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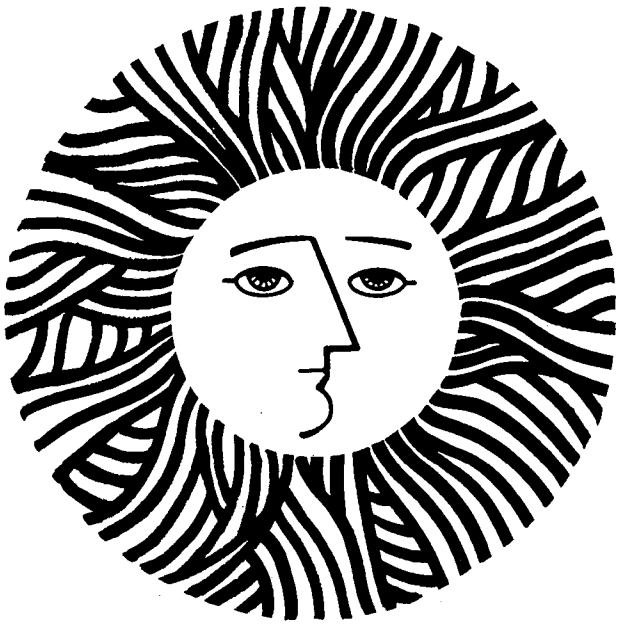

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ABSTRACT

A series of experiments involving the addition of detritus to replicate lake-water aliquots is described. The studies revealed anticipated qualitative behavior characterized by a delayed series of peaks in decomposition activity, inorganic nutrient concentrations, and subsequently phytoplankton density. The observed quantitative behavior was highly replicable and unexpected, however. In particular, a threshold effect was seen, characterized by an absence of increase in inorganic nitrogen levels until the concentration of added organic materials exceeded a certain level. This effect, and the detailed time-dependence of the concentration of NH_4^+ produced by mineralization, will constrain models used to describe decomposition; density-dependence regulation in microbial decomposer populations is hypothesized as a cause of the observed effects.

INTRODUCTION

In a series of experiments, we have studied the responses of lake water aliquots to the addition of detritus. Our approach was to add a range of amounts of autoclaved organic matter to lake-water aliquots and then measure the subsequent chemical and biological responses of the system. The expected qualitative pattern of response to such detritus additions is an increase in uptake of nutrient by microbes for growth (immobilization) and an increase in production of inorganic nutrients (mineralization), followed by increasing nitrification and assimilation of nutrients by phytoplankton (17). In the experiments reported here we followed in detail the short-term responses. As described below, we found that the quantitative patterns of response were sufficiently complex and interesting that they provide a detailed characterization of microbial-detritus interactions and in particular suggest the existence of density-dependent microbial population dynamics.

Mineralization, or the production of inorganic nutrients from organic detritus, is an end stage of decomposition. Mineralized inorganic nutrients are potentially available for primary production, but they also can be immobilized for growth of the organisms that carry out decomposition, and they can be exported from the system as, for example, in denitrification. We define net mineralization to be total mineralization minus immobilization and export. It is thus a measure of the production of inorganic nutrients that are available for primary production. Our interest here was in the relation between

net mineralization subsequent to a detritus addition and the size of that addition. Specifically, we were concerned with the production of NH_4^+, $NO_2 + NO_3$, and CO_2. We added different amounts of identical natural detritus to each member of a set of initially identical lake-water aliquots and determined the dependence of the net quantity of inorganic nitrogen (IN) and inorganic carbon that was mineralized on the amount of detritus that was added.

Subsequent to the addition of organic matter, the fraction of added organic matter that is mineralized can depend on the amount added and the time that has elapsed since the addition. In the most careful study of this to date, Williams and Gray (18) added small quantities of ^{14}C-labeled amino acids (0.1 µg/liter) and at the same time a range of larger quantities of unlabeled amino acids (100-5000 µg/liter) to sea-water aliquots and observed the resulting respiration rates over a 2-day period. They deduced the following conclusions:

i) Initially, within a few hours after the addition of substrate, the percentage of substrate respired decreased with increasing initial substrate concentration;

ii) The larger the initial substrate concentration, the later the time of maximum respiration rate;

iii) By the end of 2 days, all systems had respired 30-50% of the added substrate, independent of the amount added.

A simple mathematical explanation of these conclusions in terms of Michaelis-Menten uptake kinetics was given.

In related studies with labeled assemblages of amino acids Williams (19) and Hobbie and Crawford (9) have observed that a large fraction of decomposed substrate is incorporated into heterotrophic biomass growth (primarily bacteria). This fraction averages well over 0.5, in contrast with the much smaller incorporation fraction when the added substrate consists of a single detrital component such as glucose. Given that a large percentage of added substrate is so incorporated, it follows that the net mineralization of inorganic nutrients can be quite sensitive to factors influencing the growth of heterotrophic populations. In particular, if density-dependent regulation of heterotrophic biomass growth limits immobilization of nutrients for that growth above a certain threshold concentration of added substrate, but does not limit mineralization activity, then net mineralization should account for a larger percentage of added substrate above that threshold than below. This would be in contradiction with deduction (iii) of Williams and Gray (18) discussed above.

However, if one looks at the data of Williams and Gray, it appears that this third conclusion may have been drawn prematurely. In two of the experiments they reported, early termination before the respiration rates had levelled off makes it difficult to reach any conclusion about asymptotic mineralization (their figures 2 and 3) while in the third reported experiment with amino-acid mixtures (their figure 1) the data are manifestly at variance with the simple kinetic model used. Again, early termination of the experiment makes it difficult to reach a firm conclusion, but there is evidence from their data that the

asymptotic fraction respired is dependent on the amount of substrate added initially.

We hypothesize that the fraction which appears as net mineralization will depend on the amount of substrate added and that the dependence is of a threshold nature, with the amount of net-mineralization occurring increasing sharply above a threshold concentration of added substrate. The experiments we report here were designed to test this hypothesis. Our approach differed from that of Williams and Gray in several respects. First, we investigated water from freshwater lakes rather than marine systems. Secondly, the substrate we added consisted of dissolved and particulate fractions of freshly grown, killed, and sterilized freshwater organisms, rather than prepared assemblages of amino acids. Thirdly, we measured mineralization activity over a five-day period or longer following the addition of substrate, thus allowing opportunity to observe mineralized inorganic nutrients reach their maximum levels. Finally, we did not use ^{14}C-labeling here. Our reason for this was the perceived difficulty in obtaining large quantities of uniformly-labeled, freshly-grown and prepared detritus. Since we completed our investigations, a paper appeared by Cole and Likens (3) that describes a method for carrying out decomposition studies with detritus consisting of ^{14}C-labeled algae. While their study was restricted to considerably smaller fractional increases in detritus concentration than in ours, future application of their method to the problem at hand is intended.

In order to quantify the short-term mineralization activity in our systems, we measured daily water-column concentrations of inorganic nitrogen (NH_4^+ and NO_2 + NO_3) for 5 to 10 days subsequent to the addition of detritus. This time period was sufficient to detect a rise and then a fall in the inorganic nitrogen levels. Such measurements, alone, do not allow a separation of inorganic-nitrogen production from inorganic-nitrogen uptake by phytoplankton, and for that reason we made a number of supplementary measurements, including dark- and light-bottle CO_2 evolution and phytoplankton counts.

METHODS

The experiments reported here were carried out in 4-liter glass beakers housed in a temperature-controlled room at 19 ± 1°C. Illumination was provided by a bank of eight 1.3m very-high-output, cool-white fluorescent lights on a 12h:12h light:dark cycle; the light irradiance on the water surface of the microcosms was 7.0 ± 0.3 watts/m^2 PAR. The water in each beaker was aerated and agitated gently by air pumped at a rate of about 1 liter per minute through a capillary tube extending 15 cm below the water surface.

Each of the four experiments was carried out with water samples taken originally from lakes in the San Francisco Bay area. Except for experiments K-3 and K-4, which were conducted simultaneously on identical lake water samples, the experiments were carried out sequentially and with different lakes as a source of water. Prior to each experiment, the lake water samples were maintained in large laboratory microcosms (50-700 liters) for a period of several months,

where they served as controls for other experiments we were conducting. Because the experiments reported here were performed with lake water samples housed temporarily in laboratory microcosms, it is possible that our results reflect laboratory conditions.

Table 1 summarizes the conditions of each of the experiments carried out (labeled K-1 to K-4). In each experiment, the replicate 4-liter beaker systems were initiated from the larger laboratory microcosms three days prior to the addition of detritus, and background values of all monitored quantities were then determined. On day zero of each experiment, organic carbon was measured in all 4-liter systems and in the concentrated detritus spike. The detritus was then immediately added to all treatment systems, at relative concentrations shown in Table 1.

The detritus was prepared in several different ways, depending on the experiment. In two of the experiments, K-1 and K-2, E. coli grown specifically for the purpose were used. These dense cultures reached concentrations of 5 mM (C) (5 millimoles of carbon per liter of water). The E. coli were harvested, sonicated for 30 minutes effectively breaking cell walls, and then autoclaved for 40 minutes at 110°C and 25 psi. To prepare detritus for the other two experiments, algae consisting primarily of Scenedesmus, Chlorella, Gloeocystis, Ankistrodesmus, and unidentified small, round, green nanoplankton were grown under nutrient-rich conditions, harvested, and then sonicated and autoclaved. For one of these experiments (K-3), the fine-particle and soluble portion of the algal detritus was separated and used for

the detritus addition. Separation was accomplished by first passing the algal culture through a 5μ filter and then by letting the filtrate settle for 24 hours and decanting the top quarter of the filtrate. Hereafter, for the sake of brevity, we denote this portion of the detritus as dissolved organic matter. Immediately prior to the addition of the detritus, its sterility was examined by standard bacterial plating methods (1). No bacterial colonies developed on plates to which small amounts of the detritus were added. Because our systems, like natural lakes, are exposed to the atmosphere, sterile controls were not maintained. Having determined the sterility of the detritus, however, we used replicate sysems to which no detritus was added as controls.

With the exception of water-column phytoplankton and zooplankton (number and volume), which were measured approximately weekly, monitoring was carried out daily for periods ranging from one to several weeks. Measurements were made from water samples taken from the 4-liter systems at approximately 4 hours after the onset of light each morning, at 11:00 h. Integrated water-column samples for measurement were taken with a hollow polyethylene tube (1 cm i.d.) inserted to within 0.5 cm of the bottom of the beaker, stoppered at the top, and removed. For the sealed-bottle CO_2 and NH_4^+ evolution measurements, 50 ml bottles were used. Table 2 lists the methods used for monitoring chemical and biotic parameters.

RESULTS

The four experiments were similar in design while different in initial parameters. Different water samples were used in K-1 and K-2 and K-3, 4; the biological materials from which the added detritus was obtained in K-1, 2 differed from that in K-3, 4; and the size spectrum of the added detritus in K-3 differed from that in K-4. Therefore, identical behavior in the four experiments cannot be expected and, as discussed below, was not observed.

For the sake of clarity, some of the data presented in the accompanying figures (Figs. 1 to 10) are averaged over replicates rather than displayed separately for each replicate system; except for K-4, where measurements in replicate systems were carried out they agreed to within 20 of one another. The replication in nutrient data among the duplicate or triplicate subsystems in experiments K-1 and K-2 was particularly good, as seen in Figs. 1 and 8.

Table 3 lists the organisms other than bacteria present in the 4-liter beakers in K-1. The species list was not identical to this in the other experiments, as expected, since their source of water was different. Nevertheless, the variations were not great, with about 80 of these species present in the other experiments. The numbers of

well as during the course of each experiment. In K-1, for example, a ciliate protozoan dominated (by volume) the animal population, while

in K-2, a rotifer (Lecane sp.) and a cladoceran (Alona guttata) dominated. In K-3 and K-4, the dominant phytoplankton were Mougeotia sp. and Phacus sp., while in K-1, an unidentified flagellate dominated.

We describe the results of K-1 below in considerable detail and then point out more briefly similarities and differences in the results of K-2, 3, and 4.

K-1. Three levels of bacterially-derived detritus, corresponding to 117, 235, and 470 μM(C) organic carbon, were added to systems B, C, and D respectively. Figure 1 shows IN concentrations plotted as functions of time for all the systems. Here, and in the other experiments reported, the substrate was added on day 0 immediately after the day 0 water-column samples for measurement were taken. The IN concentrations in replicate systems for both sets A and B were identical within experimental error and only their average values are shown. Most of the increase in IN was accounted for by NH_4^+, with maximum NO_2 + NO_3 concentrations in all systems never exceeding 3 μM(N) (see Fig. 2). Measurable induced increases in IN concentrations were only seen in systems C and D, where maximum levels of 9 and 27 μM(N) respectively were measured on day 2. The 3-fold maximum increase in IN in D as compared to C is to be noted, since the amount of detritus added to D was only double that added to C. No significant increases in inorganic nitrogen levels were observed in systems B, even though 117 μM(C) of detritus was added to them. Clearly, in this experiment a threshold value of detritus needed to be exceeded before observable changes in IN concentrations appeared.

Figure 3 shows the peak IN concentrations (averaged over replicates) for K-1 (as well as for K-2 and K-3), plotted as a function of the amount of organic carbon added in the form of detritus. In K-1 the threshold effect is clearly seen, with peak IN concentration increasing rapidly only beyond a certain initial increase in organic material. The NO_2 + NO_3 concentrations and the NH_4^+ concentrations each separately exhibited threshold behavior, as can be seen from Figs. 1 and 2.

Dark and light bottle CO_2 evolution rates are plotted as a function of time in Figs. 4 and 5. The control's value has been subtracted from each treatment's values in order to display directly the relative effects of the detrital additions (see figure captions for absolute rates). Through day 3, dark-bottle CO_2 evolution rates in the systems with detritus added (B, C, D) were greater than in the controls (A). The maximum rate in each of the three spiked systems occurred on day 1. The values of these maximum dark bottle CO_2 evolution rates increased faster than linearly with corresponding increases in detritus, which is commensurate with inorganic nitrogen data. Light bottle CO_2 evolution rates showed significant uptake (negative evolution) rates of CO_2 between days 2 and 4 for the spiked systems, with the magnitude of these negative rates ordered as A < B < C < D.

In addition to the CO_2 evolution rate data, the water column phytoplankton data indicate induced primary productivity in the systems with detritus added. Figure 6 shows total phytoplankton

volume plotted as a function of time. Between days 5 and 17 the spiked systems showed increases in water column phytoplankton volume with values of 1.2, 3.4, 4.6, and 8.2 x 10^9 μ^3/liter for systems A, B, C, and D respectively being attained on day 17. We caution here that phytoplankton growing on the sides and bottoms of the containers are not included in our counts.

Over the first 10 days subsequent to the detrital additions, total zooplankton volumes in all systems remained low (<0.5 x 10^9 μ^3/liter). Between days 10 and 17 they increased in D to 2.5 x 10^9 μ^3/liter, while remaining low (<0.5 x 10^9 μ^3/liter) in the other systems. Unlike the zooplankton, protozoa exhibited significant increases over the first 4 days in those systems where detritus was added (see Fig. 7). They remained low (<0.25 x 10^9 μ^3/liter) in the control systems A. The smallest time interval between successive protozoa measurements was 4 days, which means that the peak levels might have been missed. Nonetheless, we observed apparent protozoa volume maxima in all spiked systems on day 4 with values of 2, 7.3 and 4.5 x 10^9 μ^3/liter obtained for systems B, C, and D respectively.

K-2. Five levels of bacterially derived detritus, corresponding to additions of 82 μM(C), 163 μM(C), 367 μM(C), 612 μM(C), and 1020 μM(C) organic carbon were added to systems B, C, D, E, and F respectively. Figure 8 shows IN levels plotted as functions of time for systems C, D, E, and F. The IN levels in systems A (controls) and systems B (82 μM(C) detritus added) remained constant and low (~1.0 μM(N)) and are not plotted. As in K-1, NO_2 + NO_3 concentrations

remained low (<2 μM(N)) in all systems and the IN increases were comprised largely of NH_4^+. The peak IN levels displayed the same threshold effect as in K-1 and indeed the two sets of data as plotted in Fig. 3 are nearly overlapping. It should be emphasized that K-1 and K-2 were run nearly 8 months apart and were performed with different sources of lake water. The only major difference between the IN data of K-2 and K-1 is that in the former, with increasing amounts of added detritus, increasing time intervals occurred before the maximum level of produced inorganic nitrogen was attained. A similar phenomenon was observed by Williams and Gray (18).

Figure 9 plots total water column phytoplankton volumes as a function of time. By day 9, increases were observed in systems C, D, E, and F, with the maximum volume densities being 4.5, 24.5, 24.5, 26 x 10^9 μ^3/liter) during the experiment.

K-3 and K-4. These two experiments differed from K-1 and K-2 in that the detritus spike consisted of algae rather than E. coli. In K-3, the added detritus contained only dissolved organic matter, while in K-4 the entire algal concentrate, consisting of dissolved plus particular organic mater (DOM + POM), was added. Figure 10 shows IN concentrations as a function of time for K-3, while Fig. 3 shows the results of the comparison of the measured peak concentrations of mineralized inorganic nitrogen versus the amount of substrate added. Evidence for a threshold is not observed. Although the replication in K-4 was sufficiently poor that no conclusions about a threshold can be drawn, lower IN concentrations were seen in K-4 compared with K-3. In systems C and D of K-4, for example, the increase in IN was less than half that observed in C and D, respectively, in K-3. This indicates

that DOM was more effectively mineralized than was an equivalent concentration of DOM + POM, consistent with the findings of Cole and Likens (3) at considerably lower concentrations of added substrates. In both K-3 and K-4 the fraction of measured IN in the form of NO_2 + NO_3 was considerably higher than in K-1 and K-2; in all systems over half the observed IN was NO_2 + NO_3. In K-3 and K-4, as in K-2, the larger the substrate addition, the later in time that the peak IN concentration was reached.

DISCUSSION

Our discussion focuses on the threshold effect seen in the peak IN concentrations in the two experiments K-1 and K-2. In particular, we concentrate on whether this effect actually characterizes net mineralization activity. We recall that net mineralization is mineralization minus immobilization and export. The IN concentrations that we measure do not need to be corrected for immobilization or export losses, prima facie. On the other hand, the measured IN concentrations do not necessarily indicate directly the net mineralization activity in our systems; corrections for uptake of IN by primary producers must be taken into account. It is possible that nutrient uptake by primary producers took place in such a fashion as to produce the threshold effect, even though net mineralization activity was simply proportional to the amount of substrate added. For example, if during the initial time period when the peak IN concentrations were observed, the total uptake of IN by primary producers was limited to be less than or equal to a fixed amount, independent of the amount of substrate added, then

the fractional amount of IN removed from the water column would decrease with increasing substrate addition. The observed threshold effect would then have been generated.

To understand the role that phytoplankton assimilation of inorganic nitrogen can play in influencing the relation between the amount of organic matter added and the subsequent maximum concentration of IN observed, consider the phytoplankton densities observed in the systems on each side of the IN threshold. For K-1, this would be systems B and C while for K-2 this would be systems C and D. If a threshold is observed in the phytoplankton densities--that is, phytoplankton density does not increase in the system just below threshold (B in K-1 and C in K-2) but does increase in the system just above the threshold--then that would be strong evidence that assimilation did not cause the threshold in IN production but, rather, reflected it. In contrast, if sufficient phytoplankton growth occurs in the system just below the threshold, then it is possible that phytoplankton assimilation actually produced the threshold in IN concentrations.

Based on these arguments, the data shown in Figs. 6 and 9 lessen the likelihood that nutrient uptake for water-column phytoplankton growth caused the threshold effect in K-2. These data show a threshold effect, in the sense that production in D was 8 times greater than that in C, despite the fact that only 2.25 times more substrate was added to D than C. This suggests that phytoplankton growth reflected, rather than caused, the threshold-like large difference between the maximum

IN concentration in C and D. On the other hand, the bunching effect observed for phytoplankton growth in D, E, and F suggests that a levelling off of inorganic-nitrogen uptake by phytoplankton might have cause the slightly greater-than-proportional increase in peak IN levels as the organic input increased from D to E to F. Because zooplankton densities remained very low throughout the course of K-2, we believe the phytoplankton dynamics reflected nutrient conditions and were not altered significantly by grazing effects.

The phytoplankton growth rates and absolute levels in K-1 (Fig. 6) suggest that with increasing organic additions, a roughly proportional increase in the water-column phytoplankton production took place. In order to assess whether phytoplankton assimilation in K-1 caused the IN threshold, we need to convert phytoplankton biomass into nitrogen units. Assuming that nitrogen comprises 1 percent of the wet weight of phytoplankton, the total nitrogen equivalent of the phytoplankton biomass observed in system B of K-1 increased by about 1 μM(N). Thus the observed phytoplankton do not contribute significantly to the total nitrogen budget, and on the basis of that data a definitive conclusion about the origin of the threshold in K-1 cannot be reached.

Serious objections can be raised to the arguments advanced above, which were based on observed water-column phytoplankton densities. Most importantly, these measurements do not provide information about uptake of IN by phytoplankton cells that initially grew in the water column and then subsequently sunk to the bottom of the beakers or attached to its walls. They also do not provide information about up-

take and storage of IN in pre-growing phytoplankton cells. Finally, they do not provide information about uptake of IN by algae attached to the surfaces of the beakers.

A direct indication of water column net mineralization in K-1 can be obtained from the dark- and light-bottle CO_2 evolution measurements, shown in Figs. 4 and 5. From these data an argument can be advanced to suggest that the threshold phenomenon characterized the mineralization process, itself, and was not an artifact of the uptake kinetics of IN by phytoplankton.

We write:

$$L = P + Q + R$$

$$D = Q + R$$

where L and D are the lightand dark-bottle CO_2 production rates, respectively; Q is the contribution to CO_2 production from gross mineralization (including zooplankton respiration) minus immobilization, P is the gross primary production contribution to CO_2 production in the light, and R is the phytoplankton respiration contribution to CO_2 production. Note that P will often be negative with our sign convention. It is then straightforward to show that on day 1, when CO_2 production was maximum, the Q's are a faster-than-linearly increasing function of added detritus for any fixed P/R ratio satisfying $0 \leq R \leq -P$. This is illustrated in Table 4, which gives the value of

Q on day 1 for 3 different assumed values of P/R. We cannot estimate reliably from the closed-bottle data what the net amount of algal CO_2 uptake actually was, as that quantity is very sensitive to the value of P/R.

We note that these measurements were made in water-column samples that did not include any of the added detritus which may have sunk to the bottom of the 4-liter containers. The fact that the threshold effect was seen suggests that it reflected water-column activity and was not due to the proportionally greater amount of detritus which may have settled to the bottom of D or C as compared with B.

Uptake of IN by denitrifying bacteria can also be considered as a possible cause of the loss of significant quantities of $NO_2 + NO_3$ from the water column of our beakers. A saturation phenomenon in the kinetics of this process, could have generated the observed threshold effect. As discussed in the introduction, however, denitrification of mineralized IN is not a correction that need be applied to the IN measurements in order to obtain the net mineralization rate. In addition, the O_2 saturation maintained in our beakers makes it unlikely that denitrification could have depleted a large fraction of the produced $NO_3 + NO_2$.

One other possible explanation of the threshold phenomenon deserves mention. Some NH_4^+ is known to adsorb onto the surfaces of particles (10), and this fraction of the produced NH_4^+ would escape detection by our measurement procedures. If particle-surface-area were adequate to adsorb a relatively large fraction of the NH_4^+ produced in systems B,

but not in the systems with larger amounts of added detritus, then a threshold effect would appear. The difficulty with this explanation is that the amount of particle-surface-area added to each of the systems in K-1 and K-2 was proportional to the amount of organic carbon added, and therefore such a saturation effect is unlikely.

If, as we have argued, the threshold effect characterizes net mineralization activity, it is then pertinent to inquire as to the mechanism responsible for this effect. The measurements of protozoa population density in K-1, shown in Fig. 7, provide some information in this regard. Predation on bacteria by protozoa has been widely reported (2,6). Our observed increases in protozoa population densities very likely reflect increases in bacteria population densities. Figure 7 shows that the maximum protozoa density in system D, was actually below that of C. It is unlikely, then, that in system D, with twice as much substrate added as in system C, the bacteria population grew twice as large as in system C. Saturation of bacterial biomass growth (as a function of increasing substrate) can be inferred, and as discussed in the introduction, this could have generated the threshold in net mineralization activity. In K-3, where no threshold was observed, we note that in all systems with increased organic matter, IN production began immediately, where as in K-1 and K-2, there was a lag-period of about a day before net IN production began to increase. It is reasonable to associate that lag with the period of microbial immobilization of nitrogen.

CONCLUSIONS

Our original hypothesis was confirmed in two of the four experiments (K-1 and K-2) designed to test it. In K-4, poor replication did not allow a test. In K-1 and K-2, utilizing detritus of bacterial origin, a threshold effect was observed, while in K-3, involving detritus of algal origin, no threshold effect was observed. The analysis of all the data from K-1 and K-2, particularly the IN concentrations and the sealed-bottle measurements of CO_2 changes, suggests strongly that the observed threshold phenomenon characterizes detritus-decomposer interactions and is not simply a reflection of the kinetics of uptake of inorganic nutrients by phytoplankton. A qualitative picture utilizing a simple microbial carrying capacity mechanism is one possible way of viewing the data. Above a certain population density, in this model, decomposer growth (immobilization) ceases while mineralization continues at significant rates. From this point of view, we would say that the initial conditions (water source and type of detritus) of experiments K-1 and K-2 allowed this carrying capacity to be reached within the range of detritus additions. Within this framework, we can deduce that a threshold in net mineralization in K-3 was missed because that threshold corresponded to a level of added substrate either below the lowest level added or above the largest level. The rapid onset of net IN production seen in K-3 (Fig. 10) suggests that the threshold was below the lowest level added.

The increasing delay in the time at which peak IN concentrations were seen in K-2, K-3 and K-4 as the detritus addition increased is consistent with the similar observation of Williams and Gray (18). This behavior can be simulated by Michaelis-enten kinetics for the uptake of detritus by decomposers, but that same model analysis gives rise to curves in Fig. 3 that are either linear or that bend over with a nonlinearity opposite to that seen in K-1 and K-2.

From studies of ecosystem stability, the importance of being able to measure density-dependent saturation effects in populations can be inferred (12). However, the empirical accessibility of the concept of density dependence has been a subject of controversy (5,11,14). Empirical evidence of density-dependent regulation in populations of decomposers is conflicting, at best. Hairston et al. (8) argued that decomposers are generally food-limited in nature, while Potter (13) concluded that in aquatic systems the number of benthic bacteria present limit the rate of decomposition (presumably because factors other than food limit their numbers and activity). Much of the discussion on this topic has taken place within the context of attempts to search for and quantify density dependence by correlation analysis, in which the changes in a population over a sequence of fixed time periods are examined to see whether the changes depend nonlinearly upon the population. As shown by Eberhardt (4), this approach is beset with statistical traps. It is suggested here that appropriately chosen detritus manipulations followed by measurements of mineralization products, offer a means of identifying and quantifying microbial carrying capacities in aquatic systems.

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Table 1.
THE FOUR EXPERIMENTAL CONFIGURATIONS

Experiment	Initial organic carbon concentration	Detrital material	System	Amount of detritus added, expressed as percent increase in organic carbon	Replicates
K-1	430 µM(C)	E. coli (DOM+POM)*	A	0% (control)	2
			B	27	2
			C	54	2
			D	109	2
K-2	340 µM(C)	E. coli (DOM+POM)*	A	0% (control)	3
			B	24	3
			C	48	3
			D	108	3
			E	180	3
			F	300	3
K-3	260 µM(C)	algae (DOM)*	A	0% (control)	2
			B	24	2
			C	116	2
			D	348	2
K-4	260 µM(C)	algae (DOM+POM)*	A	0% (control)	2
			B	61	2
			C	122	2
			D	366	2

* DOM = dissolved organic matter (see text)
POM = particulate organic matter (see text)

Table 2

METHODS USED FOR MEASURING CHEMICAL AND BIOLOGICAL PARAMETERS

Parameter	Method	Special Equipment	Reference
O_2	polarography	O_2 meter (YSL 57)	--
pH	electrometry	pH meter (Orion)	--
IC	infrared absorbance	IR analyzer (Beckman 865)	--
OC	combustion to IC	TOC analyzer (Beckman 915A)	--
NH_4	blue indophenol	spectrophotometer (Zeiss PM2 DL)	(15)
NO_3+NO_2	reduction, diazotization	"	(7)
CO_2 evolution	equilibria kinetics	pH meter (Orion 601) IR analyzer (Beckman 865)	(16)
phytoplankton	tube chamber	5 ml tube chamber (Wilde) inverted microscope (Lietz)	--
zooplankton	counting chamber	100 ml count. chamber (Wild) binocular microscope (Lietz)	--

Table 3
LIST OF ORGANISMS PRESENT IN THE 4-LITER BEAKERS FOR K-1

CHLOROPHYTA

- Ankistrodesmus sp.
- Chodatella quadrisets
- Closterium sp.
- Mougeotia sp.
- Rhizoclonium sp.
- LRGT I (5)
- LRGT II (5)
- Nephrocytium sp.
- Gloeocystis sp.
- Planktosphaera gelatinosa
- Quadrigula sp.
- Scenedesmus bijuga
- Scenedesmus quadracauda
- Schroderia setigera
- Staurastrum sp.
- Treubaria trippendicular

BACILLARIOPHYCEAE

- Coscinodiscus lacustris
- Cyclotella menenghiana
- Fragilaria sp.
- Navicula sp.
- Synedra radians
- Synedra ulna
- Anomoeneis sp.
- Gomphonema sp.

CYANOPHYTA

- Anabaena sp.
- Oscillatoria sp.
- Spirulina sp.

CYPTOPHYCEAE

- Cryptochrysis sp.

EUGLENOPHYTA

- Phacus sp.
- Unid. flag. I
- Unid. flag. II

PYRROPHYTA

- Unid. Dinoflagellate I

PROTOZOA

- Paramecium sp.
- Vorticella sp.
- Actinosphaerum sp.
- Monas sp.

ROTIFERA

- Ascomorpha sp.
- Discranophorus.sp.
- Keratella quadrata
- Lecane sp.
- Philodina sp.
- Polyarthra sp.
- Trichotria sp.
- Voronkowia sp.
- Unid. rotifer I

ANNELIDA

- Pristina sp.

CLADOCERA

- Daphnia pulex
- Simocephalus vetulus
- Alona guttata

COPEPODA

- Cyclops vernalis

OSTRACODA

- Cypridopsis sp.

Table 4

VALUES OF Q (THE CONTRIBUTION OF MINERALIZATION TO THE RATE OF CO_2 EVOLUTION) ON DAY-1 OF K-1, FOR 3 ASSUMED VALUES OF THE RATIO OF P TO R (See Text)

	R = 0	R = -P/2	R = -P
$Q_D - Q_A$	6.05	4.20	2.35
$Q_C - Q_A$	1.90	0.90	-0.15
$Q_B - Q_A$	1.15	0.25	-0.65
Q_A	2.70	1.30	-0.15

FIGURE CAPTIONS

Figure 1 $NH_4^+ + NO_2 + NO_3$ concentrations in the treatment (B, C, and D) and the control (A) systems in experiment K-1. Shown in parentheses next to each system label is the percent increase in organic carbon.

Figure 2 $NO_2 + NO_3$ concentrations for control and treatments in K-1.

Figure 3 The maximum $NH_4^+ + NO_2 + NO_3$ concentration plotted versus the increase in organic carbon for each system in K-1, K-2, and K-3. The dashed line shows the approximate upper bound for the IN concentraton assuming that the C/N ratio is 6, that all of the nitrogen present in the added detritus is converted to IN, and that all of the produced IN is present at the time IN concentrations reach their peak value.

Figure 4 The daily dark-bottle CO_2 production rates for K-1. the control value has been substracted from each of the treatment systems' values here in order to display directly the relative effects of te detrital additions. Replicate measurements have been averaged. For reference, the control system measurements for the 5 days of measurement presented here were -2.35, 2.7, 1.3, 2.2 and .75 μM(C)/hour respectively.

Figure 5 The daily light-bottle CO_2 production rates for K-1. The control value has been subtracted from each of the treatment systems' values here in order to display directly the relative effects of the detrital additions. Replicate measurements have been averaged. For reference, the control system measurements for the 5 days of measurement presented here were -4.75, -.15, .1, -.05, and -.65 μM(C)/hour, respectively.

Figure 6 Phytoplankton volume densities measured in experiment K-1.

Figure 7 Protozoa population volume densities measured in experiment K-1.

Figure 8 $NH_4^+ + NO_2 + NO_3$ concentrations in four of the treatment systems in experiment K-2. Results for systems A and B are not shown; their concentrations were consistently at or below that of system C. Shown in parentheses next to each system label is the percent increase in organic carbon for that system.

Figure 9 Phytoplankton volume densities measured in experiment K-2. Where two systems are represented by a common line, the results for those systems were indistinguishable within estimated measurement error.

Figure 10 $NH_4^+ + NO_2 + NO_3$ concentrations in the treatment (B, C, and D) and the control (A) systems in experiment K-3. Shown in parentheses next to each system label is the percent increase in organic carbon.

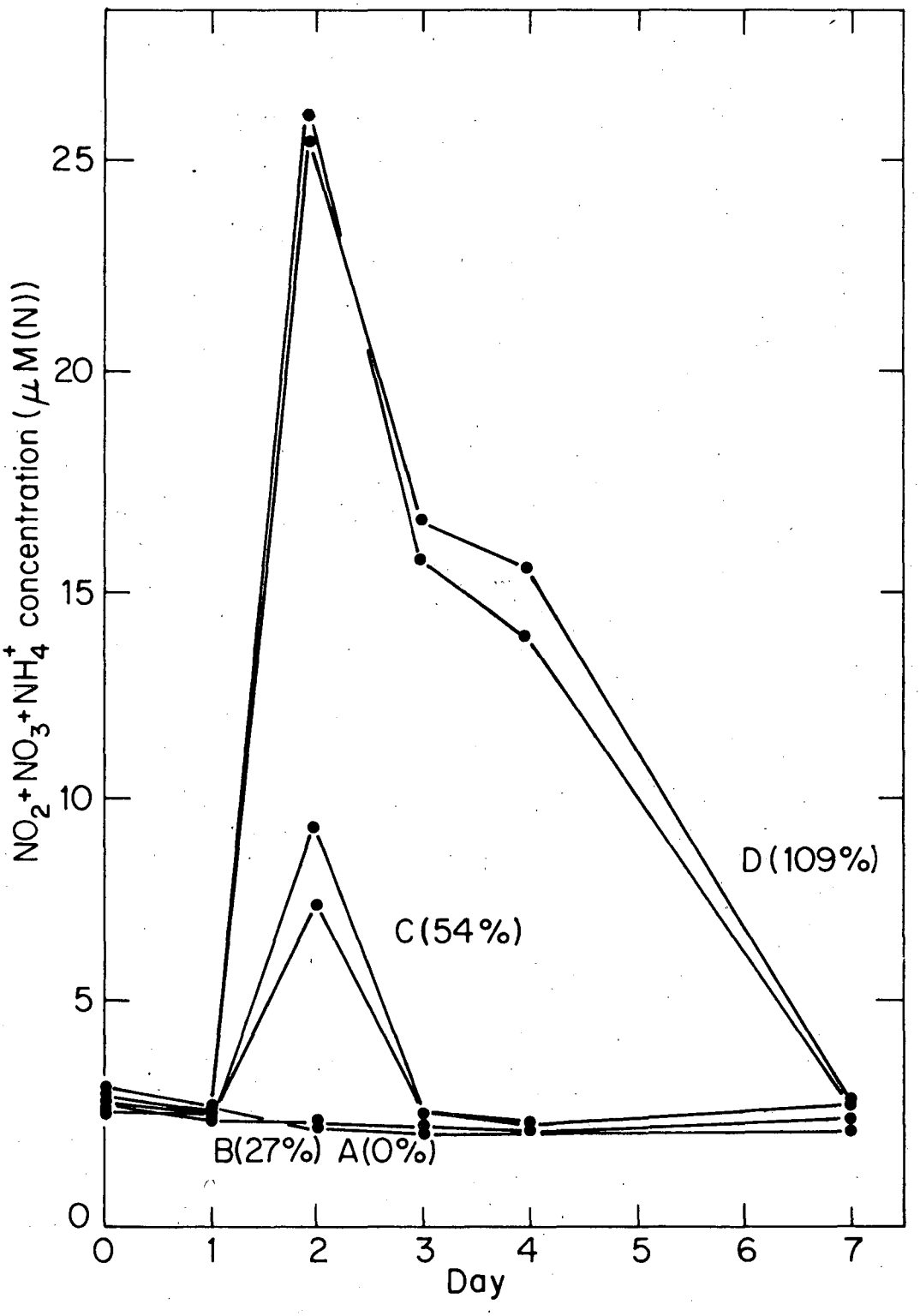


Fig. 1

XBL 794-1356

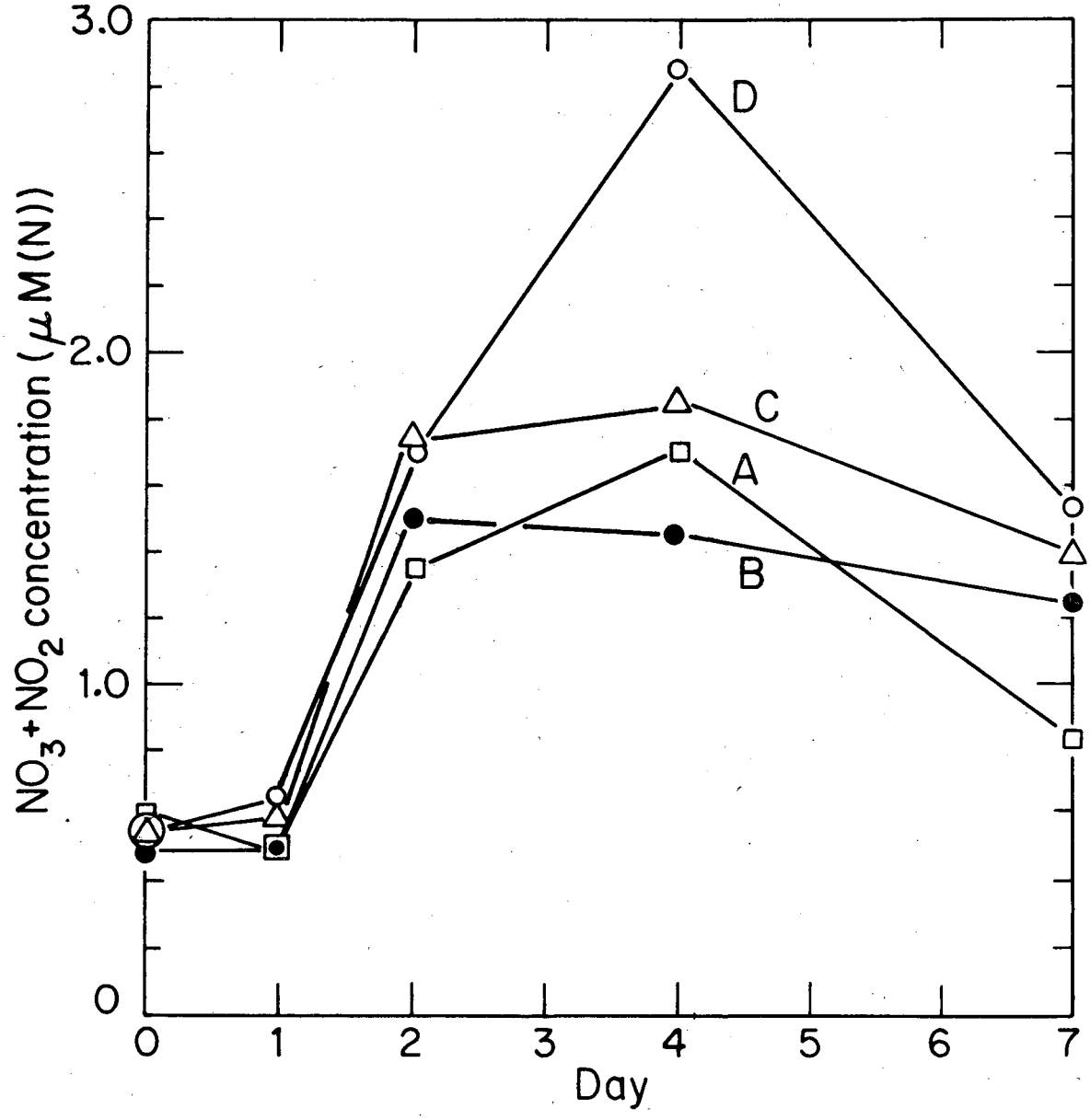


Fig. 2

XBL 7910 - 13371

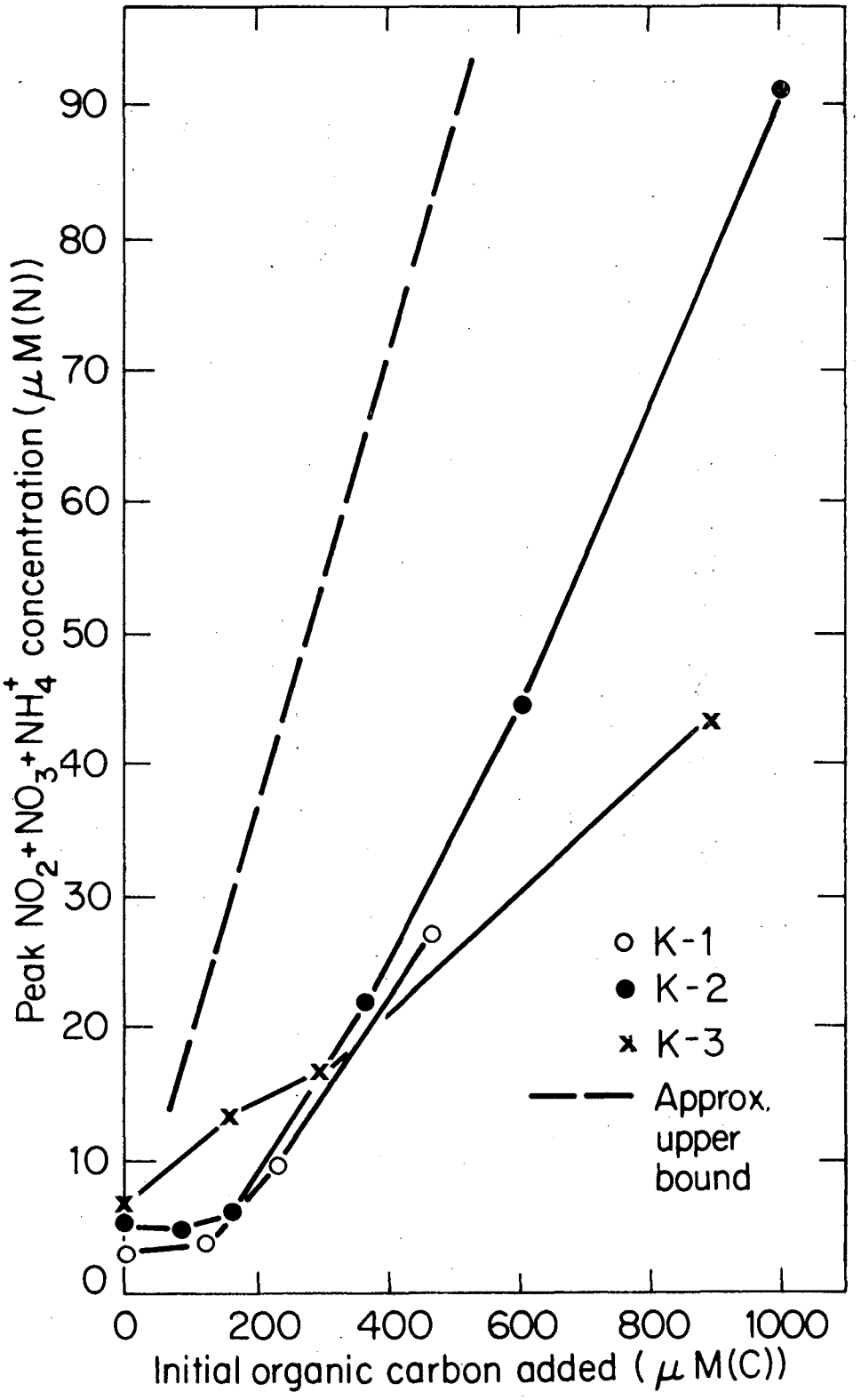


Fig. 3

XBL 795-1490

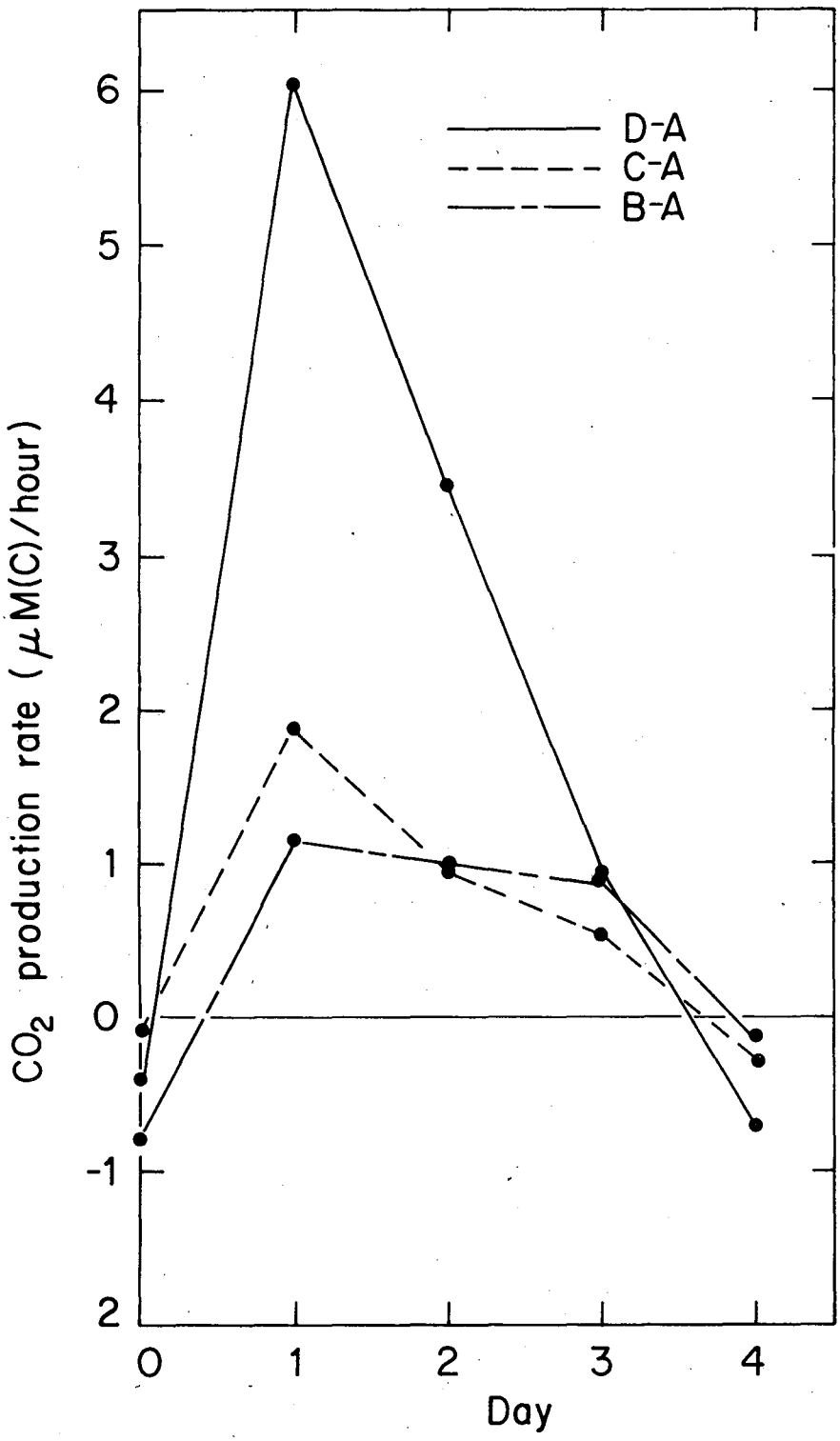


Fig. 4

XBL 795-1428

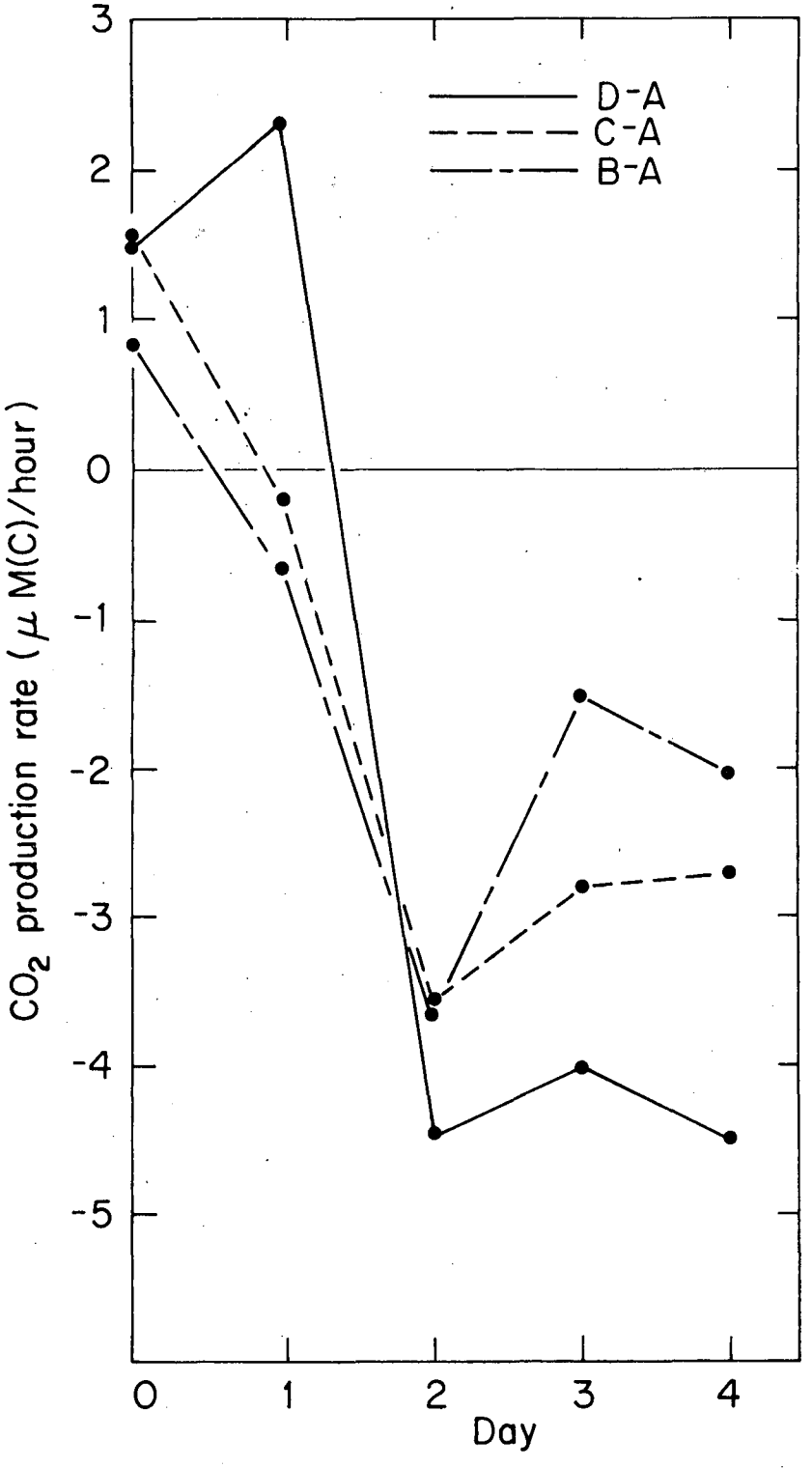
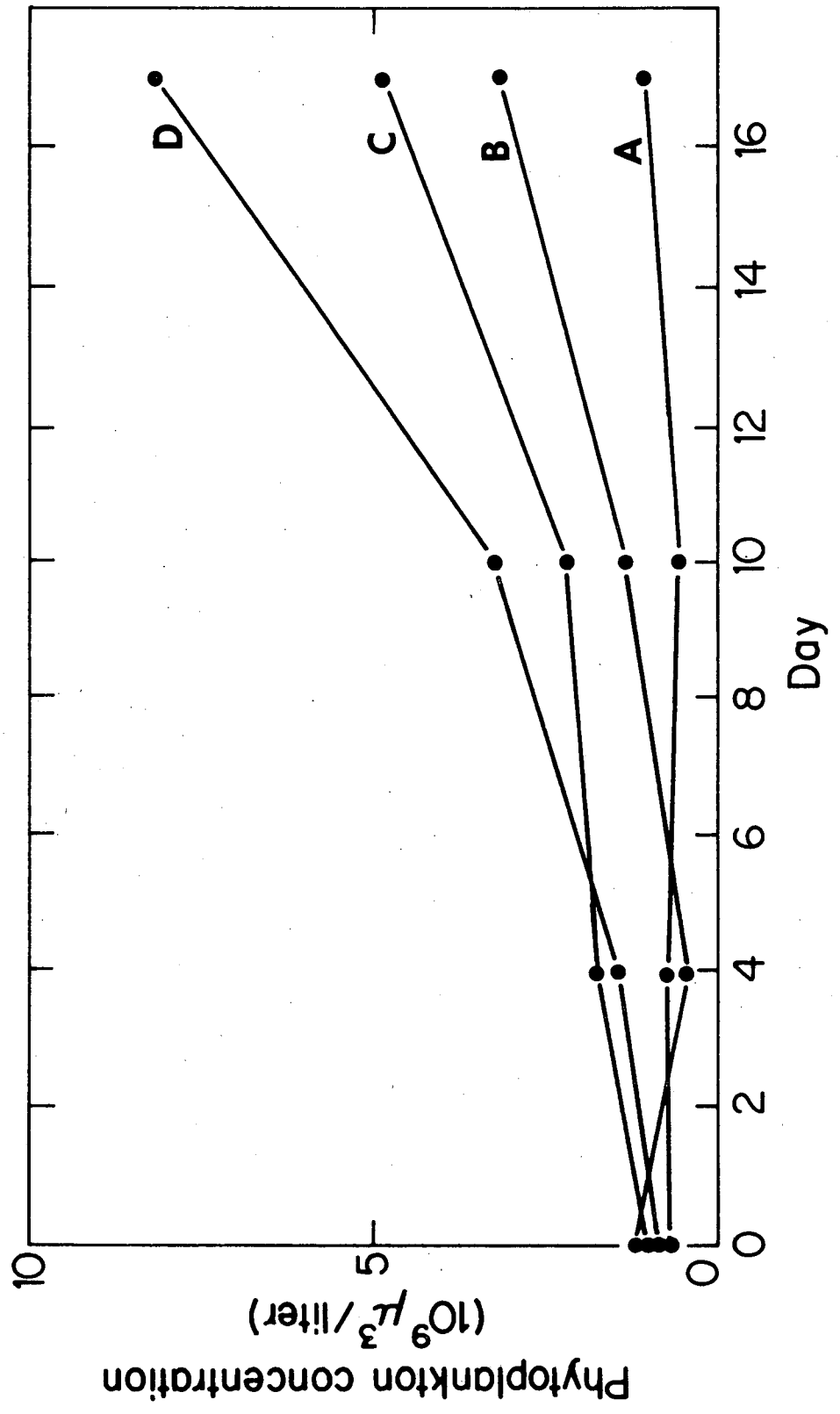


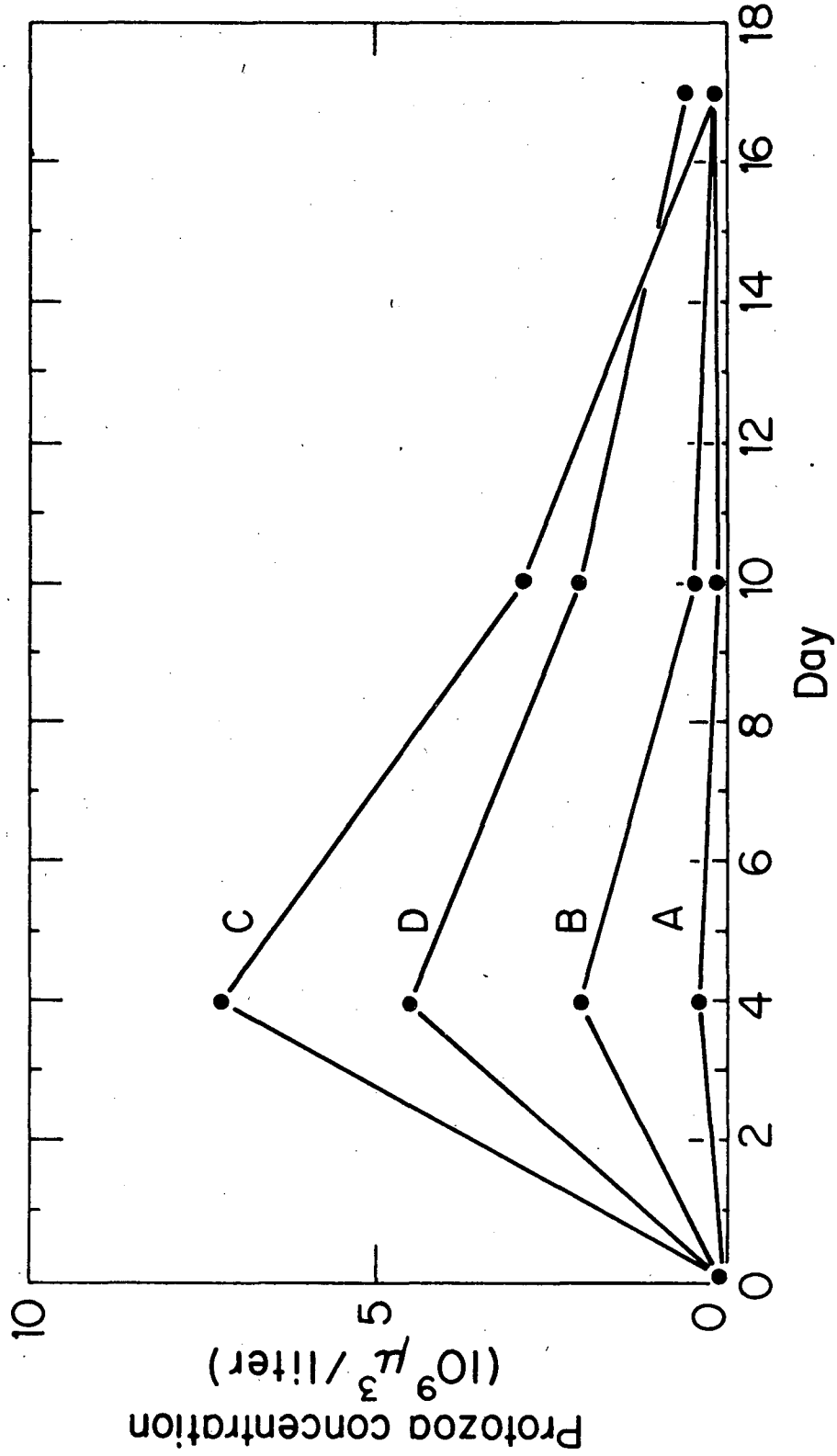
Fig. 5

XBL 795-1427



XBL 794-1357

Fig. 6



XBL 7912 - 13370

Fig. 7

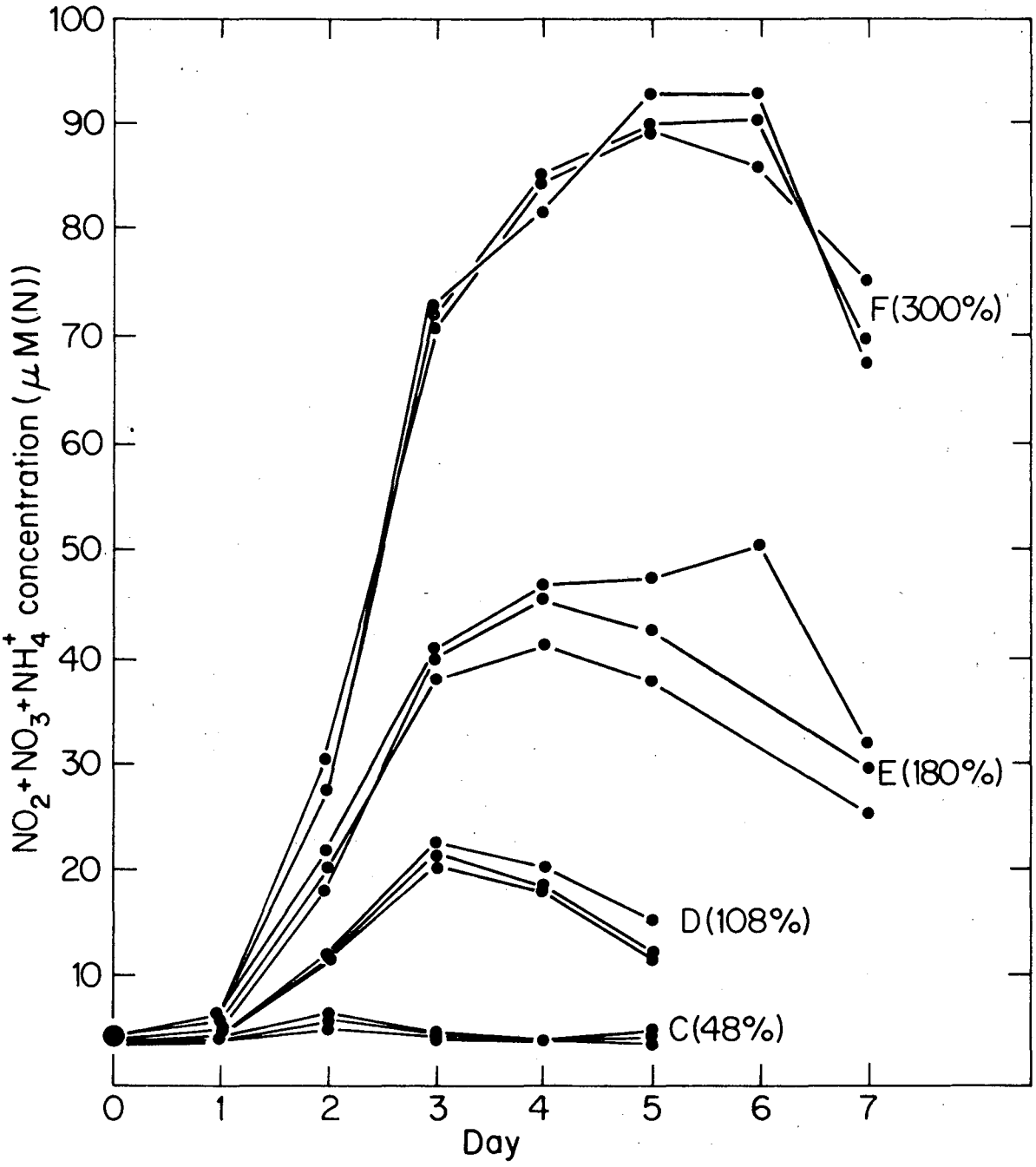


Fig. 8

XBL 794-1355

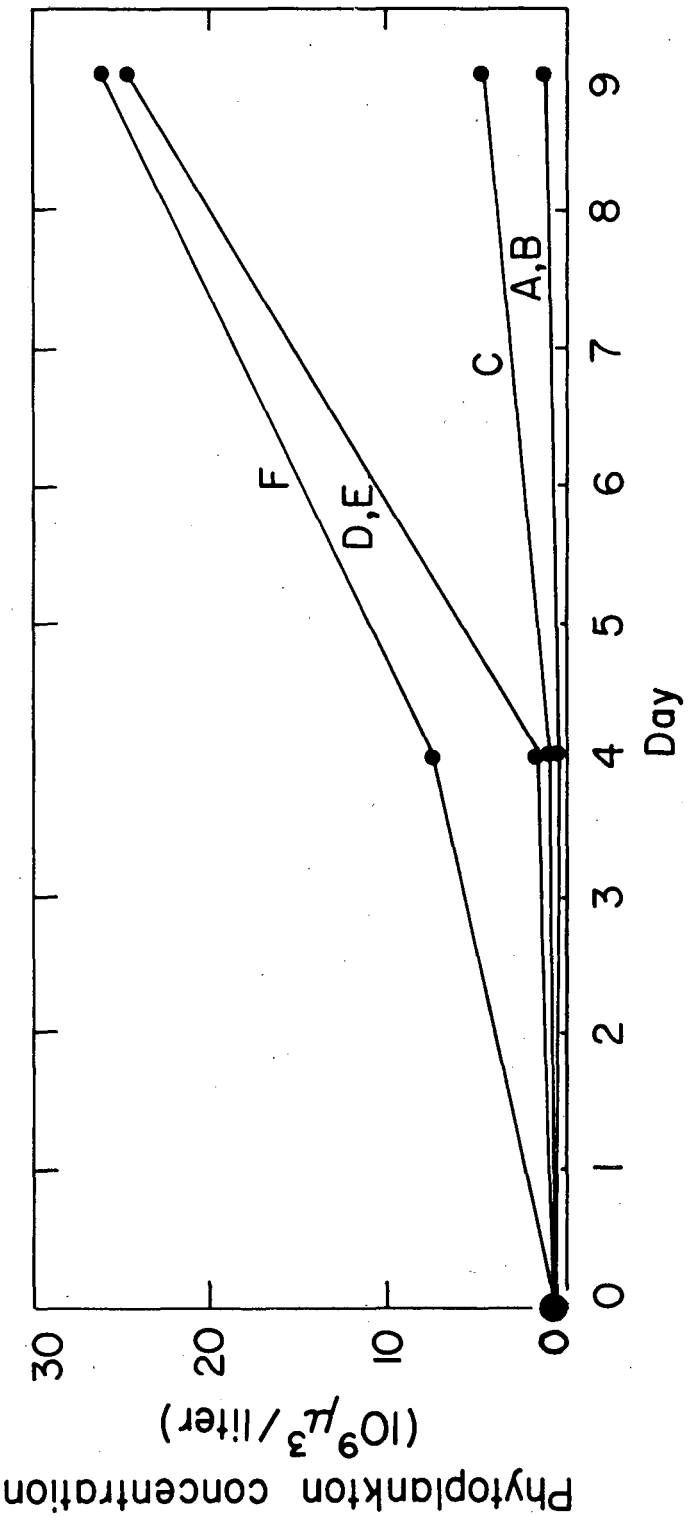


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Fig. 9

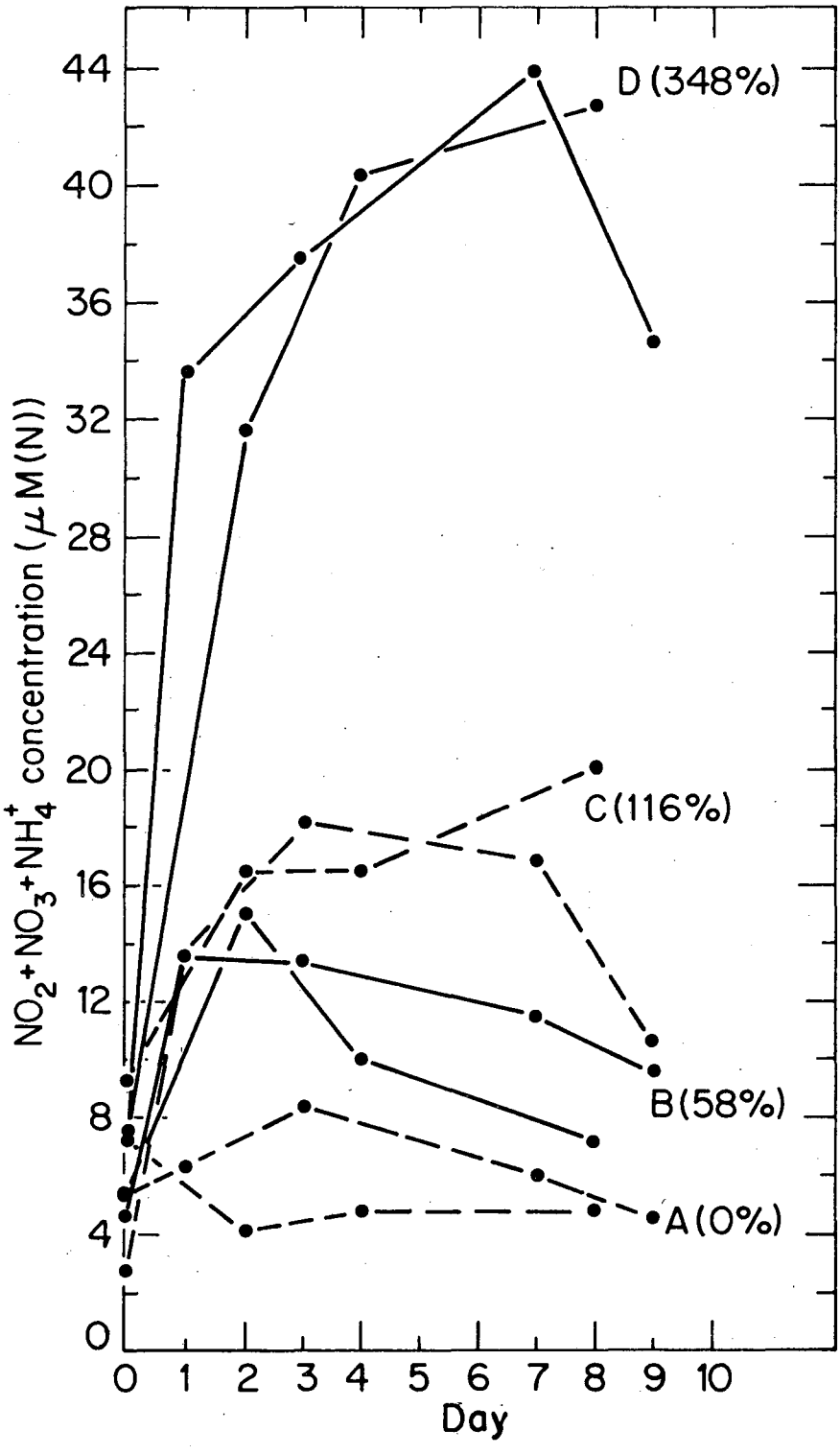

Fig. 10

XBL 7912-13377

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