This manuscript has been submitted to the

Journal of Fisheries & Aquatic Science - Special Issue

for publication and the contents are

subject to change

This copy is to provide information

prior to publication

NITROGEN TRANSFORMATIONS IN LAKE ONTARIO

by

D.R.S. Lean[1] and R. Knowles[2]

NWRI Contribution Series #86-146

December, 1986

[1]National Water Research Institute
Canada Centre for Inland Waters
P.O. Box 5050, Burlington, Ontario
L7R 4A6

[2]Macdonald College of McGill University
Ste. Anne de Bellevue, Quebec
H9X 1C0

Nitrogen Transformations In Lake Ontario

D.R.S. Lean

National Water Research Institute
Canada Centre for Inland Waters
P.O. Box 5050, Burlington, Ontario L7R 4A6, Canada

and

R. Knowles

Macdonald College of McGill University
Ste. Anne de Bellevue, Quebec H9X 1C0, Canada

MANAGEMENT PERSPECTIVES

The increasing nitrate concentration in Lake Ontario has caused the phytoplankton in 1982 to be phosphorus deficient; whereas, 10 years previously, nitrogen was most likely limiting the summer biomass. Seasonal changes in the concentration of the various nitrogen fractions are documented and related to nitrogen flux rates from one form to another. Specifically, although ^{15}N-tracer methodology shows rapid ammonium uptake, much of this is by chemoautotrophic nitrifying bacteria rather than phytoplankton. Nitrate, rather than ammonium, is the principal form of nitrogen used for algal growth. Quantities of nitrate used for protein synthesis (see Cuhel and Lean) were related to seasonal nitrate declines and provide an upper limit for protein available to support the food chain. Seasonal increases in ammonium and nitrite were shown to be related to rates of zooplankton excretion. In view of the high concentrations of oxygen and low concentrations of nitrous oxide, denitrification is probably not a significant process. Consequently, at present rates of N loading nitrate concentrations will likely continue to increase.

PERSPECTIVE-GESTION

Par suite de l'accroissement de la concentration des nitrates dans les eaux du lac Ontario en 1982, le phytoplancton souffre d'une carence de phosphore. Il y a dix ans, l'azote jouait vraisemblablement un rôle inhibiteur sur la biomasse en été. On documente les fluctuations saisonnières de la concentration des diverses fractions d'azote et on établit les rapports avec les taux de transformation d'une forme à une autre. En termes plus précis, bien que l'emploi de l'isotope ^{15}N comme indicateur révèle une captation rapide d'ammonium, le phénomène est imputable aux bactéries nitrifiantes chimiautotrophes plutôt qu'au phytoplancton. Les nitrates et non l'ammonium constituent la principale forme d'azote servant à la croissance des algues. On a relié les quantités de nitrates servant à la synthèse de protéines (voir Cuhel et Lean) aux déclins saisonniers de nitrates. Ces chiffres déterminent la production maximale des protéines utilisées par la chaîne alimentaire. On a démontré que les augmentations saisonnières d'ammonium et de nitrites sont liées au taux d'excrétion du zooplancton. Vu les concentrations élevées d'oxygène et les faibles concentrations d'oxyde nitreux, la dénitrification n'est probablement pas marquée. Par conséquent, si la charge d'azote se maintient au niveau actuel, les concentrations de nitrates demeureront vraisemblablement à la hausse.

Lean, D.R.S. and R. Knowles. 1987. Nitrogen Transformations in Lake Ontario. Can. J. Fish. Aquat. Sci. 44: ØØØ-ØØØ.

Concentrations of ammonium plus nitrite in Lake Ontario were highly correlated with ammonium regeneration from zooplankton excretion (r=Ø.966) inferring that elevated nitrite concentrations result from nitrification. Nitrapyrin-sensitive dark ^{14}C-bicarbonate assays confirmed high rates of nitrification by chemoautotrophic bacteria. ^{15}N-nitrate experiments showed that nitrate, not ammonium, was the principal form of N used for total microbial protein synthesis. Size fractionation experiments also suggested that small cells were responsible for most of the ammonium uptake while large cells used mostly nitrate. Nitrate depletion in the surface waters during summer stratification resulted from movement to particulate N, nitrite and ammonium as well as losses in particulate N due to sedimentation. At least one third however was unaccounted for (i.e. 3Ø mg $N.m^{-2}.d^{-1}$) and may have been converted to protein which would move up the food chain to larger organisms (eg. fish) not sampled during conventional water chemistry. Nitrous oxide profiles showed that nitrate losses through denitrification are unlikely to occur. Consequently, unless nitrate loading to lake Ontario is reduced, nitrate concentrations should be expected to continue to increase.

D.R.S. Lean and R. Knowles. 1987. Transformation de l'azote dans le lac Ontario. Journal canadien de sciences halieutiques et aquatiques. 44:000-0000.

On a découvert une corrélation marquée entre les concentrations d'ammonium et de nitrites dans le lac Ontario et la regénération de l'ammoniun par l'excrétion du zooplancton (r=0,966) ce qui permet de conclure que la nitrification entraîne des concentrations élevées de nitrites. Des essais de susceptibilité à la nitrapyrine dans l'obscurité au bicarbonate-^{14}C ont confirmé les taux de nitrification élevés des bactéries chimiautotrophes. Des expériences ont révélé que les nitrates et non l'ammonium représentent la principale forme d'azote qu'utilisent les micro-organismes pour la synthèse totale de protéines. Le fractionnement selon la taille permet en outre de penser que la captation de l'ammonium est surtout le fait des petites cellules tandis que les cellules de taille plus importante assimilent surtout les nitrates. L'appauvrissement de l'azote dans les eaux de surface pendant la stratification estivale a découlé de la transformation de l'azote sous forme particulaire, aux nitrites et à l'ammonium de même que des pertes d'azote particulaire dues à la sédimentation. Toutefois, le tiers de la quantité initiale (c'est-à-dire 30 mg $N.m^{-2}.d^{-1}$) n'a pas été récupéré et a pu être transformé en protéines qui sont passées dans les maillons supérieurs de la chaîne alimentaire (par ex., les poissons), organismes qui ne sont pas échantillonnés par les méthodes d'analyse chimique classiques. Les profils d'oxyde nitreux montrent que la dénitrification risque d'entraîner des pertes de nitrates. Par conséquent, à moins que la charge de nitrates du lac Ontario ne soit réduite, les concentrations continueront d'augmenter.

Our understanding of the important processes which occur as nitrogen moves through lake ecosystems is based largely on extrapolations from soil research, incomplete N-budgets, and a few kinetic measurements. Consequently, existing flow diagrams are largely hypothetical and factors which influence the seasonal concentration patterns of the nitrogenous forms are incomplete.

Because of the intermediate oxidation level of nitrite, an emphasis on factors which influence the concentration of this form is essential. Nitrite maxima have been observed in many of the world's oceans (Olsen 1981a), in Lake Michigan (Mortonson and Brooks 1980) and in Lake Ontario in 1966 and 1967 (Dobson 1984). Nitrite maxima in the ocean have been thought to result from either ammonium oxidation by chemoautotrophic nitrifying bacteria (Brandhorst 1959; Olsen 1981a; Ward et al. 1982), or phytoplankton excretion during assimilation and reduction of nitrate (Vaccaro and Ryther 1960). In the euphotic zone, where nitrate levels are often low, nitrite uptake would usually outweigh its production (Olsen 1981a). Here photoinhibition of nitrification is also possible (Olsen 1981b) and chemical photolysis of nitrite must be considered (Zafiriou and True 1979).

Based on mass balance calculations, significant nitrification can also occur in the aerobic hypolimnia of lakes (Christophi et al. 1981; Takahashi et al. 1982; Verdouw and Dekkers 1982; Hall and Jeffries 1984). Evidence for nitrification also comes from studies of oxygen depletion rates (Wezernak and

Gannon 1967). Knowles and Lean (1987) found winter nitrification to be the major factor causing oxygen depletion in Lake St. George, Ontario.

Despite the great sensitivity of ^{15}N stable isotope techniques, data on uptake of nitrate, nitrite, ammonium and urea are not without problems of interpretation and rarely can be extrapolated to ambient concentrations (Glibert et al. 1982, 1985; Wheeler et al. 1982; Laws 1984). In Lake Ontario, due to the high ambient nitrate concentrations, uptake rates are always maximal; but the low concentrations of ammonium make kinetics for this form difficult to interpret (Murphy 1980; Lean et al. 1982).

Limnologists have often considered that the nitrate decline in May-June in many temperate lakes was due to algal assimilation (eg. Burns 1976) but a corresponding increase in particulate N does not always occur. Consequently, other losses must be identified.

In this investigation, the seasonal concentration patterns of nitrogenous forms were related to uptake rates of ammonium and nitrate, estimates for nitrification, N-sedimentation and ammonium regeneration from zooplankton excretion. In this way, the major nitrogen pathways in the Lake Ontario ecosystem could be identified.

METHODS

Experiments were conducted in Lake Ontario in 1982 as part of the LONAS study along the transect extending from northshore

station 401, near Cobourg, Ontario to southshore station 405 with emphasis on transport rates measured at mid-lake station 403 (Lean 1986; Simons and Schertzer 1986) . As in previous limnological surveys on Lake Ontario (Stadelman and Frazer 1974; Dobson 1984) samples from discrete depths were filtered through 0.45 um (Sartorius 11103) membrane filters, with concentrations of nitrite, nitrate and ammonium were usually determined immediately using a Technicon II autoanalyser. Values for total Kjeldahl nitrogen were obtained on filtered samples maintained at $4^{o}C$ and analysed within 1 week. Total particulate N was measured using a Hewlett Packard CN analyser. All methods are described in the Environment Canada 1979 Methods Manual.

Dark incorporation of radioactive bicarbonate in the presence and absence of the inhibitor nitrapyrin has been used as an index of nitrification (Billen 1976; Somville 1978; Vincent and Downes 1981; Vincent et al. 1981; Hall 1982; Knowles and Lean 1987). In the following experiments, samples were obtained with 6-L Niskin bottles and transferred to 300-mL BOD bottles, keeping the tube at the bottom of the bottle and allowing water to overflow for 10-15 seconds to avoid changes in dissolved gas concentrations. Just prior to incubation, 20 uL of analytical grade nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) from Dow Chemical, dissolved in dimethyl sulfoxide (DMSO) was added to each BOD bottle giving a final concentration of 20 mg. L^{-1}. nitrapyrin. ^{14}C-bicarbonate (Amersham) was added to a final

concentration of about 10^6 dpm.mL^{-1}.

Measurements of N_2O were conducted as described by Knowles et al. (1981). Water samples were placed in 350 mL sealed bottles without a headspace along with 1 mL of saturated $HgCl_2$. Nitrous oxide was determined by multiple phase equilibrium and gas chromatography with ^{63}Ni electron capture detection.

Assimilation of ^{15}N-nitrate and ^{15}N-ammonium was determined using a Jasco emission spectrometer (NAJI-1) (Murphy 1980). Dried filters (preignited 25 mm Whatman GF/F) containing the collected particulate material were put into discharge tubes with CuO powder as a catalyst. The tubes were sealed after evacuation to 0.01 Pa. combusted at 590°C for 16 h and allowed to cool for 24 h. Each sample required only 10 ug N for good emission. Uptake velocity was calculated using the following formula:

$$v = \frac{\% \text{ excess } ^{15}N \text{ in seston} \times \text{Particulate N}}{\% \text{ excess } ^{15}N \text{ in } NH_4 \text{ or } NO_3 \times \text{time}}$$

^{15}N-ammonium chloride and sodium ^{15}N-nitrate was supplied as 97 ^{15}N atom % (Biorad). ^{14}C uptake was measured as described in Lean et al. (1986). Samples were incubated in a temperature-controlled incubator with light levels from a quartz-halogen fluorescent street light controlled by neutral density filters.

RESULTS AND DISCUSSION

Seasonal pattern for nitrogen partitioning

Concentrations of nitrite were in excess of 5 ug $N.L^{-1}$ throughout most of the water column during the stratified period from early July to September (Fig. 1). At other times, values were usually 2-3 ug $N.L^{-1}$. Nitrite concentration at station 403 peaked in August at over 10 ug$N.L^{-1}$ (max 19). Station 401 (Fig. 1) and 405 (not shown) followed a similar pattern but concentrations were slightly elevated during thermal bar conditions. Upwelling in October caused values at 401 to decline more abruptly than at other stations. Peak nitrite concentrations at each station across the lake (Fig. 2) illustrate the influence of thermal stratification patterns across the lake. The 12 ug$N.L^{-1}$ isopleth approximates the depth of the epilimnion. The mixing depth is greater at station 405 (Simons and Schertzer 1987).

Instead of being restricted to the metalimnion as in 1966 and 1967 (Dobson 1984), elevated nitrite concentrations extended to the surface in 1982. Unlike that in 1972 (Stadelman and Frazer 1974) and before, nitrate concentrations remained above 130 ug $N.L^{-1}$ (see below Fig. 4, and Stevens and Neilson 1986). As such, nitrite utilization may be reduced in the surface waters. In 1972, when nitrate levels were low (< 5 ug $N.L^{-1}$), nitrite uptake would probably outweigh its production.

At station 403, ammonium values were 2-3 ug $N.L^{-1}$ until the end of June (Fig. 3) when ammonium concentrations began to

increase. By August, metalimnetic values exceeded 10 ug $N.L^{-1}$. Through September and October, higher ammonium values extended into surface waters. Below 50 m, ammonium concentrations were usually less than 3 ug $N.L^{-1}$ throughout the season except in September and October. By November ammonium values returned to the 1-2 ug $N.L^{-1}$ range.

At 401 (upper panel-Fig. 3), the elevated ammonium values appeared earlier in the summer and, like those for nitrite (Fig. 1) declined sooner in the fall due to an October upwelling event. Station 405, which was similar to 401 at other times of the year, received warm surface waters causing high ammonium values in October at 30-36 ug$N.L^{-1}$ (not shown).

Seasonal changes in the depth distribution of nitrogenous forms are illustrated by comparing values from 8-11 June to those for September 20-23 (Fig. 4). Thermal bar conditions existed in June (Simons and Schertzer 1986) with surface temperatures of 10-11^{o}C down to 5 m at 401 and 405 while the mid-lake was isothermal at about 3 ^{o}C. Nitrate was depleted in the surface waters at 403 from 360 ug$N.L^{-1}$ to about 130 ug$N.L^{-1}$. When this decrease was integrated over the top 40 m, 6,215 mg $N.m^{-2}$ was converted to other forms. The increase in particulate N, nitrite and ammonium accounted for only 1250, 326, and 242 mg $N.m^{-2}$ respectively.

Other protential losses of nitrogen were considered. N-sedimentation, measured with sediment traps located at 40 m (Lean et al. 1987) was 1909 mg $N.m^{-2}$ during this period. No significant

changes in dissolved organic nitrogen were observed with values in surface waters remaining near 210 $\pm$ 16 ug $N.L^{-1}$. Consequently, only 3,727 of the 6,215 mg $N.m^{-2}$ can be accounted for. Input of N to surface waters was not measured so the difference, which has yet to be explained, is a conservative estimate.

Denitrification must also be considered as a potential sink for nitrate-N. However, conditions in Lake Ontario leave little opportunity for denitrification even late in the season. For example, on 23 September, at station 403, (Fig. 5) the temperature of the epilimnion was 16.1 ^{o}C, with the metalimnion extending from 15 to about 50 m. Oxygen concentrations were near saturation except at 25 m (91% saturation) and within 10 m of the sediments (87%). For reasons which are not clear, nitrous oxide concentrations (Fig. 5) were below saturation and consistent with data from October and from Lemon and Lemon (1981) (Table 1). Consequently, Lake Ontario acts as a sink for atmospheric N_2O (see Knowles et al. 1981 for discussion). This, and the high oxygen concentrations, suggests that denitrification in the water column is not a significant process for nitrate loss from Lake Ontario.

Below the euphotic zone (shaded area-Fig. 5), there was little accumulation of ammonium (Fig. 3, 4) resulting from decomposition, zooplankton excretion and release of ammonium from sediment pore waters (Fig. 4). Ammonium concentrations are little changed from those in June but nitrite and nitrate levels were

higher near the sediments (Fig. 4). These observations are connsistent with the hypothesis that in the deeper waters, regenerated ammonium is nitrified. Bioassays will be discussed below which support this view.

Cuhel and Lean (1987a,b) provided estimates of protein production based on ^{14}C-bicarbonate and ^{35}S-sulfate assimilation. Values were converted to protein based on 52% C and 1% S. These results can now be converted to N assuming that the percent N in protein was 17 (Jukes et al. 1975). Prior to thermal stratification, protein synthesis was low as was nitrate depletion. During the summer period, protein synthesis averaged about 300 mg protein.$m^{-2}.d^{-1}$ (Cuhel and Lean 1987a). This quantity times the 85 day period of stratification to 22 September (times 0.17 to convert to protein N) gives a value of 4,335 mg N.m^{-2}. If half of this quantity were transferred to larger organisms not sampled, it would account for the N "lost" from our budget. In other words the community would have a nitrogen or protein assimilation efficiency of approximately 50 %.

Zooplankton excretion

The release of ammonium through zooplankton excretion depends on the community assimilation efficiency rather than the individual or trophic level assimilation efficiency. If the zooplankton population was not expanding, i.e. growth was equal to losses through mortality only a fraction of the nitrogen

assimilated by zooplankton would be moved up the food chain. At higher trophic levels the assimilation efficiency is lower. This illustrates the important role of fish in aquatic nutrient cycles. A community that is not expanding has an assimilation efficiency of zero.

The influence of microzooplankton (Taylor and Heynen 1987) and heterotrophic microflagellates (Pick and Caron 1987) was neglected. The filtering rate of small animals (30-64 um) was small relative to the > 64 um (Lean et al. 1987). Heterotrophic microflagellates have been shown to be significant in ammonium regeneration in marine systems (Goldman et al. 1985) but their overall importance in nutrient regeneration cannot be quantified at this time. Since they are food for macrozooplankton and if their net growth is zero, their N assimilated is included in the feeding rate calculation for the > 64 um animals. Consequently, for the following calculations it is recognized that the community assimilation efficiency is unknown but initially the calculation is made assuming it is zero.

Values for particulate N parallel those for chlorophyll a in the surface waters, increasing at 15 m, then continuing proportionately higher than chlorophyll throughout the water column (Fig. 5). Assuming that all particulate N was edible and assimilation efficiency was zero, the product of zooplankton grazing (from Lean et al. 1986) and particulate N provided an estimate for ammonium regeneration by major (>64 um) zooplankton

(right panel-Fig. 5). During this period (21-23 Sept.), regeneration rates would be about Ø.2 ugN.L^{-1}.d^{-1} in the deep water and 5.8 ugN.L^{-1}.d^{-1} in the upper 2Ø m (Table 2).

Data obtained throughout 1982 were treated as above. Filtering rates were averaged over the epilimnetic zone over 24 hours and multiplied by the mean particulate N (Ø-15 m). The product was plotted against ammonium concentrations (1-15 m). This relationship (Fig. 6) was significant (r=Ø.838), but the sum of ammonium plus nitrite was even better correlated to regeneration (Fig. 6) (r=Ø.966). If uptake rates of ammonium and nitrite were small, the observed concentration represents 4 days (slope of regression) of zooplankton excretion assuming an assimilation efficiency of zero. However, if assimilation efficiency was 5Ø%, the quantity would represent 8 days excretion and can account for the "lost" N from the seasonal N budget discussed above. These are minimum estimates for the reassimilation is certainly not zero. Integrating these rates will be a challenge for future investigations.

Nitrification

Nitrification by chemoautotrophic bacteria (CAB) was estimated by the nitrapyrin-sensitive dark ^{14}C-bicarbonate incorporation. For example, samples from 1-2Ø m collected on 23 September at 4Ø3 were incubated at 16 ^{o}C while samples from the rest of the water column were incubated at 4 ^{o}C. Duplicate bottles generally compared well (Fig. 7). Data for 1-2Ø m samples

averaged Ø.31 (± Ø.11), 25-5Ø m was Ø.18, and 1ØØ-176 m was approximately Ø.1 ug $C.L^{-1}.d^{-1}$. Converting carbon to ugN oxidized, by dividing by Ø.1Ø2 (Christofi et al. 1981), gives 3.16, 1.75 and 1.Ø ug $N.L^{-1}.d^{-1}$ for the three depth categories (Table 2). Although assimilation of ammonium by phytoplankton would be expected in the trophogenic zone, nitrification can account for over half of the ammonium released by zooplankton grazing in surface waters. In the deep waters, the nitrification assay suggests that these organisms have the potential to nitrify even more ammonium than is regenerated by major zooplankton excretion.

Additional data were obtained on October 2Ø. Zooplankton grazing, in the Ø-2Ø m zone, was 8 $\%.d^{-1}$ giving a regeneration rate of 3.3 ug $N.L^{-1}.d^{-1}$. Nitrification rates at ambient temperatures ($12^{o}C$), provided values of Ø.4 to 1.7 ug $N.L^{-1}.d^{-1}$ for the Ø-5Ø m zone. At this time of year, it appears that the nitrification rate was less than the regeneration rate. This may help to explain the observed increase in ammonium (Fig. 3).

Oxygen consumption associated with the oxidation of ammonium and nitrite to nitrate is 4.33 ug O_2 per ug of N (Wezernak and Gannon 1967). In September, total epilimnetic respiration rates were 6.8 ug $O_2.L^{-1}.h^{-1}$ (Lean et al. 1986). Nitrification rates averaged 3.1 (± Ø.11) ug $N.L^{-1}.d^{-1}$ or Ø.6 ug $O_2.L^{-1}.h^{-1}$. Although further investigation is necessary, nitrification required

roughly 10% of the total respiration.

Ward et al. (1982) reported that nitrapyrin-sensitive chemoautrotrophic dark ^{14}C assimilation in a mixed population would be negligible. In our example, it was only 30% of the dark ^{14}C-bicarbonate uptake in the surface waters but over 50% in the deeper samples (Fig. 7). Filter fractionation of dark ^{14}C-bicarbonate uptake confirmed that most (ca. 70%) of the dark fixation is in the fraction 0.2-1.0 um (Table 3).

Although the extracellular release of nitrite by phytoplankton as suggested by Vaccarro and Ryther (1960) may occur, nitrification by chemoautotrophic bacteria can easily account for nitrite production. Ward (1984) cautions, however, that the nitrapyrin method may overestimate nitrification and affect dark carbon assimilation by other bacteria and phytoplankton.

Nitrate and ammonium uptake kinetics

Stable isotope ^{15}N-nitrate and ^{15}N-ammonium uptake measurements were conducted from April 7 to November 20. Examples were selected to illustrate specific conditions: when the water was cold (Fig. 8, 9); when the near-shore was stratified (Fig. 10) but the mid-lake was still mixing (Fig. 9) and late summer stratification (Fig. 11). The experimental conditions are summarized on Table 4.

On several occasions, the uptake of nitrate and ammonium was

measured on whole lake water and on water filtered through 12 um Nuclepore filters after incubation. Unfortunately, these experiments were conducted from April to the end of June (eg. Fig. 8) but illustrate that small cells (less than 12 um) were responsible for most of the ammonium uptake, especially at lower enrichments, while large cells used mostly nitrate. This partitioning precludes using any simple transformation to obtain half saturation coefficients. In the dark, almost all the ammonium uptake was by the < 12 um fraction and was equal to 27% of that at optimal light. Dark nitrate uptake by the < 12 um fraction was near zero while that for whole lake water was 10% of that in optimal light. Certainly, these experiments must be repeated during summer stratification and in other lakes before generalizations can be made. Nevertheless, partitioning of the available nitrogen seems to occur and is consistent with observations of Wheeler and McCarthey (1982) who showied that small cells (< lum) were responsible for much of the uptake of the ammonium analogue methylamine.

Common features of the uptake kinetics are illustrated on figures 9-11. It has been clearly established that ammonium is the preferred form of N for algal growth (eg. Brown and Johnson 1977; McCarthey 1980). The elevated concentrations of nitrate resulted uptake was always near the maximal rate. Early in the season, the maximum uptake of ammonium was about equal to that for nitrate (eg. Fig. 8). By 10 June, at 403, maximum nitrate

uptake was less than maximal ammonium uptake (Fig. 9) for this deep mixing population. This pattern persisted and in the September example nitrate uptake was less than 10% of maximal ammonium uptake velocity (Fig. 11). Nevertheless, because ammonium was enriched much above ambient 1-3 ug $N.L^{-1}$ concentrations, the contribution from nitrate at ambient concentrations would be much greater.

Ammonium and nitrate uptake, like ^{35}S-sulfate uptake (Cuhel and Lean 1987), reached optimal rates at lower light intensities than that for ^{14}C-bicarbonate uptake and was not photoinhibited at high light intensities (Fig. 9-11). While dark nitrate uptake was often barely significant, dark ammonium uptake was 30-70% of that at optimal light.

Rates for protein synthesis (from Cuhel and Lean 1987b) using ^{35}S-sulfate uptake were converted to protein N by assuming a weight percent N of 17 (Jukes et al. 1975). Early in the year, at 403, protein-N was equivalent to nitrate-N uptake but at 401 under thermal bar conditions some contribution from ammonium-N was required (Fig. 10). In the September example, no incubator measurements of protein synthesis were made but values from in situ experiments were equivalent to nitrate-N uptake alone (Fig. 11). The advantage of the ^{35}S-protein method (Cuhel and Lean 1987a,b) is that sulfate is never stored and the concentration can always be easily measured. Instead of guessing which form of N is being used, protein synthesis can be reliably measured.

There has been a problem in marine systems involving initial rapid ammonium uptake rates (Goldman and Glibert 1982). To observe if this also occurred in Lake Ontario, time course experiments for ^{14}C-bicarbonate and ^{15}N-ammonium were run routinely throughout the entire period (eg. Fig. 10B, 11B). Initial rapid uptake was not observed and the uptake of both forms was linear for periods of up to 4 h. This does not mean that the uptake of bicarbonate and ammonium are necessarily coupled.

At station 403 on 23 September the maximal rates for ammonium and nitrate uptake were 0.32 and 0.03 ug $N.L^{-1}.h^{-1}$, respectively. The ammonium uptake curve had a very odd shape, i.e. less ammonium was taken up at high concentrations (Fig. 11C). This has been observed by other investigators (Toetz et al. 1973; Liao and Lean 1978) and even in Lake Ontario (Murphy 1980). The uptake curves approach those expected only by assuming that the measured ambient ammonium concentration was too high. This problem adds to the complexity of interpretation of ammonium uptake at ambient concentration.

The ammonium dark uptake rate was 0.15 ug $N.L^{-1}.h^{-1}$ (or 3.6 ug $N.L^{-1}.d^{-1}$), a value strikingly similar to the potential for nitrification using nitrapyrin-sensitive dark bicarbonate assimilation (Table 2). In nitrification, a great deal of ammonium must be oxidized to produce relatively little cell growth (Ward et al. 1982). This would leave nitrate uptake

supporting much of the algal growth as postulated above.

Synthesis

The particulate nitrogen compartment (Fig. 12) contains algae, chemoautotrophic bacteria (CAB), heterotrophic bacteria (HB) with some detritus. The contribution of major zooplankton to the particulate N compartment is relatively small and is shown separately. Unfortunately, present technology does not permit estimates of the relative contribution of each of these other forms. Even neglecting the contribution of microzooplankton and heterotrophic flagellates, zooplankton excretion has been shown to be a very significant process for ammonium regeneration (Fig. 5, 6 and Table 2). Ammonium is oxidized by chemoautotrophic bacteria (Fig. 7) and some nitrite accumulates as a result of this process (Fig. 1-2). Nitrification in the deep water results in little accumulation of ammonium (Fig. 3,4).

Much of the dissolved organic nitrogen (DON) may be colloidal (Wetzel 1981) and little progress has been made in the last 55 years in characterizing the DON. With the exception of urea, noted by McCarthy (1980) and some amino acids, most of the DON must be biologically inert for it changes little throughout the season (210 $\pm$16) and from year to year (Stevens and Neilson 1986).

N_2-fixation has been considered to be relatively insignificant when compared to the direct uptake of nitrite, nitrate, ammonium and urea (McCarthy 1980). In Lake Ontario,

there were few nitrogen-fixing cyanobacteria (Gray 1987) and consequently, nitrogen fixation was considered to be insignificant. Similarly, in view of the nitrous oxide profiles and the high oxygen concentrations (Fig. 5), denitrification was also thought to be unimportant. Instead, much of the "loss" in nitrate-N (Fig. 4) occurs as protein is transferred up the food chain to larger organisms not sampled in the present study.

Acknowledgement. We thank Tom Murphy for his comments and suggestions.

References

Billen, G. 1976. Evaluation of nitrifying activity in sediments by dark ^{14}C-bicarbonate incorporation. Water Res. 51-57.

Brandhorst, W. 1959. Nitrification and denitrification in the eastern tropical north Pacific. J. Conseil. Perm. Intern. Exploration Mer. 25: 3-20.

Brown, C.M. and B. Johnson. 1977. Inorganic nitrogen assimilation in aquatic microorganisms. pp49-114. In M.R. Droop and H.W. Jannach (Eds). Advances in Aquatic Microbiology.

Burns, N.M. 1976. Temperature, oxygen, and nutrient distribution patterns in Lake Erie, 1970. J. Fish. Res. Board Can. 33: 485-511.

Christofi, N., T. Preston and W.D.P. Stewart. 1981. Endogenous nitrate production in an experimental enclosure during summer stratification. Water Res. 15: 343-349.

Cuhel, R. L. and D.R.S. Lean 1987a. Protein synthesis by lake plankton measured using in situ carbon dioxide and sulfate assimilation. Can. J. Fish. Aquat. Sci. 44: 000-000.

Cuhel, R. L. and D.R.S. Lean 1987b. Influence of light intensity and seasonal patterns of carbon dioxide and sulfate metabolism by lake plankton. Can. J. Fish. Aquat. Sci. 44: 000-000.

Dobson, H.F.H. 1984. Lake Ontario Water Chemistry Atlas. Sci. Series 139. Environment Canada Pub. p37.

Glibert, P.M., F. Lipschultz, J.J. McCarthy and M.A. Altabet. 1982. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. Limnol. Oceanogr. 27: 639-650.

Glibert, P.M., F. Lipschultz, J.J. McCarthy and M.A. Altabet. 1985. Has the mystery of the vanishing ^{15}N in isotope dilution experiments been resolved. Limnol. Oceanogr. 27: 444-447.

Goldman, J.C., and P.M. Glibert. 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. Limnol. Oceanogr. 27: 814-827.

Gray, I.M. 1987. 1987. Nearshore and offshore differences in composition of phytoplankton in Lake Ontario. J. Fish. Aquatic Sci. 44: ØØØ-ØØØ.

Hall, G.H. 1982. Apparent and measured rates of nitrification in the hypolimnion of a mesotrophic lake. Appl. Environ. Microbiol. 43: 542-547.

Hall, G.H. and C. Jefferies. 1984. The contribution of nitrification in the water column and profundal sediments to the total oxygen deficit of the hypolimnion of a mesotrophic lake (Grasmere, English Lake District). Microbiol. Ecol. 10: 37-46.

Jukes, T.H., R. Holmquist and H. Moise. 1975. Amino acid composition of proteins: selection against the genetic code. Science 189: 50-51.

Knowles, R. 1981. Denitrification, p. 323-369. In E.A. Paul and J. Ladd (eds). Soil Biochemistry, Vol. 5. Dekker.

Knowles, R., D.R.S. Lean and Y.K. Chan. 1981. Nitrous oxide concentration in lakes: variation with depth and time. Limnol. Oceanogr. 26: 855-866.

Knowles, R. and D.R.S. Lean. 1987. Nitrificaiton: A significant process causing oxygen depletion under winter ice. Can. J. Fish. Aquatic Sci. 44: ØØØ-ØØØ.

Laws, E. 1984. Isotope dilution models and the mystery of the vanishing ^{15}N. Limnol. Oceanogr. 29: 379-386.

Liao, C. F-H. and D.R.S. Lean. 1978. Nitrogen dynamics in lake ecosystems: Part II. Transformations within the trophogenic zone. J. Fish. Res.Board Can. 35: 11Ø1-11Ø8.

Lean, D.R.S. Lean, T.P. Murphy and F.R. Pick. 1983. Photosynthetic response of lake plankton to combined nitrogen enrichment. J. Phycol. 18: 5Ø9-521.

Lean, D.R.S., M.N. Charlton, R.C. Cuhel and H-J. Fricker. 1987. Integration of P and N transport in Lake Ontario related to plankton growth and deposition. Can. J. Fish. Aquat. Sci. 47: ØØØ-ØØØ.

Lemon, E. and D. Lemon. 1981. Nitrous oxide in freshwater of the Great Lakes Basin. Limnol. Oceanogr. 26: 867-879.

McCarthy, J.J. 198Ø. Nitrogen. pp 191-234. In I. Morris Ed. The Physiological Ecology of Phytoplankton. Studies in Ecology. Vol. 7. Blackwell Sci. Pub. Oxford.

Murphy, T.P. 198Ø. Ammonia and nitrate uptake in the Lower Great

Lakes. Can. J. Fish. aquat. Sci. 37: 1365-1372.

Olson, R.J. 1981a. ^{15}N tracer studies of the primary nitrite maximum. J. Mar. Res. 39: 203-226.

Olson, R.J. 1981b. Differential photoinhibition of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum. J. Mar. Res. 39: 226-238.

Pick, F.R. and D.A. Caron. 1987. Pico- and nanoplankton biomass of Lake Ontario: relative contribution of heterotrophic and phototrophic communities. Can. J. Fish. Aquatic Sci. 44: 000-000.

Simons, T.J. and W.M. Schertzer. 1987. Stratification, currents and upwelling in Lake Ontario, Summer 1982. J. Fish. Aquatic Sci. 44: 000-000.

Somville, M. 1978. A method for the measurement of nitrificaiton rate in water. Water Res. 12: 843-848.

Stadlemen, P. and A. Frazer. 1974. Phosphorus and nitrogen cycle on a transect in Lake Ontario during the International Field Year 1972-1973 (IFYGL). Proc. 17th Conf. Great Lakes Res. 92-108.

Stevens, R. and M. Neilson. 1987. Response of Lake Ontario to reduced phosphorus loading. J. Fish. Aquatic Sci. 44: 000-000.

Takahashi, M., T. Yoshioka, and Y Saijo. 1982. Nitrogen metabolism in Lake Kizaki, Japan. III. Active nitrification in early summer. Arch. Hydrobiol. 93: 272-286.

Toetz, D.W., L.P. Varga and G. Huss. 1973. Half-saturation constants for uptake of nitrate and ammonia by reservoir plankton. Ecology. 54: 903-908.

Vaccaro, R.F. and J.H. Ryther. 1960. Marine phytoplankton and the distribution of nitrite in the sea. J. Conseil. Perm. Intern. Exploration Mer. 25: 260-271.

Verdouw, H. and E.M.J. Dekkers. 1982. Nitrogen cycle of Lake Vechten: concentration patterns and internal mass-balance. Hydrobiologia 95: 191-197.

Vincent, W.F. and M.T. Downes. 1981. Nitrate accumulation in aerobic hypolimnia: relative importance of benthic and planktonic nitrifiers in an oligotrophic lake. Appl. Environ. Microbiol. 42: 565-573.

Ward, B.B., R.J. Olson, and M.J. Perry. 1982. Microbial nitrification rates in the primary nitrite maximum off Southern California. Deep-Sea Research 29: 247-255.

Ward, B.B. 1984. Combined autoradiography and immunofluorescence for estimation of single cell activity by ammonium-oxidizing bacteria. Limnol. Oceanogr. 29: 402-410.

Wetzel, R.W. 1975. Limnology. Saunders Pub. p203.

Wezernak, C.T. and J.J. Gannon. 1967. Oxygen-nitrogen relationships in autotrophic nitrificaiton. Appl. Microbiol. 15: 1211-1215.

Wheeler, P.A., P.M. Glibert and J.J. McCarthy. 1982. Ammonium uptake and incorporation by Chesapeake Bay phytoplankton: Short term uptake kinetics. Limnol. Oceanogr. 27: 1113-1128.

Wheeler, P.A. and J.J. McCarthy. 1982. Methylammonium uptake and by Chesapeake Bay phytoplankton: Evaluation of the use of the ammonium analogue for field uptake measurements. Limnol. Oceanogr. 27: 1129-114Ø.

Zafiriou, O.C. and M.B. True. 1979. Nitrite photolysis in seawater by sunlight. Marine Chemistry. 8: 9-32.

Table 1. Nitrous oxide concentrations in Ø-2Ø m and 25 m to bottom water depths (S.D).

		Nitrous oxide (ug $N_2O.L^{-1}$)	
		Ø - 2Ø m	25 m - Bottom
21 Sep	Station 4Ø5	Ø.42 (Ø.Ø8)	Ø.5Ø (Ø.Ø5)
	4Ø3	Ø.35 (Ø.Ø7)	Ø.6Ø (Ø.Ø5)
	4Ø1	Ø.39 (Ø.Ø7)	Ø.55 (Ø.Ø6)
2Ø Oct	Station 4Ø3	Ø.31 (Ø.Ø3)	Ø.46 (Ø.Ø5)
Sept 1977 near 4Ø5*		Ø.33	Ø.53

*(from Lemon and Lemon 1981)

Table 2. Rates of nitrogen transformations at station 403 on 23 September 1982.

Depth (m)	Particulate N ($ugN.L^{-1}$)	Grazing (d^{-1})	Regeneration ($ugN.L^{-1}.d^{-1}$)	Nitrification ($ugN.L^{-1}.d^{-1}$)
0-20	75	0.0775	5.81	3.16
20-50	32	0.01	0.32	1.75
50-176	30	0.005	0.15	1.0

Table 3. Size distribution of dark ^{14}C-bicarbonate uptake (3-6 hour incubations).

Station	Date	Distribution (%)		
		Ø.2-1.Ø	1.Ø-5.Ø	>5.Ø
4Ø1	9 Jun	72	18	9
4Ø3	1Ø Jun	67	26	7
4Ø5	3Ø Jun	68	2Ø	12

Table 4. Variables for kinetic experiments.

Date	Station	Conditions	Temp. (°C)	NO_3^-	NH_4^+	Part N ($ugN.L^{-1}$)	Fig. Ref.
30 Apr	401	Cold Water	2.7	316	1	39	8
8 Jun	401	Thermal Bar	10.1	280	2	65	10
10 Jun	403	Deep Mixing	3.1	360	1	25	9
23 Sep	403	Stratification	16.3	130	10	70	11

Figure Headings

Figure 1. Isopleths of nitrite concentration (ug $N.L^{-1}$) from April to November 1982 at stations 401 (top) and 403 (bottom).

Figure 2. Isopleths of nitrite concentration (ug $N.L^{-1}$) across lake at peak nitrite levels (August 24-27).

Figure 3. Isopleths of ammonium concentration (ug $N.L^{-1}$) from April to November 1982 at stations 401 (top) and 403 (bottom).

Figure 4. Depth profiles of total particulate nitrogen (TPN), nitrate, nitrite and ammonium concentrations (ug $N.L^{-1}$) measured on 8-11 June (closed symbols) and 21-23 September (open symbols) at stations 401 (top), 403 (middle) and 405 (bottom).

Figure 5. Depth profiles of oxygen, temperature and nitrous oxide (left panel). The dashed line represents saturation levels for nitrous oxide. Concentrations particulate nitrogen (X10), chlorophyll a and zooplankton grazing are provided on the right panel. The euphotic zone (shaded) extends from the surface to the depth of the 1% light level. Data from 21-23 September.

Figure 6. Discrete depths 1-15 m averaged for ammonium alone (open symbols) and ammonium plus nitrite (closed symbols) as a function of ammonium regeneration (1-15 m average) at stations 401 (▲), 403 (●) and 405 (▼).

Figure 7. Dark ^{14}C-HCO_3^- fixation for 0-20 m samples incubated at 16°C and 25-176 m samples incubated at 4°C with (closed circles) and without (open circles) nitrapyrin. The difference (shaded area) is plotted as Δ C on the right. Data from 23 September.

Figure 8. Uptake rates for ammonium (top) and nitrate (bottom) as a function of added plus endogenous ammonium and nitrate. The uptake by all the plankton (solid symbols) is compared with that through 12 um (open symbols). The experiment was conducted at 460 uEinst.m^{-2}.s^{-1} (round symbols) and in the dark (square symbols) at Station 401 on 30 April.

Figure 9. (A) Ammonium (●) and nitrate (▲) uptake (solid lines), along with the corresponding carbon (dashed line) uptake rates and ^{35}S-protein N (◆) production as a function of light intensity. Experiment was conducted at station 403 on 10 June at added substrate levels of 66 and 333 ug N.L^{-1} for ammonium and nitrate respectively.

(B) Ammonium uptake velocity as a function of added ammonium at 150 uEinst.m^{-2},s^{-1}.

Figure 10. (A) Ammonium (●) and nitrate (▲) uptake rates along with corresponding carbon (dashed line) uptake rates as a function of light intensity. Experiment was conducted at station 401 on 8 June at added substrate levels of 66 and 333 ug N.L^{-1} for ammonium and nitrate respectively. Also shown is the ^{35}S-protein N production.

(B) Time course for ^{15}N-ammonium (o) at 9Ø ug $N.L^{-1}$ added substrate and corresponding carbon (o) uptake.

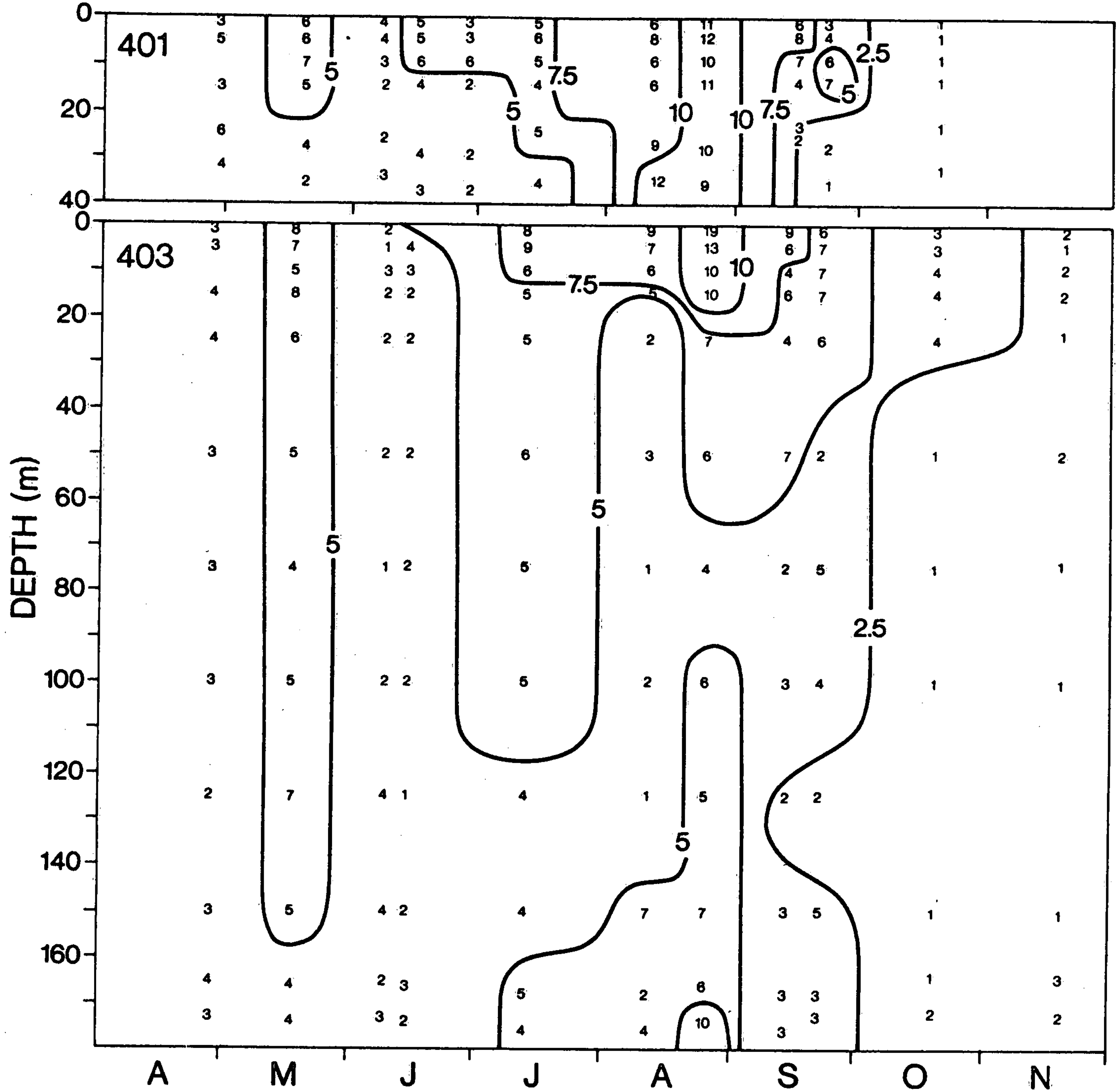
(C) Ammonium uptake as a function of added ammonium at 15Ø $uEinst.m^{-2}.s^{-1}$.

Figure. 11. (A) Ammonium (●) and nitrate (▲) uptake rates (solid lines) along with the corresponding carbon (dashed line) uptake rate as a function of light intensity. Experiment was conducted at station 4Ø3 on 23 September at added substrate levels of 4Ø and 4ØØ ug $N.L^{-1}$ for ammonium and nitrate respectively.

(B) Time course for ammonium and carbon uptake at 33Ø $uEinst.m^{-2}.s^{-1}$

(C) Ammonium uptake shown as a function of added ammonium at 33Ø $uEinst.m^{-2}.s^{-1}$ assuming ambient concentration is 1Ø ug $N.L^{-1}$ as measured (●) or zero (o).

Figure 12. Schematic model for integration of principal N-transformations at station 4Ø3 on 23 September. Thickness of arrow denotes relative amount of N flux. Zooplankton (Zp) excretion represents a significant ammonium regeneration pathway. Utilization is partly by chemoautotrophic bacteria (CAB) with the production of nitrite and nitrate. Nitrate uptake is the principal source of N for algae. The role of heterotrophic bacteria (HB) is poorly understood as is the pathway between the particulate forms and dissolved organic nitrogen (DON)


401
403
DEPTH (m)
0
20
40
60
80
100
120
140
160
A
M
J
J
A
S
O
N
2.5
5
7.5
10

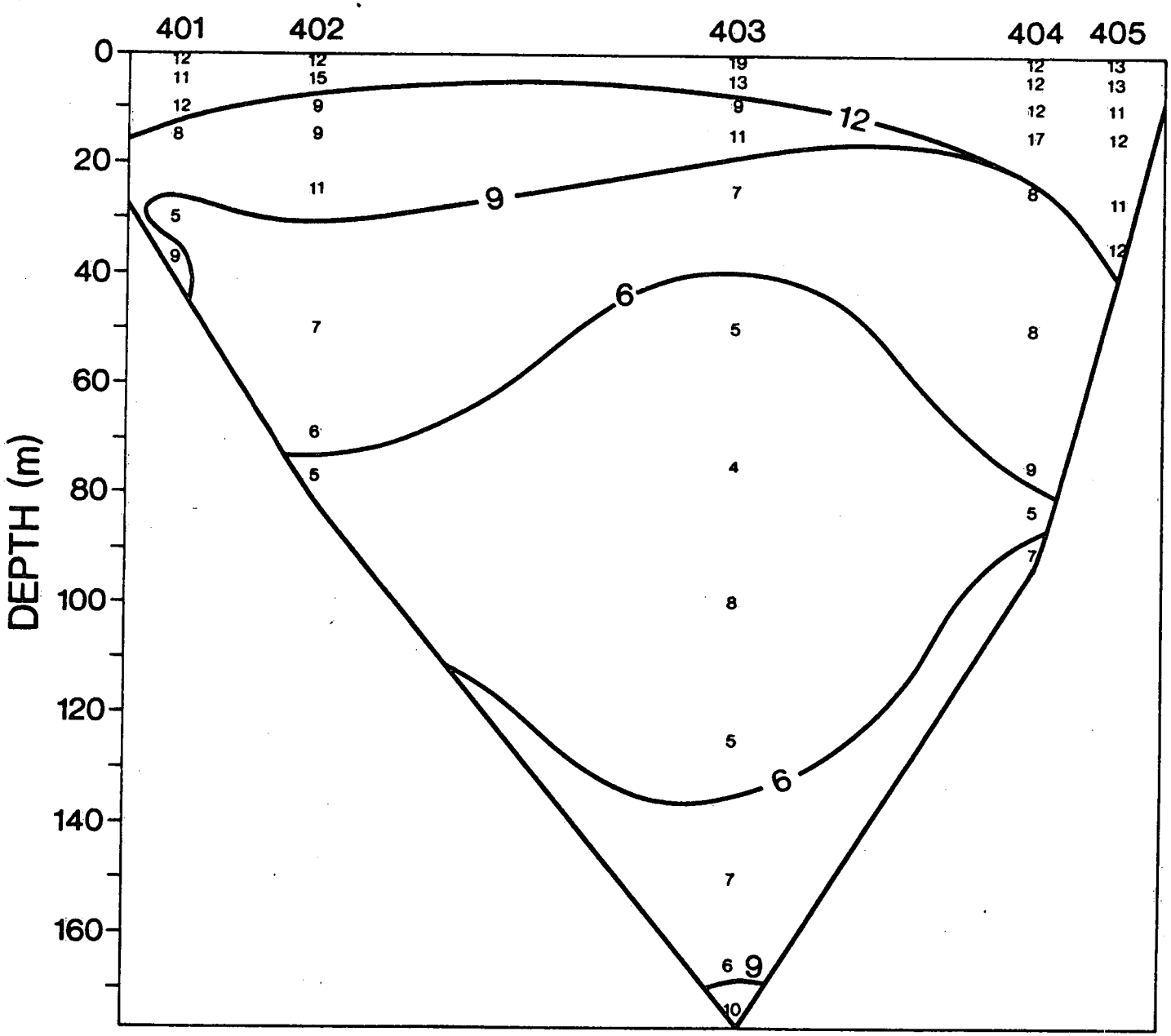


Fig 2 (a)K

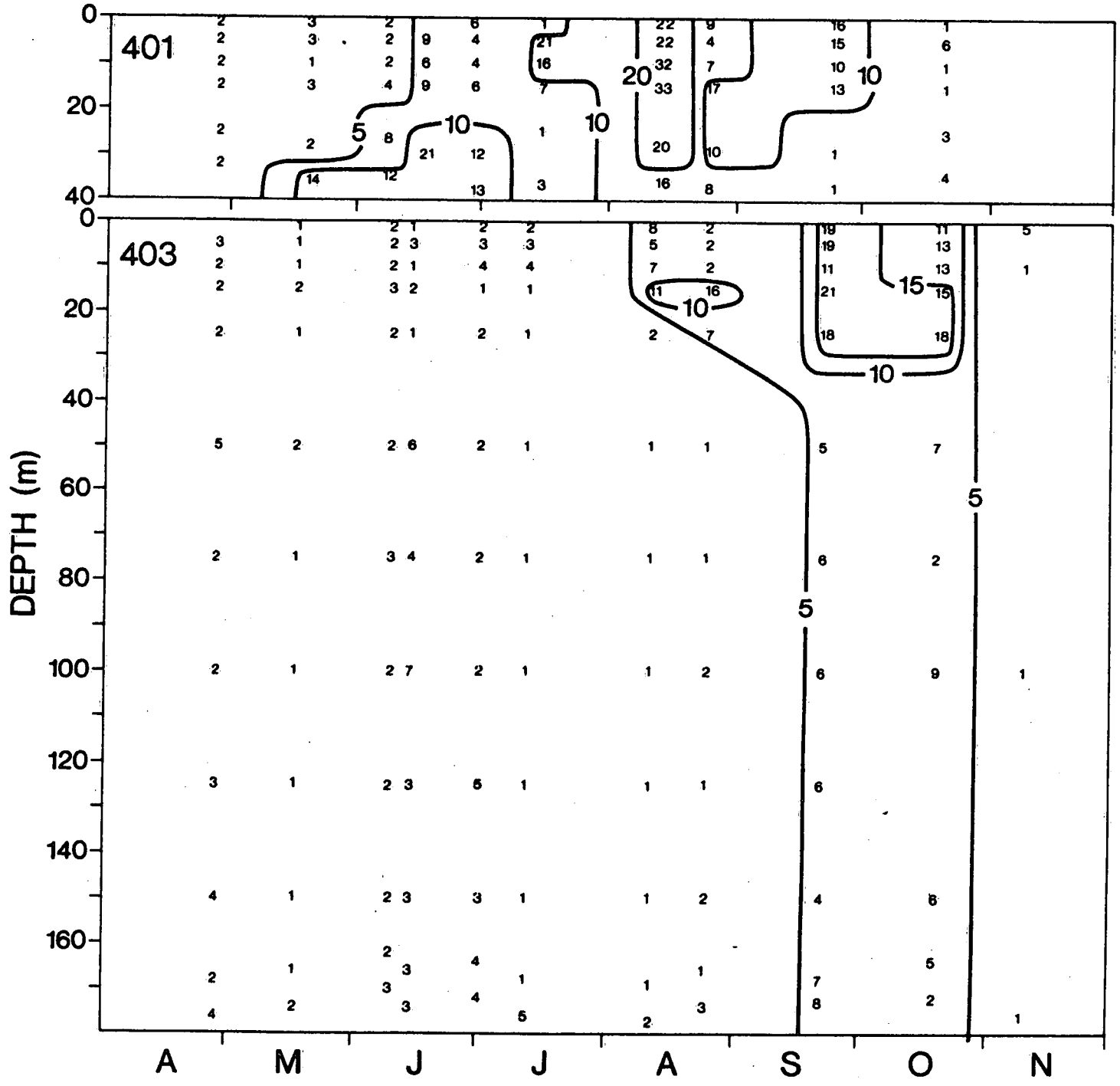


FIG. 3 b&K

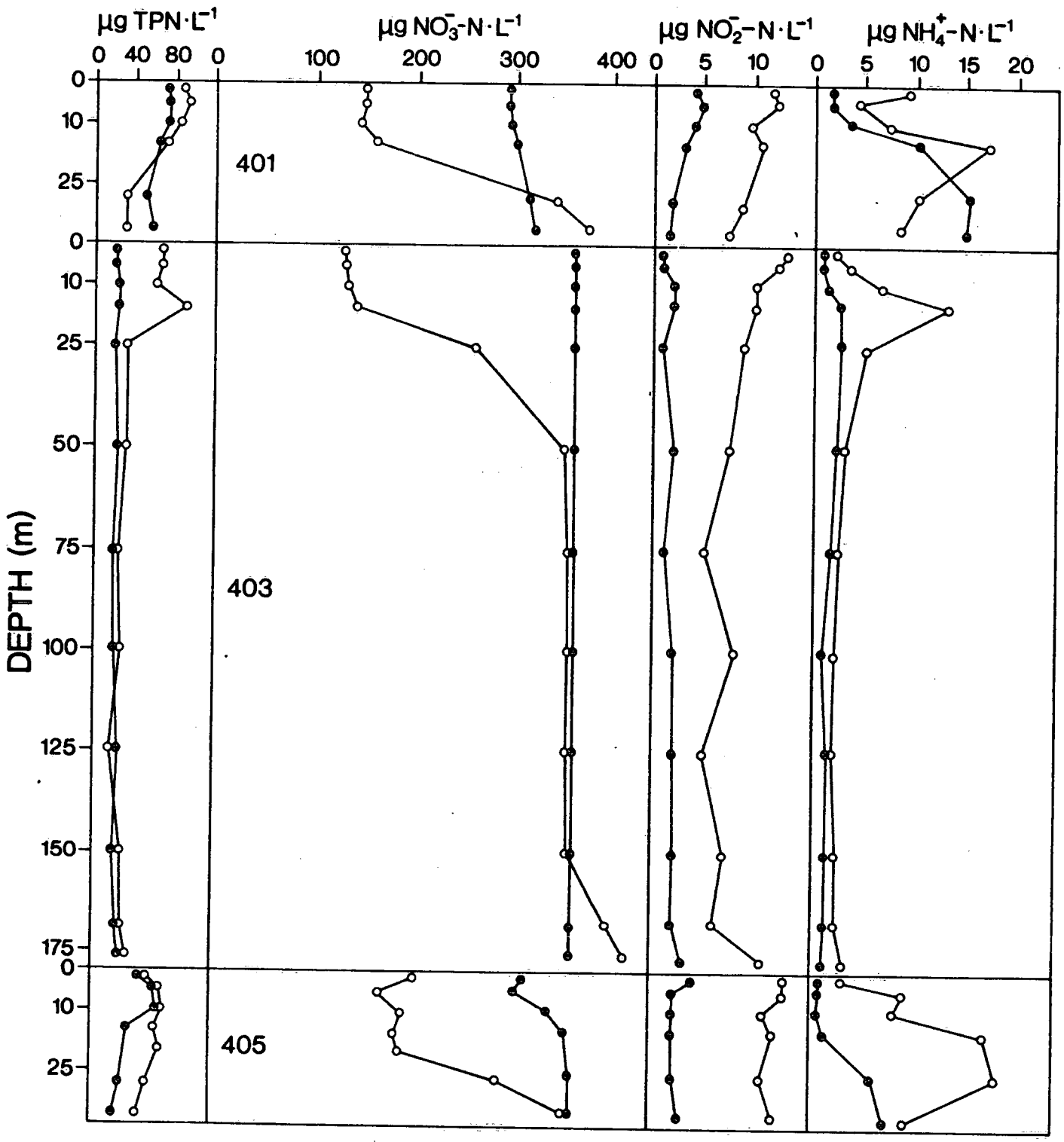

µg TPN·L⁻¹
0 40 80
µg NO₃⁻-N·L⁻¹
0 100 200 300 400
µg NO₂⁻-N·L⁻¹
0 5 10
µg NH₄⁺-N·L⁻¹
0 5 10 15 20
DEPTH (m)
0 10 25
401
0 10 25 50 75 100 125 150 175
403
0 10 25
405

FIG. 4 L+K

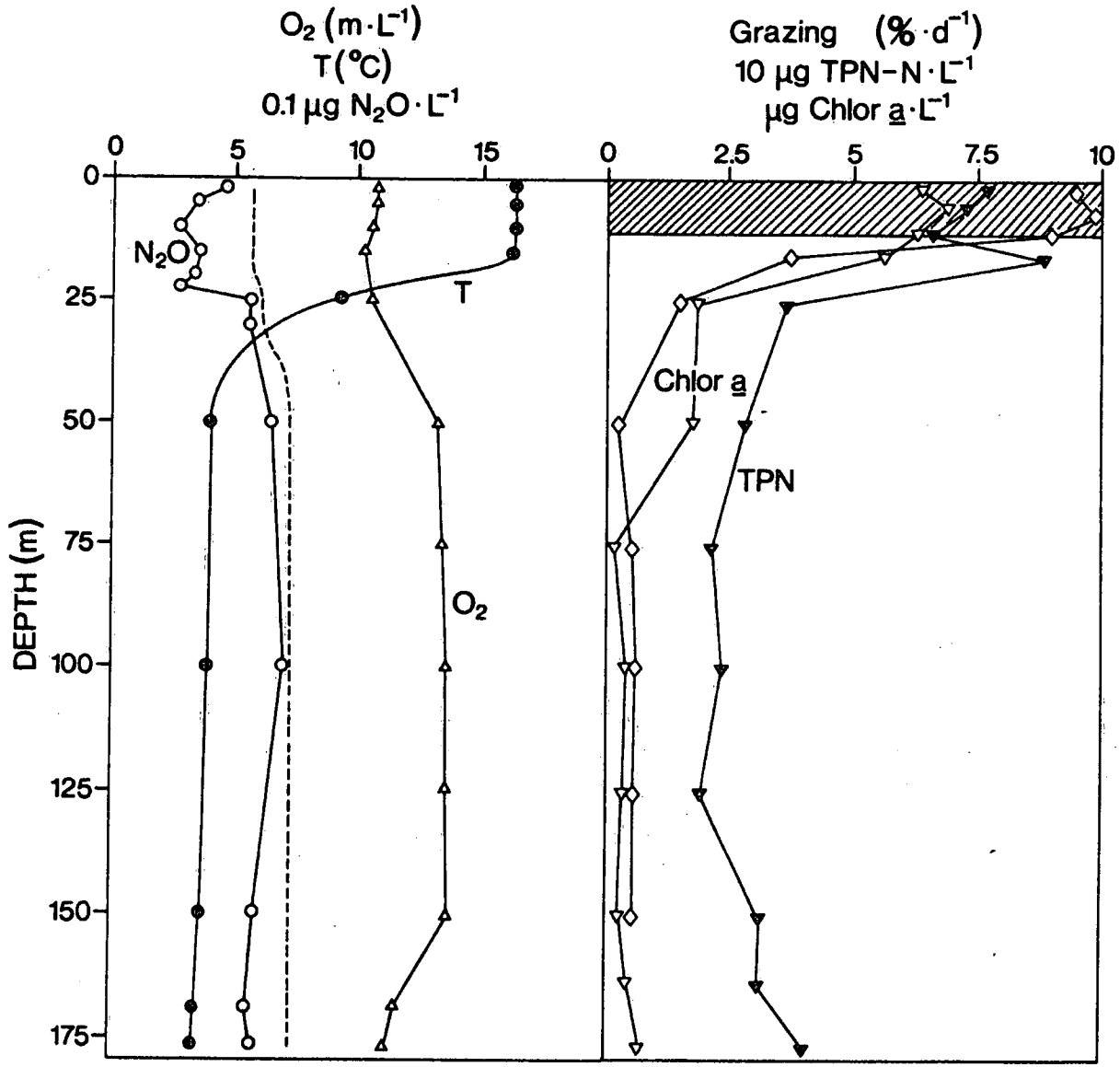

O_2 (m·L^{-1})
T(°C)
0.1 µg N_2O·L^{-1}
Grazing (%·d^{-1})
10 µg TPN-N·L^{-1}
µg Chlor a·L^{-1}
0
5
10
15
2.5
7.5
25
50
75
100
125
150
175
DEPTH (m)
N_2O
T
O_2
Chlor a
TPN

FIG. 5 L&K

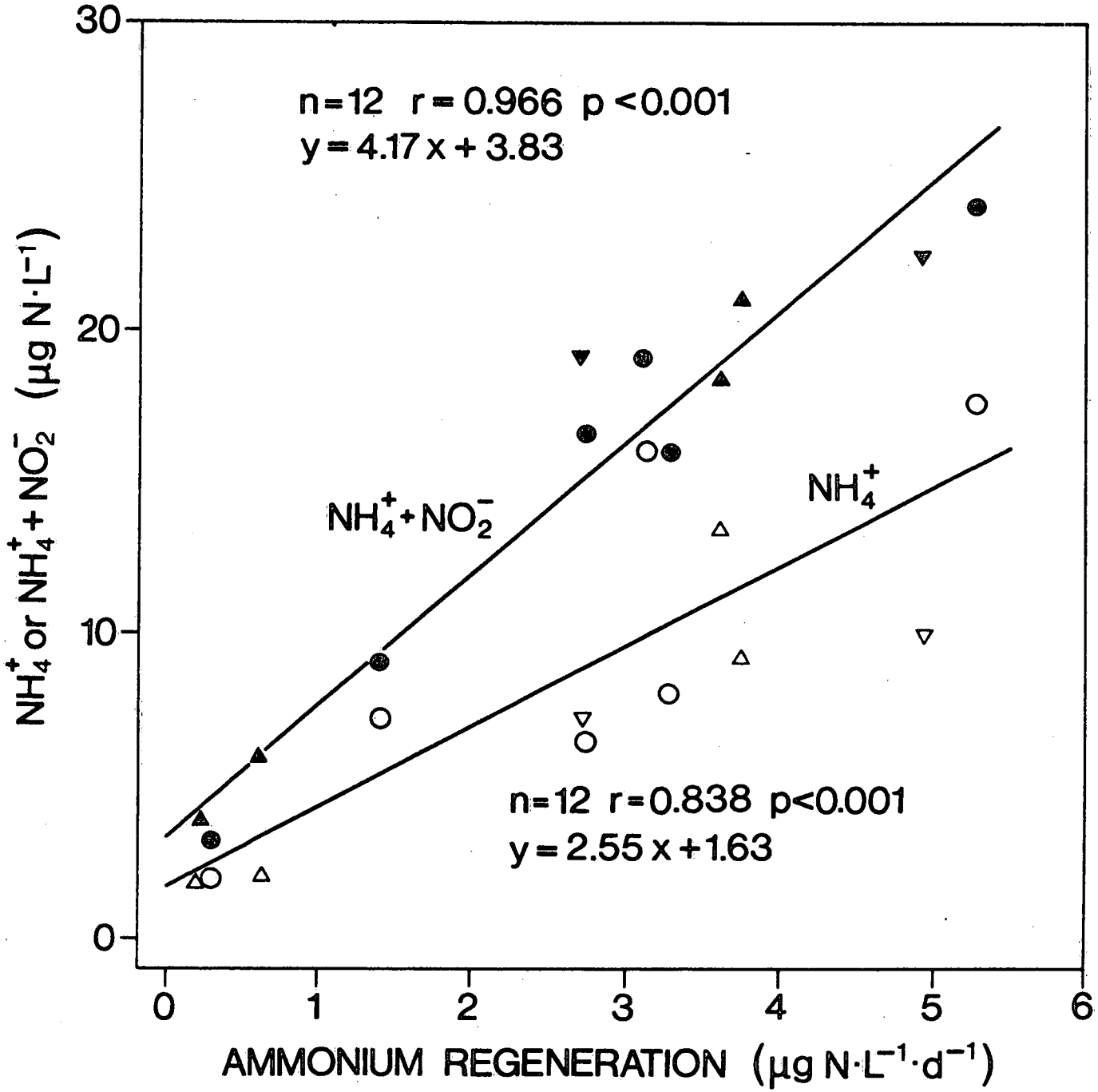
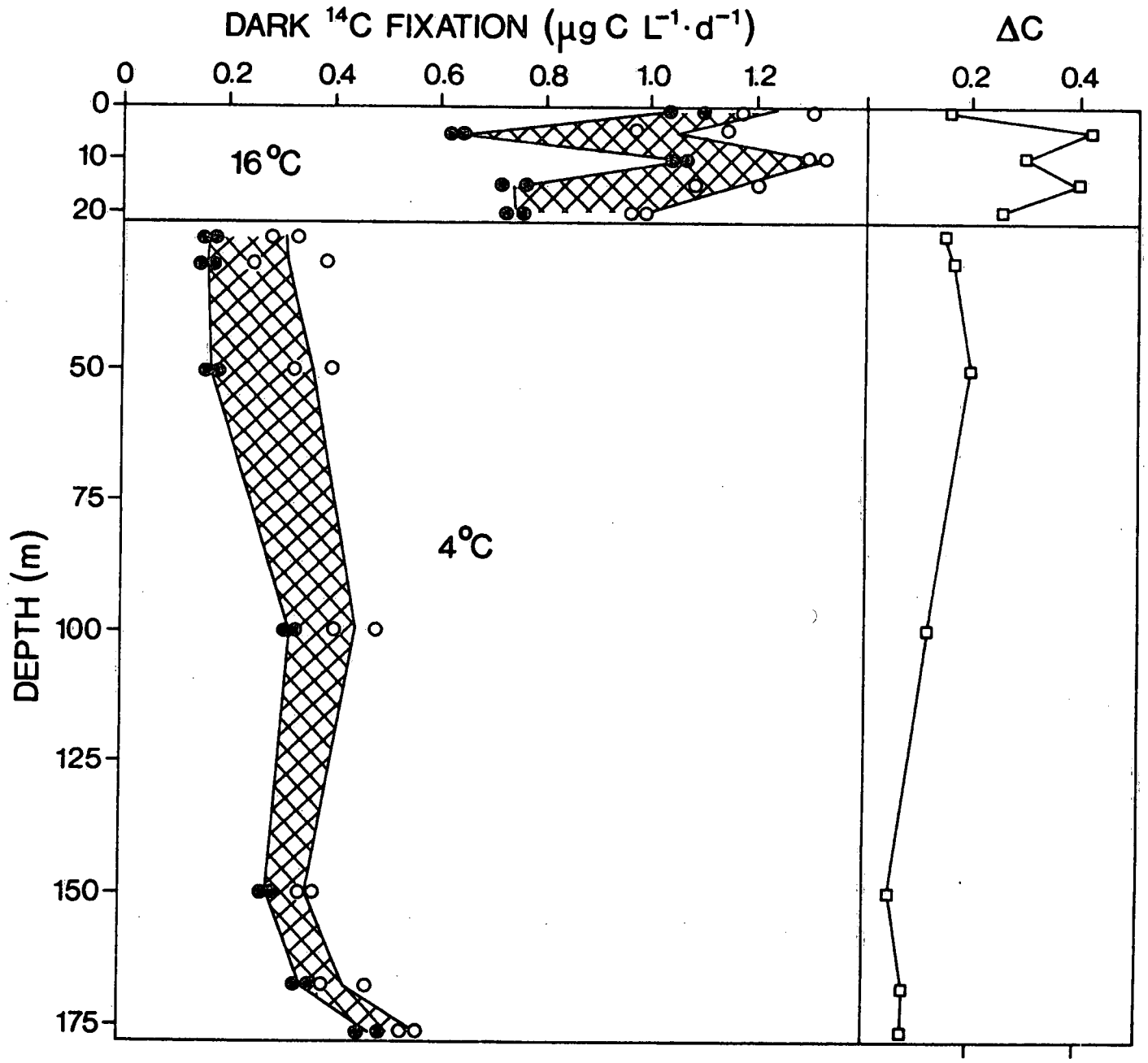
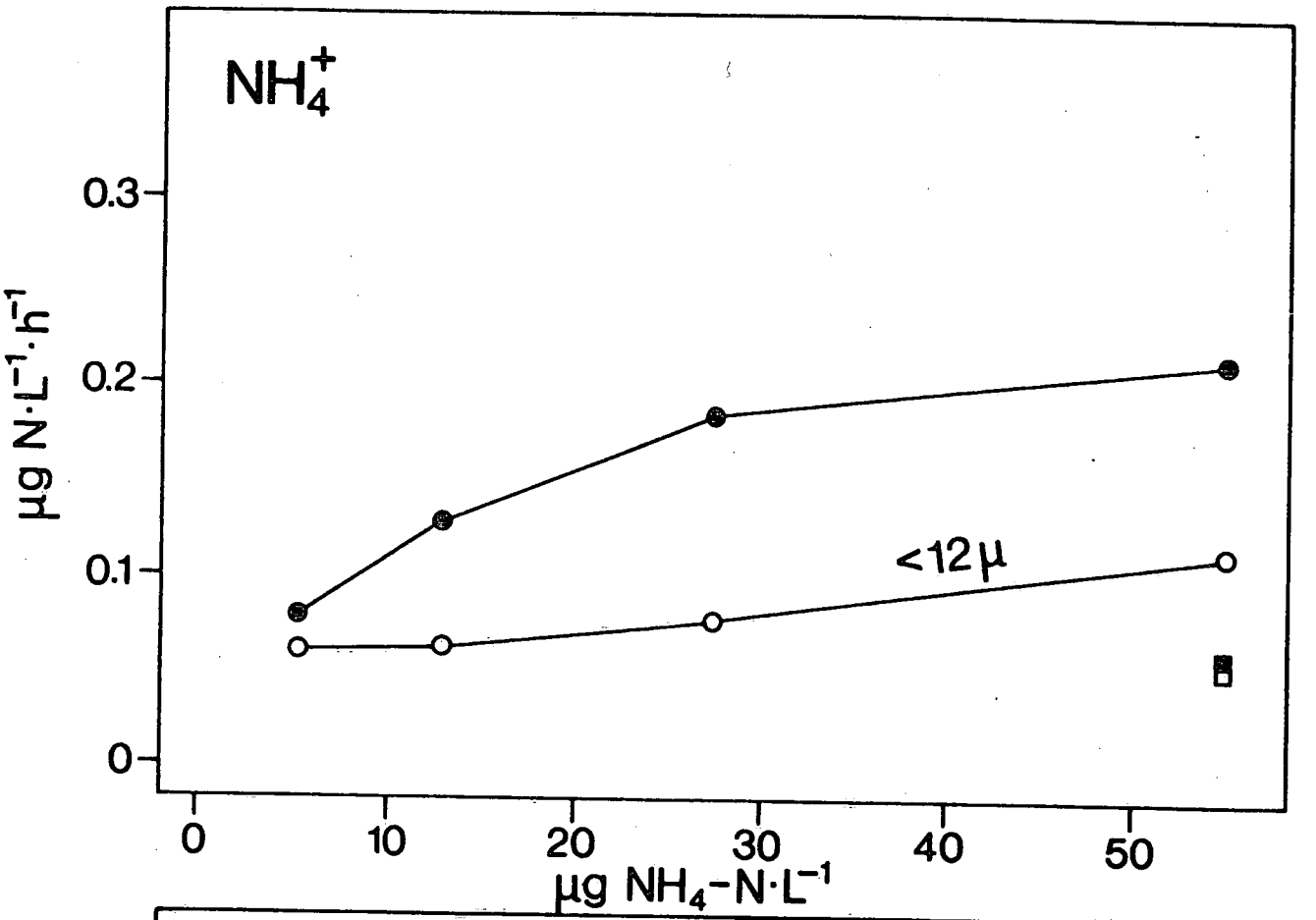

n=12 r=0.966 p<0.001
y=4.17x+3.83
NH4+ + NO2−
NH4+
n=12 r=0.838 p<0.001
y=2.55x+1.63
NH4+ or NH4+ + NO2− (µg N·L−1)
AMMONIUM REGENERATION (µg N·L−1·d−1)
0
10
20
30
0
1
2
3
4
5
6

FIG. 6 LaK


DARK ^{14}C FIXATION ($\mu g\ C\ L^{-1} \cdot d^{-1}$)
ΔC
0
0.2
0.4
0.6
0.8
1.0
1.2
0.2
0.4
16°C
4°C
DEPTH (m)
0
10
20
50
75
100
125
150
175


NH_4^+
0.3
0.2
0.1
0
µg N·L⁻¹·h⁻¹
<12µ
0
10
20
30
40
50
µg NH_4-N·L⁻¹

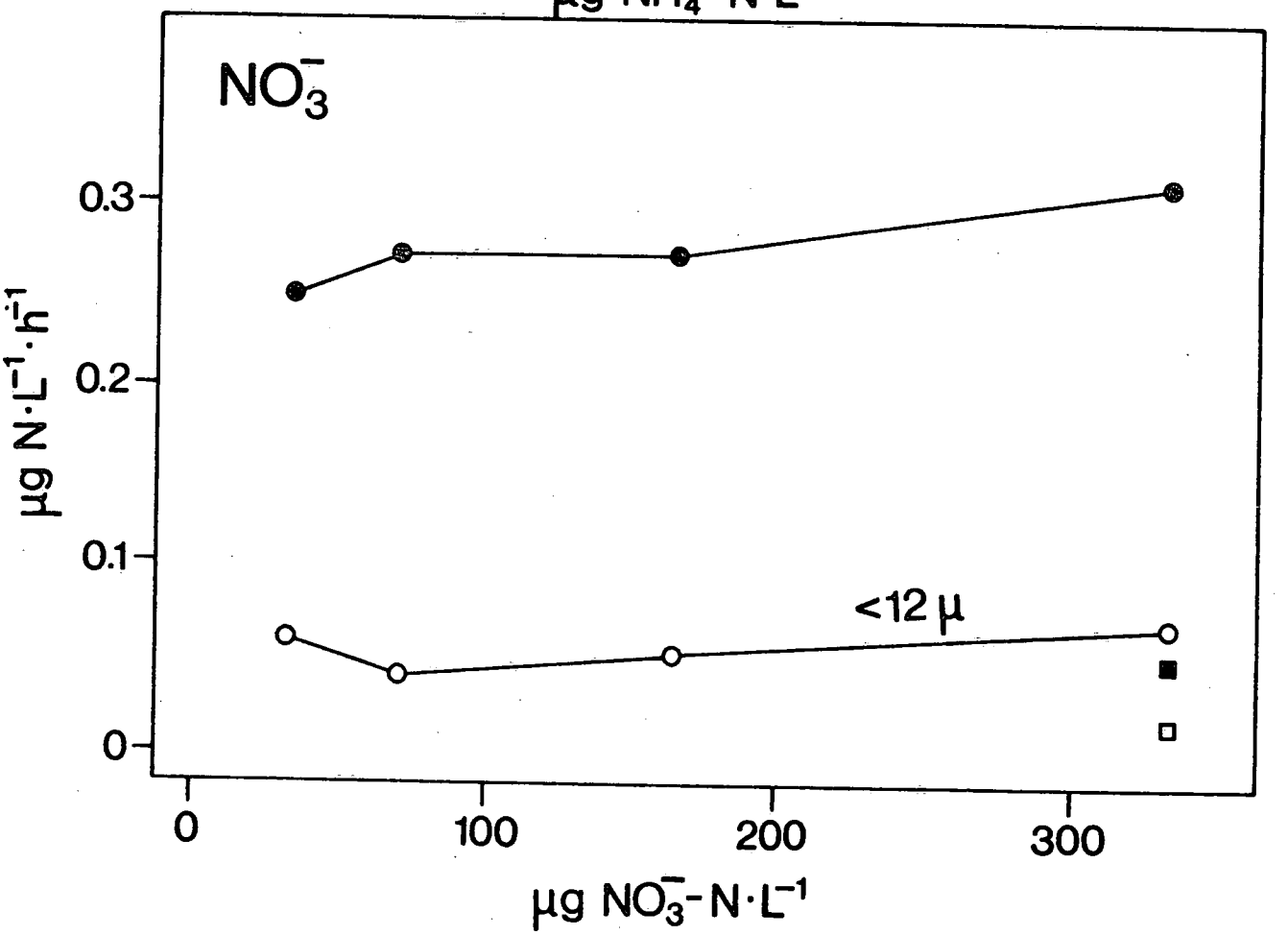

NO_3^-
0.3
0.2
0.1
0
µg N·L⁻¹·h⁻¹
<12µ
0
100
200
300
µg NO_3^--N·L⁻¹

Fig. B 1aK

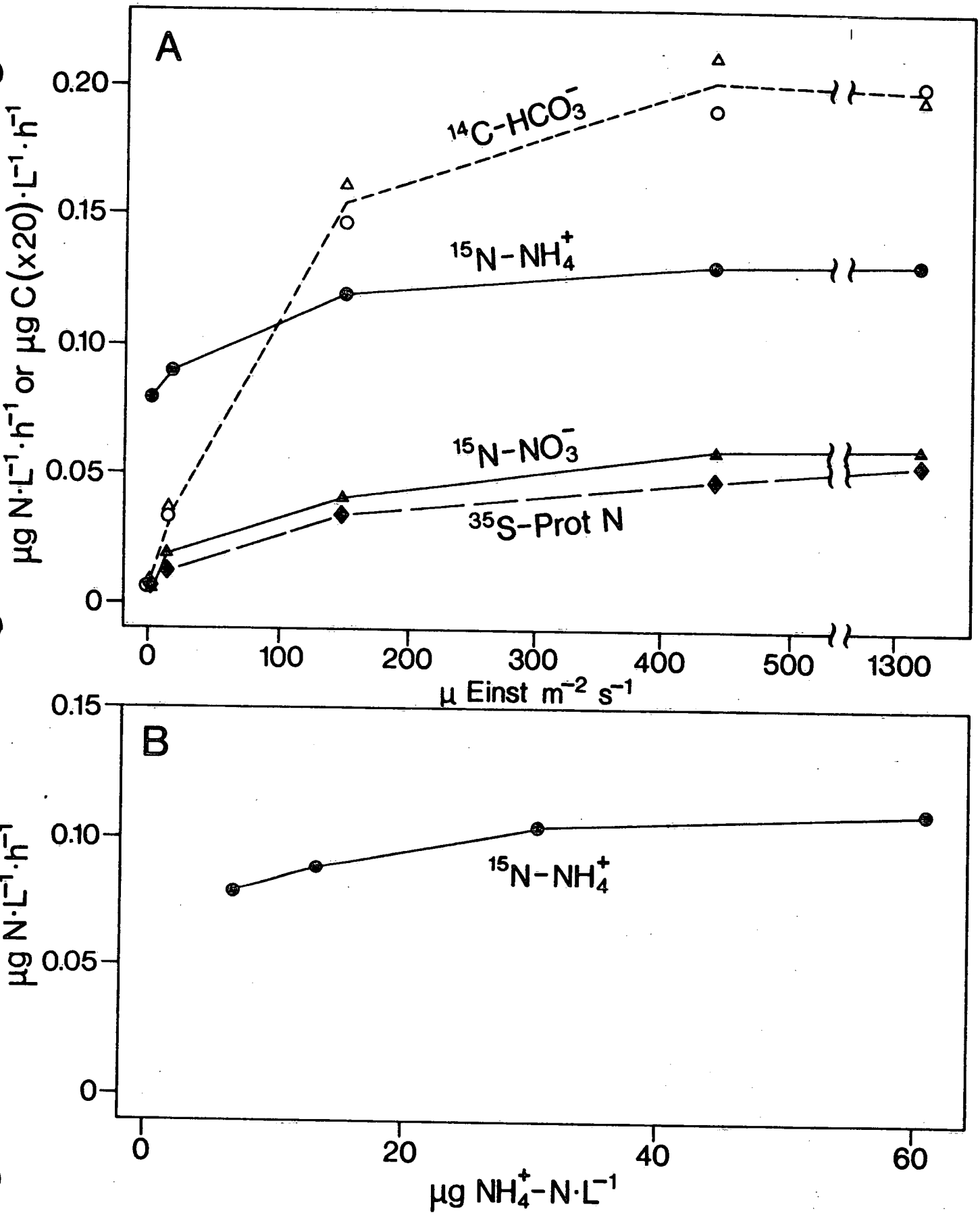

A
0.20
0.15
0.10
0.05
0
µg N·L⁻¹·h⁻¹ or µg C(x20)·L⁻¹·h⁻¹
¹⁴C-HCO₃⁻
¹⁵N-NH₄⁺
¹⁵N-NO₃⁻
³⁵S-Prot N
0
100
200
300
400
500
1300
µ Einst m⁻² s⁻¹
B
0.15
0.10
0.05
0
µg N·L⁻¹·h⁻¹
¹⁵N-NH₄⁺
0
20
40
60
µg NH₄⁺-N·L⁻¹

Fig. 9. [illegible]

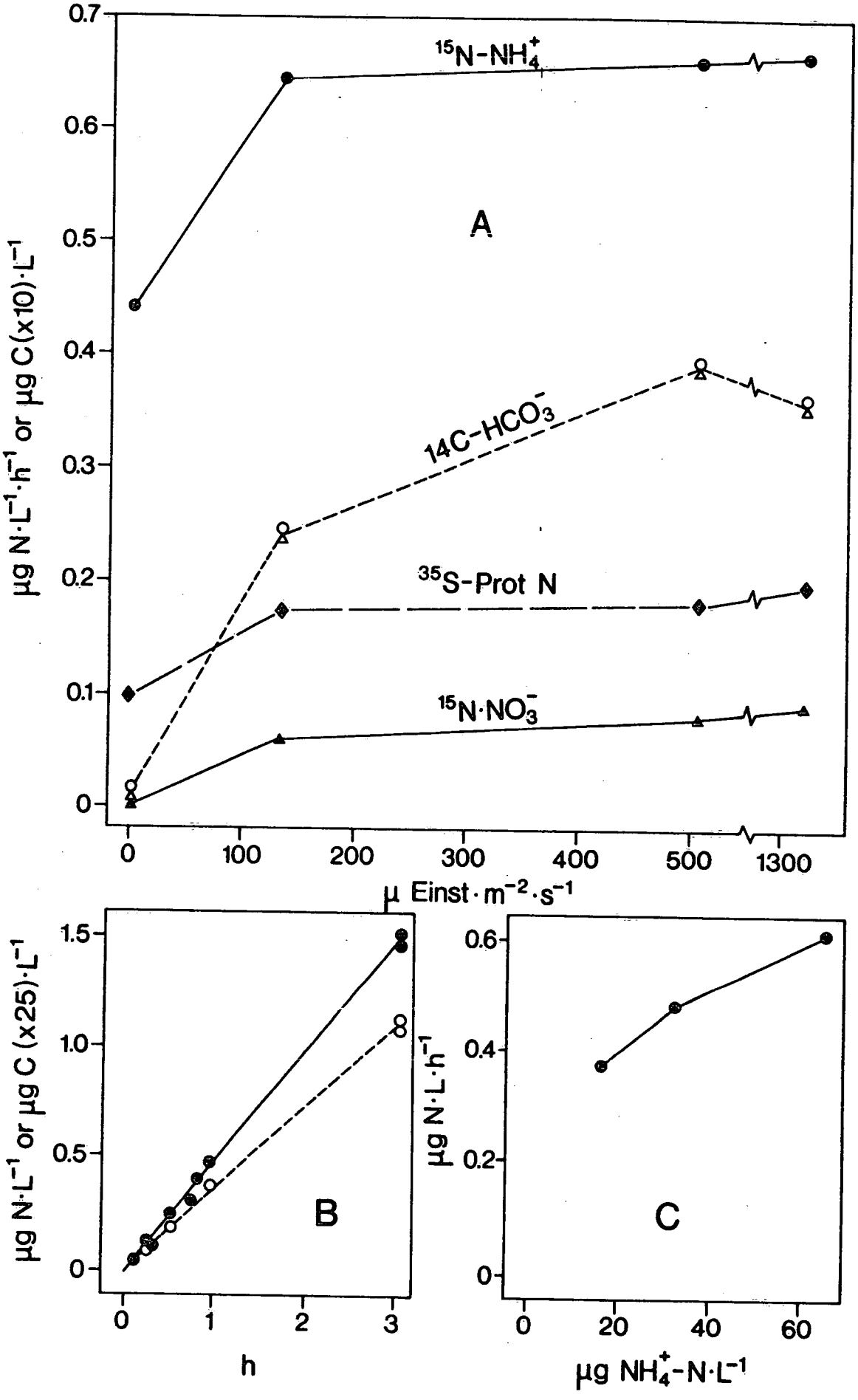

A
$^{15}N-NH_4^+$
$^{14}C-HCO_3^-$
^{35}S-Prot N
$^{15}N\cdot NO_3^-$
$\mu g\ N\cdot L^{-1}\cdot h^{-1}$ or $\mu g\ C(x10)\cdot L^{-1}$
μ Einst $\cdot m^{-2}\cdot s^{-1}$
B
$\mu g\ N\cdot L^{-1}$ or $\mu g\ C\ (x25)\cdot L^{-1}$
h
C
$\mu g\ N\cdot L\cdot h^{-1}$
$\mu g\ NH_4^+-N\cdot L^{-1}$

Fig. 10 a-k

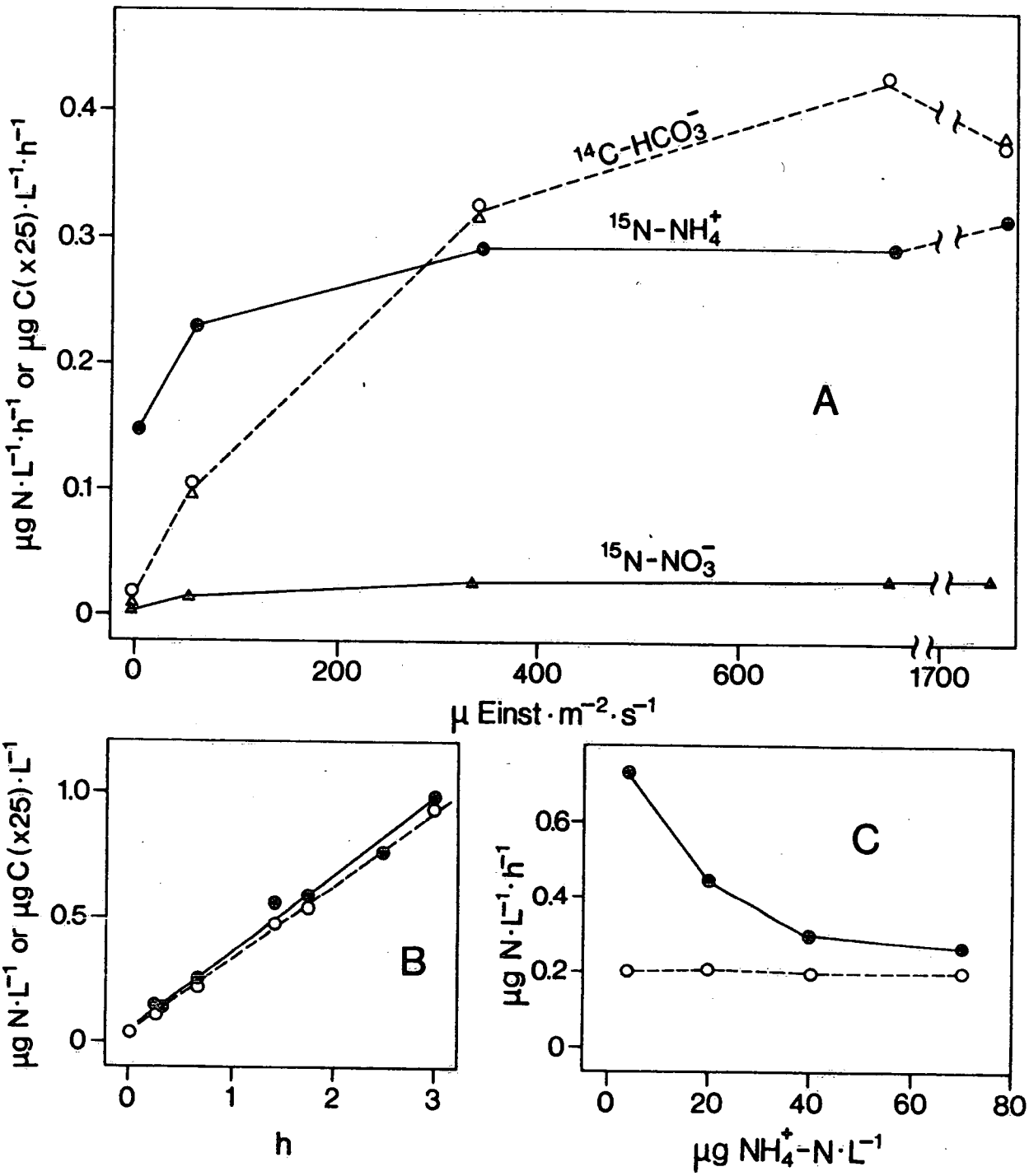

0.4
0.3
0.2
0.1
0
µg N·L⁻¹·h⁻¹ or µg C(x25)·L⁻¹·h⁻¹
¹⁴C-HCO₃⁻
¹⁵N-NH₄⁺
¹⁵N-NO₃⁻
A
0
200
400
600
1700
µ Einst·m⁻²·s⁻¹
1.0
0.5
0
µg N·L⁻¹ or µg C(x25)·L⁻¹
B
0
1
2
3
h
0.6
0.4
0.2
0
µg N·L⁻¹·h⁻¹
C
0
20
40
60
80
µg NH₄⁺-N·L⁻¹

FIG. 11 L9K

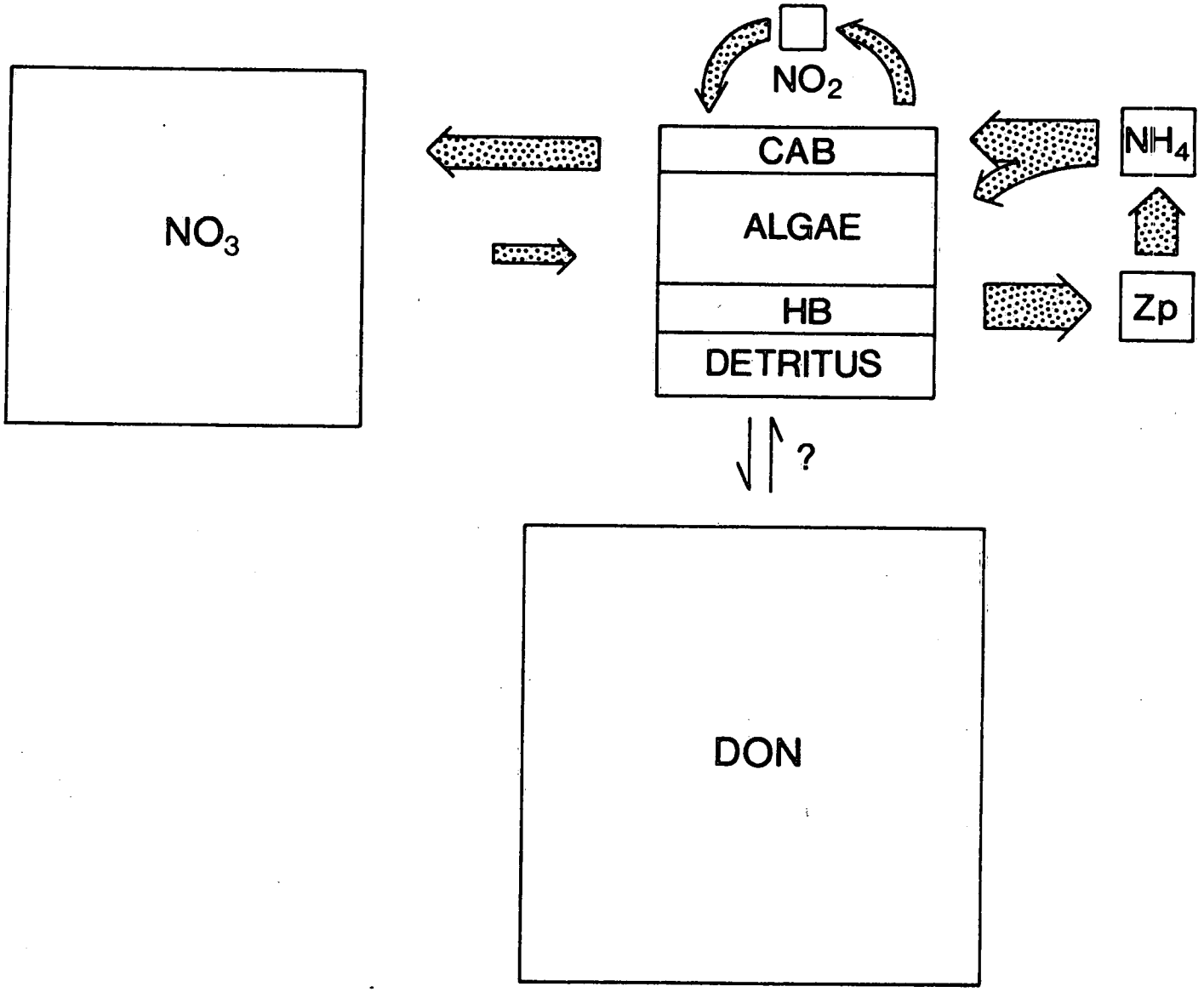


FIG. 12 LOK