in the northern U.K. on the quantitative significance of N inputs from agriculture and urbanization, and on the ways in which the added N is cycled. Particular attention is paid to key microbiological processes involved in such cycling and to the environmental factors that affect these.

INTRODUCTION

Nitrogen is essential for the growth of living cells and on average such cells contain 1–2% of their dry matter as N. Because of rapidly increasing human populations, there is a concomitant increased demand for N to sustain and indeed improve their well being. In early agriculture this N was supplied mainly by biological N₂ fixation and by the use of organic wastes. Today, in advanced countries of the world, including the U.K., such a N demand can only be met by the increased use of chemical N fertilizer. Concomitant with intensified agricultural production, there has been population drift from the land to urban environments, intensive animal production, particularly in areas peripheral to urban populations, and associated problems of waste disposal and treatment.

These two factors, increased agricultural production and urban discharges to aquatic ecosystems, are major causes of increased N loadings to freshwaters of the U.K. today, and irrespective of the precise routes whereby fixed N from land reaches aquatic ecosystems it is a fact that the nitrate concentrations in many freshwaters of the U.K. are increasing steadily to levels which, if present trends continue, will exceed the World Health Organization recommended limit for NO₃⁻-N in drinking waters (11.3 mg l⁻¹), if they have not already done so (see Foster *et al.*, this symposium, and Wilkinson & Greene, this symposium). The Royal Commission on Environmental Pollution (1979), when referring to the increases in nitrate in water supplies over recent years concluded, 'we do not doubt that the large increase that has occurred in the use of inorganic N fertilizer is directly, or indirectly, a major cause of the rising nitrate levels that are observed in many water supply sources'.

The freshwater biologist has an important role to play in ascertaining the quantities of N reaching aquatic ecosystems from different sources, and in determining what effects these may

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be having on the populations of living organisms and how the N entering such waters is cycled. With such an armoury of basic knowledge available it may then be possible to exploit various naturally occurring processes to advantage in sustaining, or indeed improving, water quality. This paper presents information on the freshwater nitrogen cycle with that in mind. The approach adopted is to consider two specific areas from the northern U.K. where we have carried out some original research on processes of aquatic N cycling. We hope that the findings will have more than local application and will complement work on other areas of the U.K. such as Loch Leven (Johnston *et al.* 1974; Holden 1976), Lough Neagh (Smith 1976, 1977), the English Lake District (Jones & Simon 1980, 1981; Jones *et al.* 1980) and elsewhere (Troake *et al.* 1976; Royal Commission on Environmental Pollution 1979).

THE STUDY AREAS AND THE APPROACH ADOPTED

One series of investigations was carried out in the Forfar Loch, Balgavies Loch and Rescobie Loch areas of the county of Angus on the East of Scotland. The other area studied was Blelham Tarn in the English Lake District (figure 1). The overall aims of the study were threefold: (1) to quantify and compare the inputs of N from agriculture and urbanization to such waters; (2) to obtain quantitative data on various processes of the nitrogen cycle; (3) to relate the results obtained to problems of water management.

Our contention was that the most satisfactory way of approaching the problem was to obtain quantitative data on a seasonal basis, which would allow us to draw up net budgets for the waters and to measure specific process rates that affected these budgets, with the use of ^{15}N tracer techniques. This approach was adopted in the Scottish loch study, and it was complemented by the Blelham Tarn study where we followed the fate of ^{15}N -labelled fertilizer N added to a large enclosure within the lake. Both approaches had advantages and disadvantages. The advantage of the loch study was that we were dealing with a completely natural ecosystem, but hydraulic load was a major fluctuating factor and process rate studies had of necessity to be made in bottle incubations. In the Blelham study it was possible to fertilize the entire enclosure with ^{15}N -labelled nitrate and trace its subsequent fate, but because it was an enclosed system it was artificial, even although it was larger than most ponds that have been studied in detail

A COMPARISON OF NUTRIENT INPUTS FROM AGRICULTURE AND URBANIZATION

The Forfar-Lunan channel area where Forfar Loch, Rescobie and Balgavies are located consists mainly of intensively farmed agricultural land. The soils are freely drained, with boulder clay just below the soil surface. Surface drainage is thus the major source of nutrient input to the lochs. Land usage in the catchment of Rescobie (2073 ha) is 78% arable, 22% rough grazing and woodland, and 0% urban. The corresponding values for the Forfar catchment (1689 ha) are 74%, 5% and 21%. The small Balgavies catchment area (320 ha), which is not part of the Rescobie catchment, is very similar in terms of land use to that of Rescobie. Because Rescobie drains into Balgavies, what happens in the large Rescobie catchment has a profound effect on what happens in Balgavies. Indeed, in earlier studies we treated both lochs as the same water body (see Stewart *et al.* (1975) and Stewart *et al.* (1976)).

In the Rescobie catchment in 1977 the main arable crop was barley, land under grass was extensive, and smaller areas were under potatoes, turnip and soft fruit (mainly raspberries)

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although soft fruit production has declined dramatically within the last few years. In the Forfar drainage basin about 10% of the land is used for nursery stock (trees and shrubs). All chemical N fertilizer applications are made in spring and early summer, applications to grassland (average 156 kg ha^{-1} annually) are higher than to other arable land (average 103 kg ha^{-1} annually) and one application is made per year except in the case of grass and nursery stock where the fertilizer is added in three applications. The average chemical N fertilizer application to agricultural land is 115 kg N ha^{-1} annually, although local farmers may add substantially

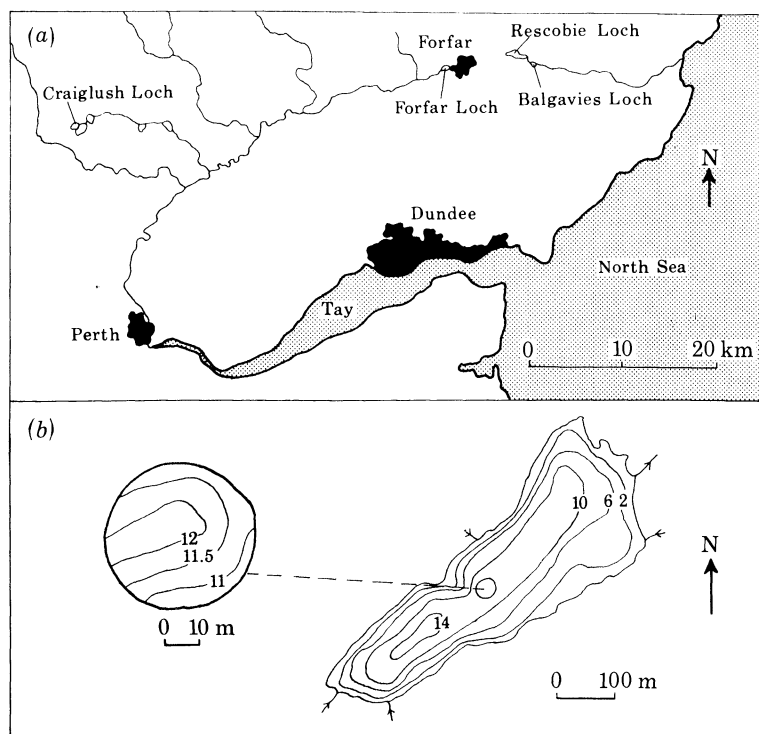


FIGURE 1. The major freshwater areas studied and some of their characteristics. (a) Study area showing the position of Forfar Loch (NO 439506), Rescobie Loch (NO 513518) and Balgavies Loch (NO 534508). Rescobie drains via Balgavies to the North Sea. Forfar Loch drains into the River Tay. Rescobie has the following features (1978): maximum depth, 7.0 m; mean depth, 3.0 m; surface area 65 ha; mean volume $1950 \times 10^3 \text{ m}^3$; catchment/loch surface area ratio, 32; average water retention time, 66 days; hydraulic load, 5.5 loch volumes per year. Forfar has the following features (1978): maximum depth, 8.8 m; mean depth, 3.5 m; surface area, 38 ha; mean volume, $1330 \times 10^3 \text{ m}^3$; catchment/loch surface area ratio, 44; average water retention time, 42 days; hydraulic load, 8.6 loch volumes per year. Balgavies has the following features (1975): maximum depth, 9.3 m; mean depth, 3.0 m; surface area, 20.7 ha; mean volume $621 \times 10^3 \text{ m}^3$; catchment (including that of Rescobie)/loch surface area ratio, 114; average water retention time, 35 days; hydraulic load 10.4 loch volumes per year. (b) Blelham Tarn (SO 366005), showing the position of enclosure A. For further details see Lack & Lund (1974).

more, and are advised to increase the rates by 25 kg N ha^{-1} after a wet winter (see E.S.C.A. 1977). In addition to this, and especially in the Forfar catchment, farmyard manure is added extensively in autumn to prepare the land for the following spring crop. Also, the spreading of sewage sludge, from the Forfar sewage works, throughout the year to the agricultural land surrounding Forfar loch is common practice. Applications of organic manure in November–December are considered to have lost 75% of their N content by the following March (E.S.C.A. 1977). The addition of such organic manure, the particularly dense network of field drains in

the Forfar catchment, the raspberry-growing regions which show high leaching rates (see Stewart *et al.* 1976) and which are common round the loch, and the continual disturbance of nursery grounds all facilitate N loss to Forfar Loch. Inputs of N from the Forfar catchment are thus substantially higher than from the Rescobie catchment.

Table 1 presents information on the amounts of NO_3^- , NH_4^+ and PO_4^{3-} (dissolved inorganic) present in the drainage water from arable land and from urbanization in Forfar and Rescobie drainage basins. It is seen that agricultural drainage waters are high in NO_3^- , have little NH_4^+

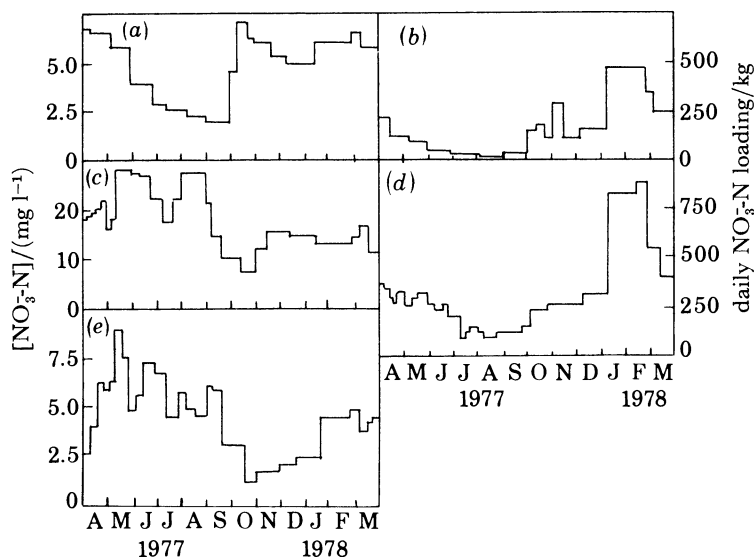


FIGURE 2. Seasonal variations in the mean concentrations of NO_3^- -N present in (a) Rescobie agricultural inflows, (c) Forfar agricultural inflows and (e) Forfar sewage works inflow, March 1977-8. (b, d) The seasonal changes in the total NO_3^- -N loadings to Rescobie and Forfar lochs, respectively.

TABLE 1. MEAN CONCENTRATIONS (MILLIGRAMS PER LITRE) OF N AND P IN THE INFLOWS TO RESCOBIE LOCH (MARCH 1977 TO APRIL 1978) AND FORFAR LOCH (JANUARY 1977 TO MAY 1978)

(Figures in brackets are the range.)

	Rescobie		Forfar	
	agriculture		agriculture	sewage
NO_3^-	5.26	(2.60-7.06)	19.63	(9.88-27.74)
NH_4^+	0.091	(0.026-0.283)	0.062	(0.035-0.124)
PO_4^{3-}	0.033	(0.010-0.058)	0.016	(0.008-0.042)

and little PO_4^{3-} , and that the sewage input discharges high amounts of PO_4^{3-} relative to NO_3^- , although the $\text{NO}_3^- : \text{NH}_4^+$ ratio is substantially lower in the sewage inflow than in the agricultural inflows. Seasonal variations in the NO_3^- concentrations in the inflows and their loadings to the lochs are shown in figure 2. The inputs per person of N and P to the loch via the treated effluent from the sewage works are 6.3 and 1.6 g per day, respectively. These values are about average for the U.K. (see Stewart *et al.* 1976). The higher N concentrations in the agricultural drainage waters of the Forfar catchment can be attributed to a combination of factors, peculiar

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to the Forfar catchment (see above), and also to the fact that the main agricultural inflow to Rescobie enters the loch via an area of marshland, which may serve to strip the water of nutrients (see, for example, Fetter *et al.* 1978). The concentrations observed for both lochs are within the ranges reported by Cooke & Williams (1970) and the Royal Commission on Environmental Pollution (1979) for drainage waters from intensively farmed agricultural land. Our data emphasize that the local situation plays a substantial role in the extent to which leaching takes place, even when soil type and crops grown are very similar. Seasonal data on the nutrient concentrations found in the agricultural drainage waters show that in Rescobie (figure 2*a*) highest NO_3^- concentrations occur in autumn and winter, although chemical fertilizer applications are made mainly in the spring. This is the typical pattern found with the use of chemical N fertilizer. In Forfar (figure 2*c*) the unusually high NO_3^- concentrations found in the agricultural drainage water in summer may be related to the extensive mineralization of organic matter applied the previous winter and spring, to associated nitrification and to reduced hydraulic loading in summer. Despite the variations between the inputs to the two lochs in terms of seasonal changes in NO_3^- concentration, the seasonal changes in nutrient loading to both lochs follow the classical pattern with pronounced winter maxima and summer minima (figure 2*b, d*).

THE FATE OF ADDED NO_3^- -N IN THE WATER BODY: THE BLELHAM STUDY

The aim of the Blelham study was to trace the way in which nitrate added from a surrounding catchment may be cycled within the water body. This was done by adding to the Blelham enclosure (18500 m³ capacity, 45 m in diameter and 12 m deep, sealed into the sediment) (see Lack & Lund 1974) 11 kg of NaNO_3 enriched with 9.63 at. % excess ¹⁵N and 789 g K_2HPO_4 (N:P atomic ratio 10:1). This material was sprayed onto the surface water around midday on 19 August 1976 during the period when the water column was thermally stratified. Its fate was subsequently monitored.

The importance of assimilation by the algae

The main effect of the addition of fertilizer was that an algal bloom resulted. Before the addition of fertilizer the chlorophyll *a* content of the water column was low (less than 5 µg chlorophyll *a* l⁻¹), but within 7 days a transient growth of green algae developed in the epilimnion to be replaced thereafter by an extensive bloom dominated by the blue-green alga *Microcystis aeruginosa*. Over 90% of the ¹⁵N added could be detected in this bloom during August–September, and when the algae sedimented in late October, after the thermocline broke down, this ¹⁵N was deposited, as a layer about 2 cm thick, mainly of algae, on the sediment surface. Incorporation of ¹⁵N into the zooplankton (mainly *Daphnia hyalina*, *Eudiaptomus gracilis* and *Mesocyclops leuckarti*) was negligible because although the zooplankton showed labelling this represented less than 1% of the total particulate ¹⁵N. That is, the algae served as the sink into which the ¹⁵N accumulated and when they sedimented in autumn the ¹⁵N label likewise sedimented (figure 3). Such results emphasize the effect of added N and P on the biota of the water column: an increase in biomass occurs and the most obvious effect is an algal bloom, as has often been noted (see Lund (1965) and Reynolds & Walsby (1975)).

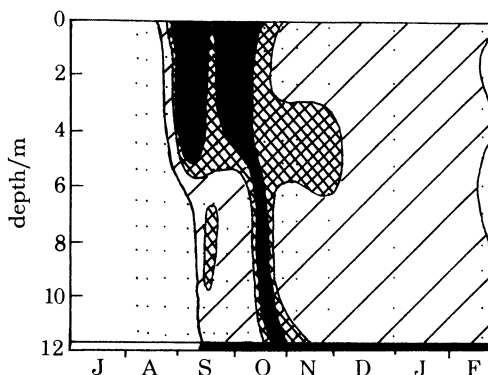


FIGURE 3. The distribution of particulate ^{15}N in the Blelham enclosure after the addition of 11 kg of ^{15}N -labelled NaNO_3 (9.63 at. % ^{15}N) on 19 August 1976. ■, over 8; ▨, 5-8; ▩, 1-5; □, 0-1 $\mu\text{g l}^{-1}$. (After Preston *et al.* (1980).)

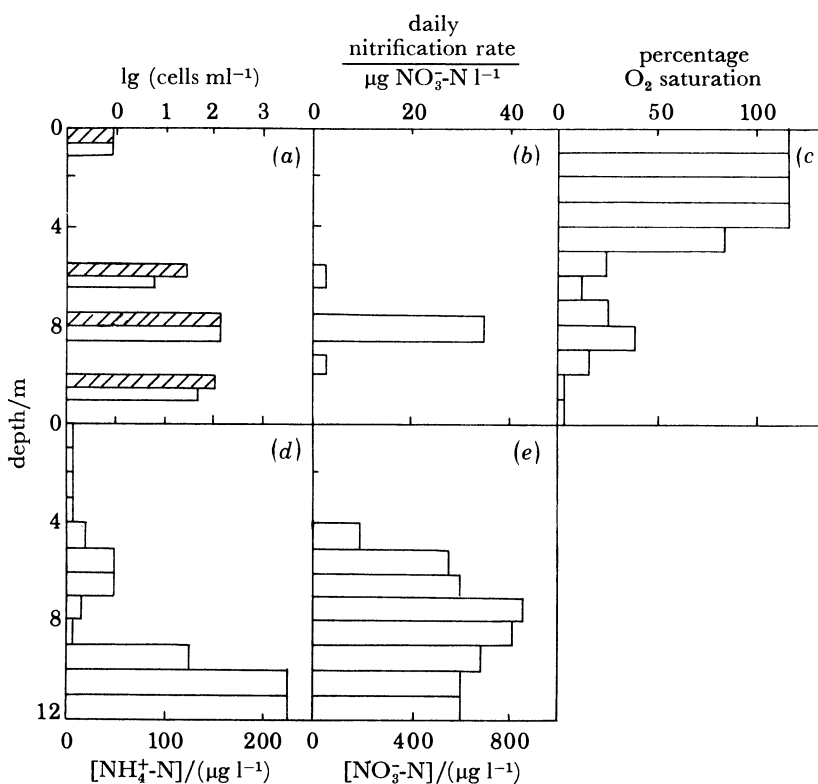


FIGURE 4. Relations between nitrification and other factors in the water column of the Blelham Tarn enclosure (8 September 1976). (a) ▨, Abundance of phase I ($\text{NH}_4^+ \rightarrow \text{NO}_2^-$) and □, phase II ($\text{NO}_2^- \rightarrow \text{NO}_3^-$) nitrifying bacteria (MPN technique); (b) nitrification rates based on N-Serve-sensitive HCO_3^- incorporation; (c) percentage O_2 saturation; (d) NH_4^+ -N concentrations; (e) NO_3^- -N concentrations. (For methodology and other details, see Christofi *et al.* (1981).)

Mineralization and nitrification

In the Blelham sediment, mineralization began almost immediately on algal sedimentation. Mineralization also occurred in the water column, but, because of reassimilation, free NH_4^+ did not accumulate in the water column. We have shown previously that when a *Microcystis* bloom sediments at the end of the summer a significant proportion of the sedimenting algal cells are appreciably less healthy and have an ultrastructural appearance characteristic of degenerate laboratory cultures, while others remain healthy and are rich in storage reserves (Stewart *et al.* 1978). It is such latter type cells that probably overwinter on the sediment and provide an inoculum for the succeeding year's *Microcystis* bloom (Preston *et al.* 1980).

In these studies, concomitant with the sedimentation of particulate ^{15}N , $^{15}\text{NH}_4^+$ began to appear in the sediment pore water. The proportion of the total ^{15}N that this represented varied with time, being about 1% in September and 8% (the maximum) in November 1976 before declining. Such NH_4^+ values represent the extent to which production exceeds consumption. By November more ^{15}N was present as NO_3^- than as NH_4^+ in the enclosure as the result of nitrification (see below); NH_4^+ assimilation by sediment microorganisms no doubt also occurred.

Nitrification, the process by which NH_4^+ is converted to NO_3^- , is carried out mainly by two genera of bacteria, *Nitrosomonas*, which converts NH_4^+ to NO_2^- , and *Nitrobacter*, which converts NO_2^- to NO_3^- . Such organisms are widely distributed in aquatic ecosystems (Niewolak 1970; Fischer 1972; Gode & Overbeck 1972; Isirimah *et al.* 1976; Larsen 1977; Christofi *et al.* 1981). The role of heterotrophic nitrifiers, such as various bacteria, fungi and actinomycetes (see Eylar & Schmidt (1959) and Verstraete (1975)) is usually of secondary importance in most ecosystems (see Painter (1970), Larsen (1977), Schmidt (1978), Belser (1979) and Christofi *et al.* (1979)). For autotrophic nitrification to occur, both NH_4^+ , the substrate, and O_2 , which acts as electron acceptor, must be available.

In general, nitrification is most active at the sediment–water interface. In the Blelham enclosure, however, in summer, when because of stratification O_2 is not available in the sediment, such conditions are provided in the hypolimnion and NO_3^- maxima were observed there at a depth of 8.0 m. Data on nitrification at this zone are presented in figure 4, and the results are discussed in further detail in Christofi *et al.* (1981). Tests for the presence of NH_4^+ -oxidizing bacteria (figure 4a) showed that both NH_4^+ -oxidizing and NO_2^- -oxidizing autotrophs were present and abundant at 8.0 m and 10.5 m, with NH_4^+ -oxidizers being detected in highest number. The rates of nitrification that occurred were examined by using a modification of the ^{14}C -labelling HCO_3^- technique of Billen (1976). The tests were carried out in the presence and absence of N-Serve (2-chloro-6-trichloromethyl pyridine), which specifically inhibits autotrophic nitrification. Typical data (figure 4b) show that substantial rates of N-Serve-sensitive HCO_3^- incorporation occurred at the 8.0 m zone, with little or no activity at the other zones sampled. Data on NH_4^+ concentrations in the water column (figure 4d) show that NH_4^+ was high on either side of an O_2 maximum (figure 4c). High concentrations of NO_3^- were observed at the NH_4^+ -oxygen chemocline (figure 4e). Such results are in accord with those of Mortimer (1942), Burns & Ross (1972) and Hall *et al.* (1978), who have observed nitrate production in the water column. The calculated rates of nitrification in a 2 m deep zone centred on 8 m, based on biological oxygen demand measurements, N-Serve-sensitive HCO_3^- incorporation, regression analyses of the daily changes in NO_3^- -N at 8.0 m, and the integrated

changes in NO_3^- -N throughout the metalimnion and hypolimnion during late 1976 were, respectively, 55.4, 45.0, 22.4 and $39 \mu\text{g N l}^{-1}$ per day (see Christofi *et al.* 1981).

The above results, especially those on N-Serve-sensitive HCO_3^- uptake, provide good evidence of autotrophic nitrification in the hypolimnion. Evidence that the NO_3^- production noted was not due to the added fertilizer N diffusing from the epilimnion to the hypolimnion was obtained on analysing the ^{15}N contents of the epilimnetic and hypolimnetic nitrate samples. The data, presented in figure 5, show that over the period 22–29 August, the decreasing

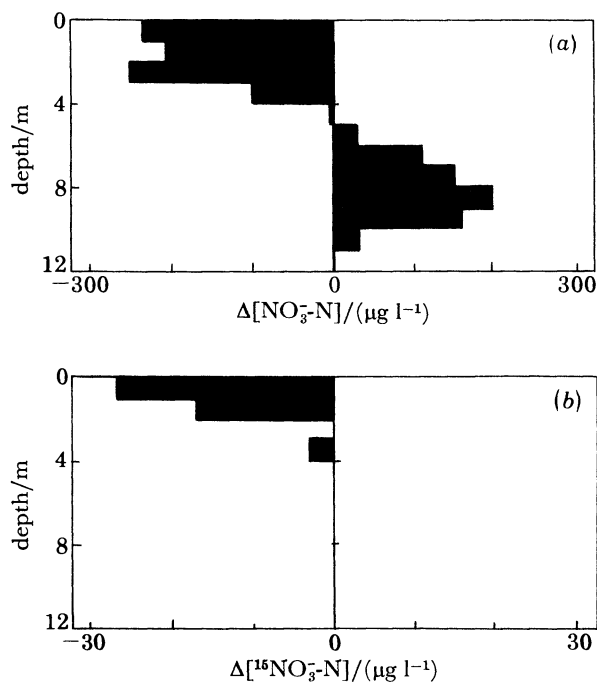


FIGURE 5. (a) Changes in NO_3^- -N concentrations in the epilimnion and hypolimnion of the Blelham enclosure water column during the period 22–29 August 1976; (b) the associated changes in ^{15}N label of the NO_3^- . (After Christofi *et al.* (1981).)

epilimnetic NO_3^- pool remained ^{15}N -labelled and was thus largely derived from the added fertilizer NO_3^- , whereas the increasing hypolimnetic NO_3^- pool had no detectable ^{15}N -labelling, i.e. it was quite distinct from the ^{15}N -labelled epilimnetic pool.

In the Blelham enclosure the zone of maximum nitrification did not remain static; we obtained clear evidence that the position of this zone varied with season. Figure 6 shows the relations between the depth at which maximum nitrification occurred with time during the period August 1976 – February 1977 and how this relates to dissolved O_2 profiles. The zone of maximum nitrification moves higher into the water column as the bottom waters become deoxygenated in summer. At overturn, in late October, when the water column becomes mixed, the zone of maximum nitrification once more reverts to the sediment–water interface. Such a moving zone of maximum nitrification associated with moving oxygen– NH_4^+ fronts has not been reported hitherto, but it is of special interest to note that Mortimer (1941, 1942) reported on seasonal changes in NO_3^- profiles in Esthwaite water that were similar in temporal trend to the results obtained here and which we have been able to correlate positively with changes

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in rates of nitrification. It is likely that such moving zones will commonly be found in deeper waters where stratification sets in in summer; they will be less likely to occur in shallower well mixed lakes where the sediment–water interface will remain the main zone of active nitrification (see Christofi *et al.* (1980), and below for Balgavies).

Nitrification in the hypolimnion and at the sediment–water interface has at least two major roles to play in freshwater N cycling. First, the NO_3^- so produced may serve as substrate for denitrification and nitrate respiration in anoxic zones. Secondly, it may serve as an important

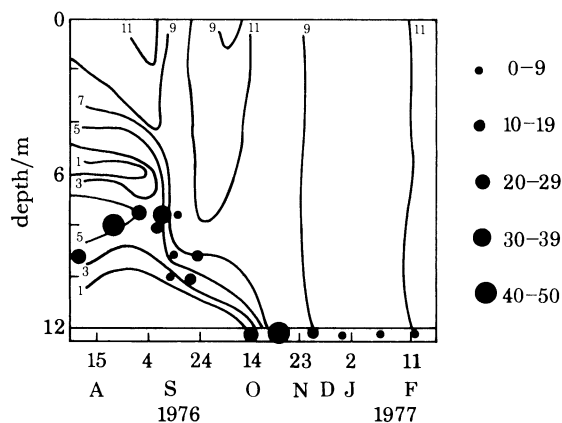


FIGURE 6. The relation between O_2 contours (mg l^{-1}) and zones of maximum nitrification in the Blelham Tarn enclosure Aug. 1976 – Feb. 1977. The diameter of the black zone at each sampling time indicates the observed rate as shown alongside the figure; units in the water are micrograms N per litre per day and in the sediment are milligrams N per square metre per day. During the period 15 August to 13 October, no sediment nitrification was detectable; in winter no water column nitrification was detectable. The water column nitrification zone at each sampling time extended over a depth of ca. 2 m. (See Christofi *et al.* (1981).)

N species for the transfer of N from deoxygenated bottom waters or sediments to oxygenated waters above. Mortimer (1941, 1942) drew attention to the fact that at the oxycline Fe^{3+} is precipitated from solution and that NH_4^+ would absorb onto such precipitates and resediment. Nitrate, however, is able to pass readily across the oxycline and may be a major route whereby inorganic N derived from mineralization in the anoxic sediment is recycled by nitrification to the oxygenated water column.

Denitrification and dissimilatory nitrate reduction to NH_4^+

Denitrification is the microbial conversion of NO_3^- to the gaseous products of N, N_2O or N_2 . Dissimilatory nitrate reduction to NH_4^+ is a process in which NO_3^- acts as a terminal electron acceptor for energy production by certain fermentative bacteria. Detailed by Woods (1938), this latter process is now arousing renewed interest because of its ability to conserve fixed N concomitant with energy production in natural ecosystems (Dunn *et al.* 1977, 1979; Sorenson 1978; Caskey & Tiedje 1979; Herbert *et al.* 1980). Figure 7 distinguishes between NO_3^- reduction to N_2 and to NH_4^+ , on a seasonal basis (1976–7) in the Blelham Tarn sediment. N_2 production (figure 7a) was highest in early August and late September; there was negligible activity in winter. N_2O production from NO_3^- was negligible in such studies (less than 0.5% of NO_3^- dissimilated appeared as N_2O). Numbers of denitrifying bacteria (figure 7c) were high

during the study period. Trends in NH_4^+ production from NO_3^- were rather similar to those for N_2 production (figure 7*b*) but almost a magnitude lower in activity, except in late August/early September when dissolved NO_3^- concentrations were at a minimum; under such conditions of N deficiency the $\text{N}_2:\text{NH}_4^+$ production ratio was at a minimum (figure 7*d*). The annual denitrification rate from the Blelham tube, based on mass balance and ^{15}N studies, was calculated as 38 kg N ha^{-1} (Preston & Stewart 1982).

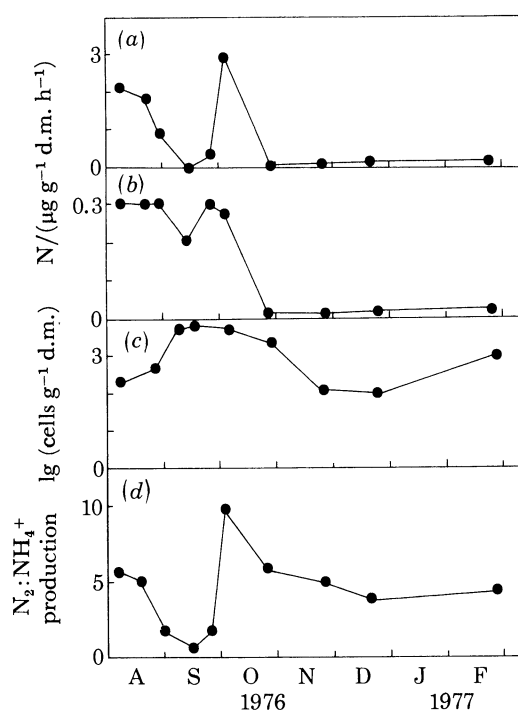


FIGURE 7. Seasonal variations in (a) N_2 production from NO_3^- , (b) NH_4^+ formation from NO_3^- , (c) numbers of denitrifying bacteria, and (d) $\text{N}_2:\text{NH}_4^+$ production ratios in the Blelham Tarn enclosure, August 1976 to February 1977. D.m., dry matter.

THE BALGAVIES NITROGEN CYCLE

The Blelham Tarn enclosure study provided one approach to N cycling in freshwater ecosystems. An alternative approach is to obtain a N budget for inputs and outputs, based on the rates at which various processes occur. This we have attempted to do for Balgavies Loch. The data will be detailed elsewhere. Here some relevant aspects are summarized.

Hydraulic loading and N inputs

In Balgavies Loch, as in other lochs examined (see also, for example, Smith 1976, 1977) there is a strong positive correlation between hydraulic load and NO_3^- load ($p < 0.001$ in the case of Balgavies). This nitrate load dominates the total N pool except in summer and exerts an important effect on the biota and on the various N cycling processes of the loch. Nitrate loading is highest in winter and spring and lowest in summer and autumn (see figure 2). The total annual N input to Balgavies via the inflows is 507 kg N ha^{-1} . Other inputs of fixed N are negligible compared with inputs in the inflowing waters. The average input in direct precipitation is

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approximately 16 kg N ha^{-1} annually (*ca.* 2% of the average in drainage water). Our calculated value for N in precipitation compares well with that quoted by Batey (this symposium) for other upland areas in Scotland (14 kg N ha^{-1} annually). Inputs from biological N_2 fixation likewise are low, with annual inputs from N_2 fixation, mainly by cyanobacteria, being $5\text{--}10 \text{ kg N ha}^{-1}$.

The major N pools of Balgavies are dissolved inorganic N, dissolved organic N and particulate organic N (figure 8*a-d*). The dissolved inorganic N pool is dominated by NO_3^- except for a short period in late summer when NH_4^+ dominates. The dissolved organic pool of N is large, no doubt because it is a poorly utilized N source; it varies little throughout the year. The

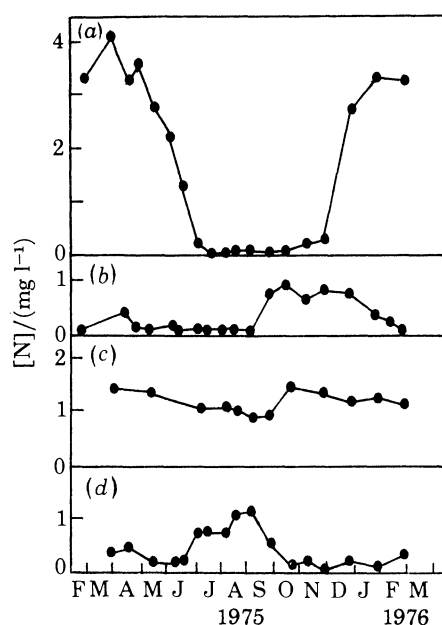


FIGURE 8. Seasonal variations in the concentrations of (a) NO_3^- -N, (b) NH_4^+ -N, (c) dissolved organic N and (d) particulate N in Balgavies Loch, February 1975-6.

particulate pool of fixed N is highest in summer and autumn and is lower at other times. In Balgavies the phytoplankton is dominated by diatoms and cyanobacteria, with the latter being particularly important in summer and early autumn. In summer, when concentrations of dissolved inorganic combined N are low, N_2 fixation by cyanobacteria may be important in sustaining biomass. Nevertheless it has to be noted that although two of the dominant cyanobacteria that occur in Balgavies (*Anabaena* and *Aphanizomenon*) do fix N_2 , the third co-dominant genus, *Microcystis*, does not. Thus, a capacity to fix N_2 , although beneficial to those organisms that possess it, should not be regarded as the overriding reason for the dominance of cyanobacteria in summer. It may be that the capacity to accumulate fixed N, as sediment-derived *Microcystis* is able to do (see above), is equally important. It may be noted that when fixed N, and other nutrients, are available in excess, as in Forfar Loch, cyanobacteria seldom develop, the algal flora then being dominated by green algae and diatoms. The dominant zooplankton present are *Daphnia hyalina* var. *lacustris*, *Eudiaptomus gracilis* and *Cyclops abyssorum*; fish present are eels (*Anguilla anguilla* L.), perch (*Perca fluviatilis* L.), brown trout (*Salmo trutta* L.), rainbow trout (*Salmo gairdneri* Richardson) and pike (*Esox lucius* L.).

Nitrate assimilation by Balgavies phytoplankton

Figure 9 shows the rates at which populations of natural phytoplankton, present at different times of the year, assimilate added $^{15}\text{NO}_3^-$. Figure 9a shows potential rates because the concentrations added may be in excess of those that would be available in Nature. Assimilation potential was low in winter and greatest during the period late June – early August, when a maximum of 47 kg N reduced per day was recorded per loch. The rate of assimilation *in vivo* (figure 9b) was calculated by using these figures and that of NO_3^- supply within the loch based on mass balance. The calculated annual rate of NO_3^- reduction was 136 kg N ha^{-1} .

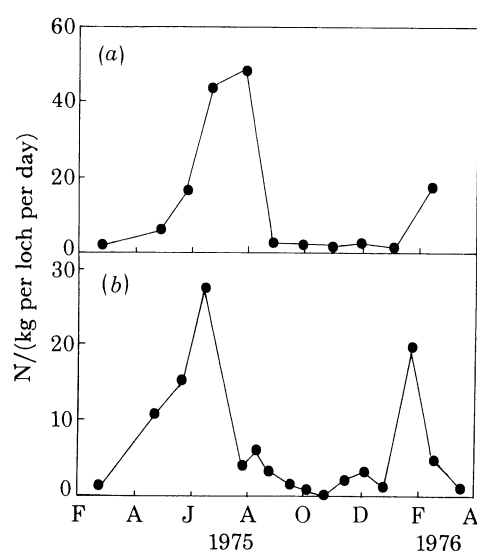


FIGURE 9. (a) Seasonal variations in potential rates of NO_3^- assimilation by the phytoplankton of Balgavies loch based on $^{15}\text{NO}_3^-$ uptake studies; (b) calculated rates of NO_3^- assimilation at *in situ* NO_3^- concentrations, February 1975–6.

Sedimentation of the particulate N

The rate of sedimentation of the particulate N in the loch was quantified by ^{137}Cs profiles of sediment cores (Pennington *et al.* 1973; Ritchie *et al.* 1973; Edberg 1980) and checked by using mass balance. An annual deposition rate of 0.57 cm was calculated, which when taken together with data on N contents gave a total annual N deposition rate of 1.25 t N per loch, a value that is increasing at approximately 4% per year.

Mineralization and nitrification

The rate at which mineralization occurred in the Balgavies sediment is not known with certainty. An estimate can, however, be obtained by a sediment NH_4^+ balance, and that has been done. The annual NH_4^+ production so calculated was 76 kg N ha^{-1} (Preston & Stewart 1982). Nitrification was readily measurable in the Balgavies surface sediment but was negligible in the water column except during a very short period of stratification in summer. As in the Blelham study, both phase I and phase II autotrophic nitrifiers were present but rates of heterotrophic nitrification were negligible. An annual nitrification rate of 105 kg N ha^{-1} was calculated for the sediment.

Denitrification and dissimilatory nitrate reduction to NH₄⁺

The sediment–water interface of Balgavies Loch is an active site of denitrification. Denitrifying bacteria require NO₃⁻ as substrate, fixed carbon as energy supply, and anoxia or very low O₂ concentrations. Various organisms, all capable of NO₃⁻ reduction to N₂ rather than to N₂O, have been detected in Balgavies (Stewart *et al.* 1976). Seasonal data showing potential rates of N₂ production from added NO₃⁻ are presented in figure 10*a*, and figure 10*b* shows seasonal trends in NH₄⁺ production from NO₃⁻. We have examined the extent to which the denitrification potential of Balgavies exceeds the rates based on the quantities of NO₃⁻ available for reduction. The potential rates, as expected, are substantially higher than those occurring *in situ* because of NO₃⁻ limitation. The rates *in situ* are calculated to be 151 kg N ha⁻¹ annually for denitrification; the corresponding rate for NH₄⁺ production from NO₃⁻ is 33 kg N ha⁻¹.

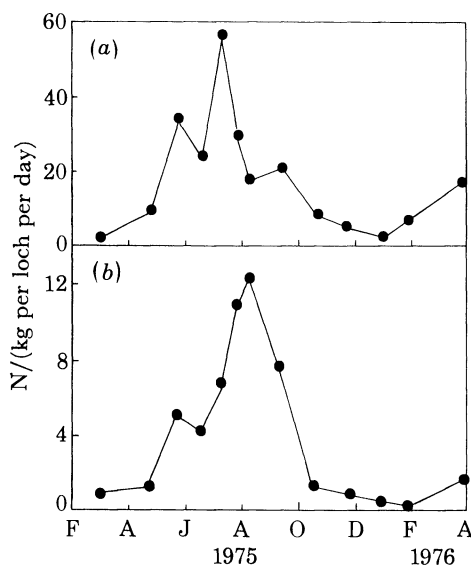


FIGURE 10. Seasonal variations in potential rates of (a) denitrification and (b) NO₃⁻ dissimilation to NH₄⁺ in Balgavies Loch, February 1975–6.

DISCUSSION

The Blelham enclosure and Balgavies Loch are different examples of freshwater bodies of water that have been subjected artificially to eutrophication. They show many differences, yet the N cycling processes going on within them differ only in quantitative detail (table 2).

The addition of fertilizer to both systems resulted in the development of algal blooms. In Blelham, a comparable bloom did not develop outside the fertilized enclosure and neither were there comparable algal biomasses to those found in Balgavies in other Scottish lochs not receiving a major input of nutrients from agriculture or urbanization (e.g. the oligotrophic Craighush). The development of algal blooms in response to nutrient addition should not be surprising. Fertilizers are added to the land to increase plant production. If these nutrients leach into freshwaters they increase production there as well. A crop response to fertilizer is not a prerogative of terrestrial ecosystems only!

Only a relatively small N addition was made to the Blelham enclosure (46 kg N ha^{-1}) compared with an annual addition to Balgavies of 507 kg N ha^{-1} . Nevertheless the net biomass of primary producers was rather similar in both ecosystems. This is because the endogenous N pool is important: for example in Blelham the N biomass greatly exceeded the amounts of N added in the summer of 1976. The fact that the biomass did not correspondingly increase in response to added N in Balgavies emphasizes that some factor other than N limits productivity there or that average N loadings do not reflect the availability of N. In Balgavies, NO_3^- was lowest when it was most required for algal growth (i.e. in summer) and then the surface waters

TABLE 2. A COMPARISON OF THE IMPORTANCE OF VARIOUS N-CYCLING PROCESSES IN THE BLELHAM TARN ENCLOSURE AND IN BALGAVIES LOCH

(All values are kilograms N per hectare per year.)

	Blelham	Balgavies
NO_3^- load	46	507
primary production	146	136
net mineralization	55	76
deposition	91	60
denitrification	38	151
dissimilation to NH_4^+	7	33
total NO_3^- dissimilation	45	184
total NO_3^- reduced	191	320
sediment nitrification	68	105
water column nitrification	85	5
total nitrification	153	110

were as nutrient-depleted as the surface waters of the Blelham enclosure. It is not the overall total N in a freshwater that is important but the supply available when N is a limiting factor.

Algal growths have at least three important roles to play in gross freshwater N cycling. First, by assimilating NO_3^- they reduce the NO_3^- concentrations *in situ*. For example, in Balgavies, algal assimilation removed more than a quarter of the added NO_3^- load and in Blelham it removed NO_3^- in excess of that added by us to the enclosure. Secondly, sedimentation of particulate algae is an important route whereby fixed N reaches the sediment, where it is rapidly mineralized. Thirdly, the primary producers provide the fixed carbon so essential as an energy source for heterotrophic N cycling organisms, especially denitrifiers which hold the key to overall NO_3^- removal from freshwaters. The NO_3^- concentrations in freshwaters today would be substantially higher than they are if it were not for the presence of algal growths and other aquatic primary producers.

The relative rates of mineralization in the two ecosystems (table 2) do not differ substantially, with N equivalent to 36% of that in primary production being mineralized in a year in Blelham compared with 56% in Balgavies. The higher rate in Balgavies is almost certainly related to the fact that Balgavies is a shallow, well mixed lake with an oxygenated sediment and a higher sediment surface temperature in summer, factors that allow rapid catabolism of organic matter. Of the N mineralized, some is lost as NH_4^+ to the deeper sediments, assimilated or nitrified. NH_4^+ loss to the deeper sediment is minimal compared with the loss of deposited particulate material in this way. In the Blelham sediment, deposition accounts for 91 kg N ha^{-1} annually, a value that compares with 100 kg N ha^{-1} calculated by Pennington (1978) for sedimentation in Blelham Tarn. The corresponding rate for Balgavies is 60 kg N ha^{-1} a value lower than that

for Blelham, largely because of the greater mineralization that occurred in the Scottish loch. N sedimentation rates reported by others for fertilized lakes are 46 kg N ha⁻¹ annually for Lake 227 in Canada (Schindler *et al.* 1976) and 41 kg N ha⁻¹ for Lake Mendota in the U.S.A. (Brezonik & Lee 1968). Only exceptionally when storms ensue in shallow, well mixed lakes will such sedimented N be returned to the water column. Such resuspension is likely to be greater in Balgavies than in Blelham.

The sediment–water interface is the engine-room of the aquatic nitrogen cycle, and foremost among the events of importance that occur there is nitrification. A high rate of nitrification is central to efficient N cycling, and its quantitative significance in lakes has been largely ignored in the past, possibly because of difficulties in measuring the rates *in situ*. By producing NO₃⁻, nitrification serves as an important source of substrate for denitrification. Indeed, NO₃⁻ production annually by nitrification in Balgavies is equivalent to 22 % of that entering in the inflow waters or about 80 % of the N present in the phytoplankton biomass; in Blelham it is significantly greater than that present in the primary producers. Perhaps in the past too much attention has been paid to the NO₃⁻ levels entering in the inflows without adequate attention being paid to that produced *in situ*. The greater nitrification noted in Blelham than in Balgavies is due to the fact that water-column nitrification occurred in Blelham; without this there would have been substantially less nitrification in Blelham than in Balgavies.

Denitrification has long been regarded as an important route of nitrate removal from freshwaters. There is no doubt that N losses by denitrification occur; the problem is in accurately quantifying these losses. What are required are accurate measurements of N gas release from the concentrations of nitrate that occur *in situ*. This is no easy matter as Jones & Simon (1981), who have made a valuable start in this connection, have shown. Like Ahlgren (1979), we believe that ¹⁵N-tracer methodology offers one of the most satisfactory approaches and in this study an attempt has been made to relate the rate processes to what happens at concentrations occurring *in situ*. The first observation from our studies is that, despite what may happen in terrestrial ecosystems, neither the freshwater isolates that we used nor natural samples produced significant amounts of N₂O as a denitrification product; N₂ was always the major end-product of the denitrification tests. This accords with the recent results of Denmead *et al.* (1979) and McKenney *et al.* (1980) for rice paddy soils. Secondly, NH₄⁺ production from NO₃⁻ was observed, as workers in the laboratory (Woods 1938; Dunn *et al.* 1977), on soils (Caskey & Tiedje 1979) and on marine ecosystems (Sorenson 1978; Dunn *et al.* 1979; Herbert *et al.* 1980) have noted. This is a minor but important source of nitrate reduction for several reasons. First, it emphasizes that the finding of NH₄⁺ in the sediment cannot necessarily be attributed to mineralization; in anaerobic sediments nitrate respiration supplements that derived from mineralization. Secondly, while beneficial to the bacteria that produce the NH₄⁺ in that it is an energy-yielding process, a problem from the point of view of water management is that it leads to N retention within the ecosystem. The N conserved in this way in Balgavies is equivalent to a quarter of the total N present in the primary producers; in Blelham the corresponding figure is 15 %. In terms of the use of denitrifying bacteria to remove NO₃⁻ from sewage works by denitrification it will be essential to ensure that strains and conditions leading to N₂ rather than NH₄⁺ are used. In both water bodies the percentage of the NO₃⁻ reduced to NH₄⁺ rather than to N₂ was very similar.

Our measurements of denitrification show clearly that denitrification to N₂ is a major source of NO₃⁻ removal from the ecosystem with the loss of N₂ in Balgavies exceeding that removed

from the water column by the primary producers. Nevertheless the Balgavies data, if typical of eutrophic waters in the northern U.K., also show that denitrification rates in such waters, while substantial, cannot cope adequately with NO_3^- inputs from agriculture, and that NO_3^- concentrations in such waters are likely to continue to increase, unless inputs to the waters are reduced, or techniques of manipulating the processes *in situ* to advantage are exploited. These latter processes may include algal stripping, placing NO_3^- inputs near anoxic sediments to enhance denitrification, particularly in summer, and sustained turbulence to disperse algal blooms. The appropriate approach will depend on the body of water under consideration, the extent of the NO_3^- problem, the purpose for which the water is to be used, and, most important, economic and health considerations.

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Discussion

W. B. WILKINSON (*Water Research Centre, Medmenham, Marlow, U.K.*). I am interested in the application of Professor Stewart's work on denitrification in natural lakes to water supply reservoirs. Generally the water industry does not wish its reservoirs to stratify in summer as this may lead to deoxygenated poor-quality bottom waters. Provision may be made, at relatively low cost, to overturn the water artificially in some reservoirs and so break down thermal stratification (Tolland 1977). Professor Stewart suggested introducing water into a reservoir at the sediment level to increase denitrification. However, with existing reservoirs this could involve costly engineering works. To maximize denitrification without such expense is it better to let the reservoirs stratify or to mix them artificially? The same question may be asked in relation to troublesome algal blooms.

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W. D. P. STEWART. I would not wish to give a generalized answer to these questions. What is best may well vary, depending on the water body under consideration. Our basic experimental findings show that algal blooms do develop during stratification and I agree that mixing the water column is advantageous in preventing algal developments. However, for denitrification to occur anoxic or near anoxic conditions are required. These are found at, or just below, the sediment surface in most well mixed waters. We have good experimental evidence that in waters such as Balgavies the potential for denitrification by the sediments is not reached because there is insufficient NO_3^- where it is most required, i.e. at the site of denitrification. It would be worth investigating whether greater denitrification could be achieved by releasing the NO_3^- -rich inflows near the sediment surface and retaining anoxia there, even though the bulk of the water column remains mixed. A problem with inflows entering at the lake surface is that the inflow waters are often warmer than the waters of the lake; that being so, the NO_3^- -rich waters tend to accumulate initially near the surface of the lake, where of course the algae are most abundant. There may be merit in locating the NO_3^- nearer to the denitrifying sediments and further away from the epilimnetic algal growths. I believe that there is a need for further research on the placement of inflowing pipes and on how to maximize denitrification concomitant with oxygenation of part, or all, of the water column.

J. A. COLE (*University of Birmingham, U.K.*). Professor Stewart reminded us about the dominant role of denitrification in the anaerobic part of the biological nitrogen cycle. Nevertheless, many anaerobic bacteria are not denitrifiers but reduce NO_3^- rapidly to ammonia. The ammonia so formed is largely excreted into the environment. Like denitrification, this is an extremely rapid process but it provides a short circuit to the nitrogen cycle because it bypasses the rate-limiting step, N_2 fixation.

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Some of these fermentative bacteria use only a soluble enzyme to reduce nitrite with NADH as the source of electrons: because this enzyme is soluble, none of the energy released during the reaction is conserved by oxidative phosphorylation.

A few years ago, Chippaux and his colleague detected a second pathway for the reduction of nitrite to ammonia by formate. Enzymes of the formate pathway are bound to the cytoplasmic membrane, and recently we have demonstrated, with an ion-selective electrode, that energy is conserved by oxidative phosphorylation during this reaction. The formal evidence for this assertion will be published in the near future. The knowledge that energy is conserved during the reduction of nitrite to NH_4^+ is significant because it means that we now have good reason to believe that it should be possible to regulate how much NO_3^- -N is lost by denitrification or conserved as ammonia in any specific ecosystem because the two processes are in direct competition.

To be able to select one or other process at will, we must first identify differences in their biochemical and genetic regulation. This is the work that is currently in progress in Birmingham.