EFFECTS OF TEMPERATURE AND AMMONIUM TO NITRATE RATIO ON MICROBIAL RESPONSES TO FUMIGATION IN ORGANIC SOIL

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ABSTRACT

EFFECTS OF TEMPERATURE AND AMMONIUM TO NITRATE RATIO ON MICROBIAL RESPONSES TO FUMIGATION IN ORGANIC SOIL

by Fang Hui Liao

Laboratory studies were conducted to investigate the effects of rising temperature and initial ratios of ammonium to nitrate on microbial numbers and activities in organic soil fumigated with chloropicrin or dichloropropene. These chemicals were used at rates and for exposure periods recommended by the manufacturer for field application.

Treated and control lots of Houghton muck were subjected to exhaustive leaching, followed by aeration, freezing and periods of low temperature ($5^{o}C.$) to simulate environmental conditions to which fall fumigated organic soils are subjected during the winter and spring months. Soils were amended with ammonium and nitrate in varying proportions before being subjected to different sequences of rising temperature.

Heterotrophic microbial populations and activities were characterized by measurements of CO_2 evolution and by enumeration of bacteria and fungi by dilution plate counts. Changes in levels of ammonium, nitrate and total mineral nitrogen were followed by microdiffusion from soil extracts.

In fumigated soil, bacterial numbers at low temperatures and respiratory losses of CO_2 at higher temperatures were directly related to the level of nitrate initially supplied. At the same time, nitrate disappeared initially in fumigated soil, whereas in unfumigated soil nitrate accumulated at rates related directly to temperature.

From these observations it was concluded that heterotrophic bacteria capable of adapting to nitrate utilization are among the first to recover in fumigated soil. Observed delays in nitrate accumulation appeared to be due to utilization of nitrate by these heterotrophes rather than to retarded recovery of nitrifiers. Longer delays in nitrate accumulation with chloropicrin than with dichloropropene were associated with larger numbers and longer persistence of nitrate dependent bacteria.

Utilization of nitrate in fumigated soil appeared to be due to assimilation by microbial cells rather than to denitrification, since no permanent differential losses of mineral nitrogen occurred.

The initial assimilatory disappearance of nitrate appeared to be a function of available energy substrates and was essentially the same at temperature of 5°, 20° and $30^{\circ}C$. Recovery and activity of nitrifiers, on the other hand, was greatly enhanced at 20° and $30^{\circ}C$. Consequently, initial delays in nitrate accumulation in fumigated soil

were greatly reduced by temperature increases imposed the 16th day after fumigation.

When the temperature was maintained at 5°C. for 51 days after fumigation, large numbers of bacteria with apparent nitrate dependence were observed. Increasing the temperature to 20°C. at this time resulted again in temporary disappearance of nitrate and markedly extended the delay in nitrate accumulation. Similar effects, though less striking, were observed when the temperature was increased from 20° to 30°C. at this time. This behavior is similar to that observed during periods of rising temperature in spring and early summer in the field following fall fumigation.

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INTRODUCTION

The primary object of soil fumigation, whether by heat or volatile chemicals is to kill pathogenic micro-organisms. The use of fumigant chemicals for the control of weeds and various pathogenic organisms in the soil has become a wide spread practice within recent years. The action of many of these chemicals is frequently not specific and may affect organisms which have a beneficial influence on plant growth.

In general, microbial numbers are initially decreased by fumigation, but certain forms quickly reinhabit or develop in the soil, and shortly after treatment overall numbers are usually in excess of those in untreated soil. Spore forming bacteria are relatively resistant to fumigants, the organisms which oxidize ammonia to nitrite and nitrite to nitrate appear to be relatively sensitive. Organisms which release ammonium from organic nitrogen complexes quickly return to soil following treatment, whereas the nitrifiers are apparently slow to develop. The apparent inhibition of nitrification has resulted in an accumulation of ammonium nitrogen in fumigated soil, particularly in organic soils. In situations where a plant species can utilize ammonium nitrogen as readily as nitrate nitrogen, retarded nitrification will have very little effect on the availability of nitrogen to the plants. If the concentration is too

high, ammonium sensitive plants may be injured. Under conditions where considerable loss of nitrogen through leaching of nitrate nitrogen might occur, temporary inhibition of nitrification may be beneficial.

Laboratory studies have shown that the intensity and duration of the suppression of nitrification vary with the chemical and with the numerous mechanical, soil and environmental factors which influence the effectiveness of the chemicals as fungicides or nematocides. Field studies have shown that nitrate accumulation in organic soils is drastically delayed in the spring and early summer, even when the soil was fumigated the previous fall and no detectable traces of the chemical remain in the soil. In these field studies, disappearance of applied nitrate was observed during periods of wet weather or after irrigation. Nitrate disappearance was also associated with rising soil temperature. These field results suggest that reduction or consumption of nitrate by an altered heterotrophic microflora may contribute to retarded nitrate accumulation in fall fumigated soil.

The present laboratory study was undertaken to investigate the effects of varying proportions of ammonium and nitrate nitrogen on nitrogen transformations and microbial numbers in fumigated organic soil under conditions of high moisture and rising temperature.

LITERATURE REVIEW

The main purpose of soil disinfestation is to kill detrimental microflora and to control weed seeds. In the search for an explanation of the remarkable increase in plant growth following soil treatments with heat or fumigants, many earlier investigators have pronounced a number of ingenious theories. These have been well reviewed by DuBuisson (14) and Kopeloff and Coleman (29). DuBuisson concluded that the beneficial influences obtained by treating the soil with volatile antiseptics can not be ascribed to a change in physical condition, to a suppression of some toxic material, or to a development of acids from the action of the antiseptics. The closely coordinated stimulation of plant and bacterial activity due to the treatment of the soil with volatile antiseptics points strongly toward a biological interpretation, with due regard for chemical effects of altered or enhanced microbial activity. Kopeloff and Coleman ascribed the beneficial effects of "partial sterilization" to increased amounts of plant food, especially nitrogen, made available for plant use as a result of increased bacterial activity.

Newhall (36) indicates that many of the earlier investigators have expressed a concern for the useful microorganisms that must inevitably suffer the same fate as their parasitic associates following any effective soil treatment.

It was well established by the workers in the late 1800's that treatment of soil, either by heat or by antiseptics, has the following results:

1. Non-spore-forming, nitrogen-fixing, nitrite forming and nitrate forming bacteria, as well as parasitic organisms are destroyed, and nitrification is thereby inhibited. The spore-forming ammonifiers survive, and ammonification goes on almost uninterrupted for weeks, especially in soils high in organic matter.
2. Soluble salts are often liberated, in some cases chlorides and sulfates of ammonia, and sometimes soluble manganese.

Microbiological properties of soil fumigated with steam or chemicals were extensively studied by Waksman and Starkey (54). They confirmed by experiment that "partial sterilization" results in an increase of the bacterial population and in ammonium nitrogen accumulation. They attributed these changes to a combination of factors, namely, changes in physical and chemical conditions of the soil, destruction of soil microorganisms thereby making their cell constituents available as a source of energy, changing the equilibrium of the microbiological flora, and efficient conversion of organic nitrogen to ammonium nitrogen.

The apparent inhibition of nitrification by soil fumigants has been observed by several investigators. Stark, Smith and Howard (45) studied the effect of chloropicrin fumigation on nitrification and ammonification and found that low dosages of chloropicrin had little effect on nitrate formation, but as the dosage was increased, nitrification, as measured by nitrate accumulation, was inhibited. The total amount of nitrogen made available for plant growth was not materially increased except where high dosages of chloropicrin were used.

Tam and Clark (48), working with pineapple plants, showed that increased growth and nitrogen composition following chloropicrin fumigation were related to restriction of the plants to predominantly ammonium nutrition as compared to the ordinary nitrate nutrition in unfumigated soil. Tam (47) also reported that D-D soil fumigant applied at the rate of 20 gallons per acre, suppressed nitrate accumulation in Hawaiin pineapple soils for a period of 8 weeks at greenhouse temperature.

Kincaid and Volk (24), working with cigar wrapper tobacco in Florida, used various fumigant chemicals for controlling root knot. They found that, following fumigation, there was a prolonged retention of ammonia nitrogen in the soil. Aldrich and Martin (1) also found that "partial sterilization" with chloropicrin and D-D mixture produced an

initial increase in ammonium nitrogen in soil. On incubation, the ammonium was oxidized to nitrate.

Recently Winfree and Cox (58, 59) reported that fumigation of an organic soil, either by chloropicrin at a rate of 43 gallons per acre or methyl bromide at 21 pounds per 100 square feet, exerted a decided influence on the kinetics of nitrogen mineralization. Ammonium nitrogen accumulated at least 10 fold following the fumigation, at the expense of nitrate nitrogen.

Soil fumigation effectively altered the activity and population of other microorganisms present in the soil. Wensley (55) investigated the action of methyl bromide, ethylene dibromide and D-D mixture on certain organisms involved in nitrogen transformations in soil. His data indicated that the population of nitrifiers is reduced more than that of the ammonifiers. Methyl bromide was shown to be much more potent than ethylene dibromide in reducing the population and activity of the nitrifiers, while ethylene dibromide was proved to be more effective against root knot nematodes than methyl bromide.

Martin (32) reported that treatment of soil with fumigant chemicals markedly altered the nature of the fungal population. After initial or near destruction of the fungal population of the soil, fungi again established themselves, although the kinds and numbers established varied

greatly between treatments. Klemmer (26) found that fumigation of soil produced moderate inhibition of fungi and increased bacterial numbers, as determined by dilution plate counts.

Overman (38), using allyl alcohol as a soil fungicide at the rate of 25 gallons per acre, showed that nitrifying bacteria were inhibited for 3 weeks after the allyl alcohol was applied. Actinomycete populations were unaffected at this rate. Colony counts of Trichoderma increased greatly following the treatment with allyl alcohol. Yatazawa (62) also reported that application of allyl alcohol caused a nearly instantaneous replacement of the native fungal population by Trichoderma viride. Populations of bacteria and actinomycetes were decreased at rates higher than 100 gallons per acre. The use of allyl alcohol apparently creates conditions in soil favorable for the development of Trichoderma.

McCants et al. (35) have presented data which showed that certain of the soil fumigants currently used for nematode control can have a significant influence upon the response of tobacco to applications of nitrogen in the ammonium or nitrate form. There was a greater yield response to nitrate applied with fumigant treatments which had the most suppressing effect on nitrification. However, where nitrification was inhibited and ammonium was applied, yields

and quality were reduced and there was a high ammonium and halogen content in the leaves.

Koike (27) conducted laboratory experiments to determine the effects of eight fumigants on nitrification of $(NH_4)_2SO_4$ and NH_4OH. Results indicated that under the conditions of the experiment the chemicals markedly inhibited nitrification from 4 to 8 weeks. The delay in nitrification was longer with $(NH_4)_2SO_4$ than where nitrogen was supplied as NH_4OH. Kirkwood (25) also found that addition of $(NH_4)_2SO_4$ delayed the recovery of nitrification in fumigated soil.

Wolcott et al (61), using Telone as a fumigant applied at recommended nematocidal rates, found nitrification to be delayed in the laboratory about 8 weeks at soil temperatures above 60°F and for longer periods at lower temperatures. In the field, following fall fumigation, they found that nitrification was delayed 6 to 8 weeks after the soil warmed to 60°F in the spring.

Concomitant development of herbicidal and insecticidal chemicals and control programs has aroused interest in possible effects of these materials on microbial activity in the soil and resulting influences on soil fertility. A great amount of work has been done on the effect of DDT, BHC and 2,4-D on biological processes in soil.

Smith et al (42) worked on the effect of certain herbicides on soil microorganisms and concluded that herbicides varied greatly in their effect on the various

groups of soil microorganisms. In some cases, they are definitely toxic, in others, stimulatory. They found that concentrations of 2,4-D up to 100 ppm. had no significant effect on total plate counts for bacteria, actinomyces, fungi or protozoa. The nitrite and nitrate forming organisms were definitely injured by 100 ppm. Jones (22) reported that 2,4-D at 25 pounds per acre had no detrimental influence upon nitrate production in a soil to which no nitrogen had been added, but 2,4-D in combination with sodium nitrate apparently inhibited the formation of nitrate during the first 2 to 3 weeks. The nitrate content of soil increased very rapidly after this initial period. This indicated that nitrate forming organisms may be temporarily inhibited by the addition of 2,4-D.

Koike and Gainey (28) observed that 2,4-D at the usual field rate did not appreciably reduce total nitrate. Although there were marked temporary reductions in total nitrate accumulation with high concentrations of 2,4-D, the accumulation of nitrate nitrogen was not completely inhibited, and within 8 to 16 weeks the rate of accumulation had again reached that in untreated soil. Newman and Downing (37), working with 2,4-D and related phenoxyacetic acid herbicides, found that normal rates of treatment are noninjurious to the general soil microbial population, do not injure Azotobacter, and are noninjurious or only slightly injurious

to nitrifiers. Extremely high amounts of 2,4-D are required to inhibit ammonification. Evolution of CO_2 from soils in the presence of herbicides may be used as a criterion of influence on the microbial population as a whole.

Wilson and Choudhri (57), in laboratory studies, showed that DDT and BHC in amounts considerably exceeding practical field applications had no significant effect on development of bacteria and molds, nor on certain of their physiological activities important in soil fertility.

Smith and Wenzel (43) found that 5 to 200 ppm. DDT were not definitely injurious and in some cases were stimulative to heterotrophic bacteria. BHC proved definitely fungicidal and also toxic to nitrifiers. Chlordane was found less toxic than BHC, toxaphene was stimulating to bacteria and molds as shown by plate counts and apparently was utilized as a carbon source.

Jones (23) studied the stability of DDT in soil and observed that no injury to nitrifiers, ammonifiers, and sulfur-oxidizing microorganisms was noted from concentrations of DDT ordinarily added to soils. No injury to the nitrogen-fixing bacteria was observed in soils containing concentrations of DDT as high as 1%. DDT added to the soil was remarkably stable during the first year of storage, but by the end of the second and third years appreciable breakdown had occurred.

Bollen et al (7,8) studied various insecticides in the field and concluded that no immediate harmful effects on soil microorganisms were caused by field applications of any of the insecticides studied. None of the treatments resulted in either an approach to sterility or a manifold increase in molds, bacteria or streptomyces, nor were marked changes in proportions of these microbes produced.

In solution media inoculated with soils, Gray (17,18, 19) observed that BHC and its gamma isomer were toxic to bacteria that oxidize ammonia to nitrite and those that oxidize nitrite to nitrate. They were not toxic to nitrifying bacteria in soil, nor to bacterial inocula from a vegetable compost in solution cultures. They were toxic to bacteria that oxidize thiosulfate in solution cultures containing inocula from mineral soils. BHC, but not the gamma isomer, inhibited the growth of phenol-decomposing bacteria and prevented starch hydrolysis by amylolytic bacteria. It also depressed the action of urea hydrolyzing bacteria in soil extracts.

New chemicals are being continuously released for use as fumigants, fungicides, insecticides and herbicides. There are numerous contradictions among published reports dealing with the microbiological effects of these chemicals. This is to be expected, considering the varied circumstances under which the work was undertaken.

Studies on nitrogen transformations are further complicated by the fact that several processes are involved, each responding independently to changes in environmental conditions of aeration, temperature, moisture, pH and nutrients.

The biochemical heterogeneity of the microflora bringing about nitrogen mineralization is a critical factor in determining the influence of environmental factors upon the transformation. Robinson (39) indicates that the ammonifying population includes aerobes and anaerobes. Consequently, organic nitrogen is readily mineralized, at moderate or at excessively high moisture levels. Ammonium is slowly formed at water levels slightly below the permanent wilting percentage, but improving the moisture status stimulates mineralization. The optimum for ammonification generally falls between 50 and 75 percent of the water-holding capacity of the soil (52).

Alexander (3) points out that mineralization is influenced by the pH of the environment. All other factors being equal, the production of inorganic nitrogen, - ammonium plus nitrate,- is greater in neutral than in acid soils. Acidification tends to depress but does not eliminate mineralization.

Temperature likewise affects the mineralization sequence, since each biochemical step is catalyzed by a

temperature-sensitive enzyme produced by microorganisms whose growth is in turn conditioned by temperature. Thus at $2^{o}C$, the microflora slowly mineralize soil organic complexes, but there is no increase in ammonium or nitrate when soil is frozen. Elevation of the temperature enhances the mobilization of nitrogen in proportion to the greater warmth (3).

Nitrogen immobilization is a consequence of the incorporation of ammonium and nitrate into proteins, nucleic acids and other organic complexes contained within microbial cells. The rate of immobilization is related to the availability of the organic molecule, very rapid with readily oxidized carbohydrates, moderate with less suitable materials and particularly slow with resistant tissue components such as lignin or well-rotted manure (3). Immobilization is also correlated with pH and soluble soil phosphate, results that are not unexpected because of the qualitative and quantitative effects of pH and phosphorus on the size and biochemical capacity of the microflora (60).

The termination of the reactions involved in organic nitrogen mineralization occurs at the point where ammonium is formed. This, the most reduced form of inorganic nitrogen, serves as the starting point for a process known as nitrification, the biological formation of nitrate or nitrite from compounds containing reduced nitrogen. Physical and chemical factors affect the rate of ammonium oxidation.

Chief among the environmental influences is acidity. In acid environments, nitrification proceeds slowly even in the presence of an adequate supply of substrate, and the responsible species are rare or totally absent at great acidities. An exact limiting pH cannot be ascertained since a variety of physico-chemical factors in soil will alter any specific boundaries (3). Wilson (56) notes that the acidity affects not only the transformation itself but also the microbial numbers, neutral to alkaline soils having the largest population.

The effect of temperature on nitrification in soil has been studied by many investigators (6,16,40). Recently, Sabey et al. (41) initiated a study to determine the influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. In soils incubated at field capacity, the maximum nitrification rates increased from immeasurably low values to as great as 900 ppm. per week in some soils and the delay periods decreased from about 32 weeks to less than 1 day, as temperatures increased from 0 to 25°C. Increase in initial population of nitrifying organisms caused decreases in delay periods but did not appreciably affect the maximum rate above 10°C.

In solution culture, temperature and the size of nitrifying populations do influence delay periods of nitrification (2). Aeration is another factor that affects the

nitrification rate. Nitrifying bacteria are autotrophes and obligate aerobes. Nevertheless, nitrifying activity is sharply curtailed only at very low partial pressures of oxygen (5). In soil systems, nitrification may proceed unhindered by increasing moisture content to levels near saturation (30). Aleem and Alexander (2) recently observed an increase in delay period of nitrification at excessive levels of aeration in solution culture.

Certain transformations of nitrogen lead to a net loss of the element from the soil through volatilization. The sequence of steps that results in gaseous loss is known as denitrification, the microbial reduction of nitrate and nitrite with the liberation of molecular nitrogen and, in some instances, nitrous oxide. Denitrification is not the sole means by which microorganisms reduce nitrate and nitrite. In the utilization of the two anions as nitrogen sources for growth, microorganisms reduce them to the ammonium level. Alexander (3) defines denitrification as essentially a respiratory mechanism in which nitrate replaces molecular oxygen, ie. a nitrate respiration. The utilization of nitrate as a nutrient source may be termed nitrate assimilation.

The rate of denitrification of the nitrate added to flooded fields is far more slow in soils low in carbon than in land rich in organic matter. The effectiveness of organic

nutrients in promoting denitrification in waterlogged soils is proportional to their availability (3). The addition of organic substances to well drained soils diminishes the nitrogen losses, the conserving action resulting from an immobilization of inorganic nitrogen (11,31).

Oxygen availability is another of the critical environmental determinants. Aeration affects the transformation in two apparently contrasting ways: on the one hand, denitrification proceeds only when the oxygen supply is insufficient to satisfy the microbiological demand; at the same time, oxygen is necessary for the formation of nitrite and nitrate, which are essential for denitrification. Decreasing the partial pressure of oxygen enhances the denitrification of added nitrate. In well-drained soils, nitrogen volatilization is related to the moisture content. Denitrification of added nitrate is appreciable at high water levels and in localities having improper drainage. The effect of water is attributed to its role in governing the diffusion of oxygen to sites of microbiological activity. (3)

Alexander (3) points out that the bacteria which bring about denitrification are sensitive to high hydrogen ion concentration. The population becomes large only above a pH of approximately 5.5. Denitrification is markedly affected by temperature. The transformation proceeds slowly at $2^{o}C$, but increasing the temperature enhances the rate of biological loss. The optimum for the reaction is at $25^{o}C$ and is

still rapid at elevated temperatures and will proceed to about 60 to 65°, but not at 70°C.

OBJECTIVES

Laboratory studies were conducted to investigate the effects of two fumigant chemicals, dichloropropene and chloropicrin, on microbial numbers and activities in organic soil amended with ammonium and nitrate in varying proportions and incubated under different controlled temperature regimens.

Specific objectives of the experiments were to:

1. Observe effects of rising temperature and NH_4 to NO_3[1] ratio on post-fumigation adjustments in respiratory activity and numbers of heterotrophes.
2. Observe effects of rising temperature and NH_4 to NO_3 ratio on post fumigation patterns of accumulation or disappearance of NH_4, NO_3 and total mineral nitrogen.
3. Test the hypothesis that adjustments in heterotrophic metabolism during periods of rising temperature contribute to delayed accumulation of nitrate in fumigated soil.

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[1] $NH_4=NH_4^+$, $NO_3=NO_3^-$. This convention is followed throughout the thesis.

EXPERIMENTAL PROCEDURES

Because of previous field and laboratory experiences associated with its use, dichloropropene was the chemical of primary concern. Chloropicrin was used as a reference chemical with general, non-selective sterilizing action. Lots of soil material treated with these two chemicals and a control lot from the same homogeneous organic soil source were amended with ammonium and nitrate in varying proportions. Amended soil systems were incubated for 94 days under three controlled temperature regimens. All samples were frozen for three days and equilibrated at $5^{o}C.$ for eight days prior to incubation. Two temperature regimens involved abrupt temperature increases at the beginning of incubation from the $5^{o}C.$ imposed prior to incubation. One of these involved a second abrupt temperature increase midway through the incubation. Under a third regimen, pre-incubation low temperature was maintained until the middle of the incubation period and then increased abruptly.

Effects of the three treatment variables on the general microbial population were characterized periodically by collecting CO_2 evolved and by enumeration of bacteria and fungi. Changes in level of ammonium, nitrate and total mineral nitrogen were followed.

Details of treatment and analytical procedures are described in the succeeding sections.

Fumigation and Preliminary Treatment of Soil

A bulk lot of Houghton muck (75 to 80 percent organic matter, pH 6.3) was collected from a recently irrigated area in the field in late June, 1962. Soil from the surface eight inches was taken, forced through a 4-mesh screen and thoroughly mixed. The moisture content of the soil was found to be 232 percent, dry soil basis. Previous experience in the same muck area indicates that this is approximately field capacity for this soil.

The bulk lot was divided into three parts. Two lots were fumigated with dichloropropene[2] or chloropicrin[3] at rates and for exposure periods recommended by the manufacturer for field application. A third lot was untreated.

Dichloropropene was used at the rate of 32 gallons per acre (0.53 ml. per Kg. dry soil). Chloropicrin was used at the rate of 70 gallons per acre (1.16 ml. per Kg. dry soil). The fumigants were dribbled on the surface of the soil in a drum mixer which was sealed while the soil was thoroughly mixed. The soils were then transferred to plastic bags and sealed for the appropriate exposure period (2 weeks for dichloropropene, 2 days for chloropicrin).

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[2] Telone, Dow Chemical Co. (90 to 95% 1, 3 dichloropropene).

[3] Picfume, Dow Chemical Co. (99% chloropicrin).

The addition of chloropicrin was delayed so that exposure periods for both chemicals terminated on the same date. This terminal exposure date is used as zero on the time scale for all subsequent observations.

All three lots of soil (control and fumigated with dichloropropene or chloropicrin) were kept in sealed plastic bags at 20°C. during the two-week period required for exposure to dichloropropene.

After exposure to the fumigants, the soils were placed in tall metal cylinders and leached with a constant 6-inch head of distilled water until no nitrate could be detected with diphenylamine in the leachates. This required four days.

After drainage by gravity had ceased, the soils were forced through a 4-mesh screen and spread out on large canvas sheets to aerate and partially dry for 24 hours. The soils were stirred periodically with a rake during the daylight hours. Moisture content after aeration ranged from 150 to 165 percent in the three soil lots. After aeration there were no detectable fumes of either fumigant in treated soil.

After thorough mixing, the soils were placed in sealed plastic bags. These were placed in a deep freeze (-10°C.) for three days, then equilibrated at 5°C. for eight days prior to incubation. This was done, in part, to simulate temperature experiences to which fall fumigated

soils may be subjected during the winter months. It was also necessary to minimize microbial activity during the time necessary to carry out chemical determinations and calculations on which mineral amendments were based, to add the amendments and to dispense soil samples for incubation.

Mineral Amendments

A major objective of the experiment was to determine to what extent disappearance of nitrate in fumigated soil might contribute to delayed accumulation of nitrate such as is commonly observed in the field. It was recognized that both dissimilatory and assimilatory reduction of nitrate might be involved. It was anticipated that the presence of other reducible ions, such as sulfate, might compete with nitrate as terminal electron acceptors in facultative metabolism leading to nitrate reduction. For this reason, ammonium was added as $(NH_4)_2HPO_4$, rather than as $(NH_4)_2SO_4$. Nitrate was added as KNO_3.

Ammonium and nitrate found in the soil after fumigation, leaching and aeration was augmented by salt additions to give 400 ppm. total mineral nitrogen and ratios of NH_4-N to NO_3-N equal to 9, 3, 1, and 1/3. The resulting variations in P and K were compensated by adding KH_2PO_4 or K_2HPO_4 in quantities calculated to give minimal variations in P, with both P and K in excess. The schedule of mineral amendments is presented in table 1.

Mineral amendments were sprayed on thin soil layers in water solutions of concentration and volume calculated to bring moisture content up to 230 percent, dry soil basis. Previous experience in the field had indicated that this was close to field capacity for this soil. It would represent the maximum moisture that could be taken up by the soil without serious loss of aggregate integrity. Beyond this point, it would be difficult to establish uniform aeration characteristics for the different treatments without increasing water to saturation levels. To promote nitrate reduction under conditions approaching those observed in the field, maximum moisture consistent with stable aggregation and uniform aeration properties appeared to be a desirable characteristic common to all experimental soil systems during subsequent incubation.

As noted earlier, the soil at the time of collection in the field had contained 232 percent moisture. However, it was found that the soil, -after fumigation, leaching and aeration, - could no longer absorb this amount of water without serious loss of aggregation. It was, therefore, necessary to force the amended soils again through a 4-mesh screen to reestablish aggregation. Additional drying in a stream of warm air was necessary before the aggregated soils could be homogenized satisfactorily by mixing. The amount of drying was gauged by the visual appearance and mixing

Table 1.--Description of mineral amendments added 12 days after fumigation, 7 days after leaching and areation.

Fumigation treatment	N treatment	Ratio NH_4/NO_3	NH_4-N		NO_3-N		P	K	Moisture content/
			Found*	Added**	Found*	Added**	Added**	Added**	
			ppm	ppm	ppm	ppm	ppm	ppm	%
None (F_1)	N_1	9	11	349	33	7	835	1050	182
	N_2	3	11	289	33	67	835	1050	194
	N_3	1	11	189	33	167	835	1050	178
	N_4	1/3	11	89	33	267	835	1550	186
Chloropicrin (F_2)	N_1	9	48	312	0	40	970	1215	190
	N_2	3	48	252	0	100	970	1215	178
	N_3	1	48	152	0	200	970	1350	190
	N_4	1/3	48	52	0	300	970	1910	183
Dichloropropene (F_3)	N_1	9	42	318	2	38	895	1125	186
	N_2	3	42	258	2	98	895	1125	178
	N_3	1	42	158	2	198	895	1240	192
	N_4	1/3	42	58	2	298	895	1795	182

* Found after leaching and aeration (5 days after fumigation).

** Nitrogen added as $(NH_4)_2HPO_4$ or KNO_3, excess P and K as KH_2PO_4 or K_2HPO_4.

/ Final moisture content, oven dry soil basis.

properties of the aggregates. The moisture contents of amended soils after thorough mixing are given in the last column of table 1.

It is not known to what extent water holding capacity of the soils was reduced by losses of soluble dry matter during leaching or by irreversible dehydration during aeration. The final moisture content of amended soils shown in table 1 is certainly high on the scale of available moisture, but it is probably less than field capacity. The variations between treatments are relatively minor. However, at these high moisture levels, such relatively small variations may have influenced oxygen diffusion to sites of microbial activity within aggregates or in thicker moisture films associated with meniscuses around points of contact between aggregates (9). No direct relationships between moisture content and observed indices of microbial activity could be observed. Nevertheless, variations in moisture, as well as in salt concentrations apparent in table 1, may have contributed to variation in data taken during subsequent incubation. No consideration was given to pH buffer effects of the varying proportions of $HPO_4^=$ to $H_2PO_4^-$ used. Phosphate was added in total amounts equivalent to 0.01 molar in the soil solution. The proportions of $HPO_4^=$ to $H_2PO_4^-$ used with the two higher NH_4/NO_3 ratios (treatments N_1 and N_2) would have tended to raise pH above the initial 6.3. With the two lower NH_4/NO_3 ratios (treatments N_3 and N_4), the compensating salt was principally

KH_2PO_4, which would have tended to buffer at a pH lower than the initial 6.3 (50). There was evidence that initial buffer effects may have influenced early transformations of ammonium and nitrate. The extent to which pH was altered by mineral amendments was not determined.

Conditions of Incubation

After moisture adjustment and mixing, the twelve lots of soil amended as shown in table 1 were returned to the 5°C. storage temperature for the balance of the 8-day equilibration period (3 to 4 days). During this time, weighed aliquots containing 10 g. oven dry soil were dispensed in 60 ml. plastic cups. Fifteen cups of soil were placed in each of six gallon jars for each treatment. Thus, there were 72 jars in all, each containing the equivalent of 150g. oven dry soil. The jars were sealed and maintained at 5°C. for the remainder of the equilibration period.

On the sixteenth day after the end of the fumigation exposure period, duplicate jars of each treatment were placed in each of three constant temperature rooms maintained at 5°, 20° and 30°C., respectively. The jars were connected to the aspiration apparatus described by Kirkwood (25). Except for periods during which CO_2 was collected (see next section), the jars were aerated for 30 minutes every fourth day using water-saturated air purified by passing through NaOH and H_2SO_4 to remove CO_2 and NH_3.

On the 51st. day after fumigation, the temperature in the 5° room (T_1) was raised to 20°C., that in the 20° room (T_2), to 30°C. The temperature in the 30° room (T_3) was not changed and was held constant from the 16th. through the 110th. day after the end of the fumigation exposure period.

No attempt was made to determine losses of dry matter or moisture during the incubation period. Estimates based on carbon loss suggest that up to 20% of the initial dry weight may have disappeared due to organic matter decomposition where the temperature was maintained at 30°C. over the 94-day period of incubation. Water loss was minimized by discontinuous aeration (4 hours each 4 days, at most) and by saturating the incoming air with water in two stages. This was done by bubbling air through a 12-inch column of water at ambient temperature before entering the controlled temperature room, and again through a 12-inch column of water at incubation temperature inside the room. Moisture losses may have tended to parallel dry matter losses. Nevertheless, at the two higher temperatures, soils were sensibly drier at the end of incubation than at the beginning.

Respiration Measurements

During major portions of the incubation, the general level of microbial activity was characterized by measurements of CO_2 evolution. Carbon dioxide formed during a 4-day period was displaced by passing water-saturated, CO_2-free

and NH_3-free air through the jars for four hours. Displaced CO_2 was collected in NaOH, after passing first through KI and Ag_2SO_4 to remove volatile chlorine or chlorides (4).

Six-inch (100 ml) plastic centrifuge tubes containing 2½ inches of fine glass beads were used for collecting CO_2. Appropriate quantities of N/2 NaOH were measured into the tubes and CO_2-free distilled water added to cover the glass beads. Effluent air from the incubation jars was introduced into the bottom of the tube through a pin hole in a flexible plastic tip which could be inserted through the column of glass beads. Unspent NaOH was titrated in the same tubes, using a similar arrangement for bubbling a vigorous stream of CO_2-free air through the column of beads for agitation and mixing. Excess $BaCl_2$ was added to remove carbonate for titration with standard acid to the phenolphthalein end point. Final standardization of NaOH was based on titration of a blank tube of NaOH aspirated simultaneously from a common manifold with each group of six incubation jars.

Carbon dioxide production was calculated from the difference in acid required by blank and sample tubes. Respiration rates were recorded as mg. C per 100 g. oven dry soil per day for the median day of each 4-day collection period. Ten-gram soil samples sacrificedfrom time to time for plate counts and mineral N determination were accounted for in these calculations. No allowance was made for losses

in soil weight which occurred by decomposition of organic matter during incubation.

Microbial Counts

Periodic plate counts for bacteria and fungi were facilitated by the fact that known quantities of soil with known moisture content had been weighed prior to incubation and dispensed in plastic cups. Initial 1:10 dilutions were made in wide-mouth, pint freezer jars containing a volume of sterile water calculated to allow for the water contained in the soil sample as determined prior to incubation. The use of wide mouth jars made it possible to transfer a plastic cup and its contained soil aliquot directly from the incubation jar into the initial water blank contained in the freezer jar. Subsequent dilutions were made in standard milk dilution bottles.

Thornton's standardized medium (49) was used for bacterial counts, no distinction being made in counting between bacterial and streptomycete colonies. Martin's rose bengal medium with streptomycin (33) was used for fungi. Duplicate plates were poured for each of the following dilutions: 1 to 10^4, 10^5 and 10^6 for bacteria, and 1 to 10^3, 10^4 and 10^5 for fungi. Plates were incubated in the dark for 5 days at room temperature (22° to 25°C.). Colony counts were recorded for plates containing 20 to 300 bacterial colonies or 10 to 100 fungal colonies.

Stastical analyses were performed on the log transformations, and geometric, rather than arithmetic, means are reported (13). Results are reported as numbers per gram of oven dry soil initially present, no allowance being made for losses of dry matter or moisture during incubation.

Nitrogen Determinations

Periodic extractions for ammonium and nitrate were made in a manner similar to the initial 1:10 dilutions for microbial counts. Plastic cups and their contained soil aliquots were transferred directly to pint freezer jars containing 100 ml. of $\underline{N}$ K_2SO_4 in N/10 H_2SO_4. These were placed on a rotary shaker for 30 minutes. Ammonium and nitrate were determined on suitable aliquots of the filtered extract by distillation at room temperature in Conway microdiffusion cells, as described by Bremner and Shaw (10). The formulation of titanous sulfate distributed by the British Drug Houses, Ltd., and recommended by the above authors was used for reduction of nitrate.

Ammonium, nitrate and total mineral nitrogen (NH_4+NO_3) were calculated to ppm. N, oven dry soil basis, allowing for dilution by the water initially contained. No allowance was made for losses of dry matter or moisture during incubation.

Statistical Treatment

Only three controlled temperature rooms were used. Consequently, there was no replication of temperature

treatments. The three rooms were side by side, and it was suspected that systematic temperature variations might arise within a given room by conductance from adjacent rooms at different temperatures. For this reason, duplicate jars were assigned to random positions, one on each side of each room. Data for each room was analyzed separately in accordance with a randomized block design. Data for the three temperature rooms were then combined as locations, as described by Snedecor (44). Multiple range and multiple F tests as described by Duncan (15) were used to test for the significance of differences between observed means for various treatment combinations.

Significant variations in microbial numbers and in the different forms of nitrogen were associated with blocks. The precaution taken to associate within-room temperature differentials with replications was, therefore, justified. Within-room relationships to inferred temperature gradients were consistent with those observed between rooms during the first half of the incubation period. After temperatures were raised in two rooms at mid-incubation, significant variations associated with replications were less frequent, and trends with temperature were occasionally in a direction opposed to those observed between rooms.

RESULTS

Respiratory Activity and Microbial Numbers

Main effects of fumigants

Main effects of fumigation treatments on CO_2 evolution and microbial numbers are shown in table 2. When interactions with temperature and nitrogen treatment were ignored, neither chloropicrin nor dichloropropene influenced the respiration rate. Data for 0 days show that, during the exposure period, dichloropropene stimulated both bacterial and fungal numbers, whereas, chloropicrin depressed them both. During subsequent incubation, numbers of bacteria increased sharply in both fumigated soils but declined during the latter half of the incubation period. The population of bacteria in fumigated soil was higher than that in check soil throughout the incubation. There was some recovery of fungal numbers, during incubation in soil treated with chloropicrin, but numbers remained lower than in the controls or in soil treated with dichloropropene.

Main effects of nitrogen treatments

CO_2 evolution was not affected by any of the nitrogen treatments, when interactions with temperature and fumigation were ignored (table 3). There were no consistent main effects on microbial numbers over the major portion of the

incubation period. However there was a tendency for bacterial numbers to be increased, fungal numbers to be decreased, by increasing nitrate level, at the beginning of the incubation. These relationships with initial nitrate level were reversed at the end of the incubation period. Both at the beginning and at the end of incubation, fungal numbers tended to be inversely related to bacterial numbers. This is a characteristic competitive relationship between these two groups (3).

Relationships with temperature

Respiration rates and microbial numbers as related to temperature are shown in table 4. Significant differences in respiration rates for the three different temperatures were expressed. The highest rate was noted in the 30°C room at the beginning of the incubation, but the rate declined during subsequent incubation at this temperature. Low respiration rates were observed at 5° and 20°C. prior to the temperature changes on the 51st. day. Immediately after the temperature in the T_1 room was raised to 20°C. and that in the T_2 room was raised to 30°C., rates were quantitatively similar to those observed earlier at the same temperatures in the T_2 and T_3 rooms. During subsequent incubation, rates declined from these new levels. Bacterial numbers increased dramatically during incubation at low temperatures, whereas numbers of fungi were depressed at the lowest temperature and increased at the two higher temperatures. In both cases,

Table 2.--Respiratory activity and microbial numbers as related to fumigation treatment.

Fumigant	Days after fumigation*							
	0	35-47	49	58-70	75	66-78	80-92	100
				Respiration rate, mg.C/100g./day				
None (F_1)		5.8 a		8.5 a		7.8 a	6.8 a	
Chloropicrin (F_2)		5.9 a		8.3 a		7.4 a	6.1 a	
Dichloropropene (F_3)		5.5 a		7.9 a		7.0 a	6.1 a	
				Bacteria x 10^6				
None (F_1)	5.3 b		4.9 b		5.1 c			5.3 b
Chloropicrin (F_2)	2.5 c		20.9 a		11.0 b			9.7 a
Dichloropropene (F_3)	9.7 a		23.1 a		14.7 a			8.4 a
				Fungi x 10^4				
None (F_1)	4.6 b		8.3 b		9.3 ab			10.5 a
Chloropicrin (F_2)	0.0 c		5.1 c		7.7 b			5.9 b
Dichloropropene (F_3)	13.4 a		12.3 a		11.1 a			11.2 a

* Two days' exposure to chloropicrin, 2 weeks' exposure to dichloropropene.

a, b, c Ranges of equivalence. Within each vertical group of three, means accompanied by the same letter are not significantly different at 5 percent.

Table 3.--Respiratory activity and microbial numbers as related to nitrogen treatments.

Nitrogen treatment	NH_4/NO_3	Days after fumigation							
		0	35-47	49	58-70	75	66-78	80-92	100
	*	Respiration rate, mg.C/100g./day							
N_1	9		5.5 a		8.0 a		7.2 a	6.7 a	
N_2	3		5.7 a		8.1 a		7.3 a	6.1 a	
N_3	1		6.0 a		8.9 a		7.9 a	6.4 a	
N_4	1/3		5.6 a		7.9 a		7.2 a	6.4 a	
		Bacteria x 10^6							
N_1	9	5.8 a		11.7 a		9.7 a			8.5 a
N_2	3	5.8 a		12.2 a		11.0 a			7.1 ab
N_3	1	5.8 a		13.8 a		8.6 a			8.2 a
N_4	1/3	5.8 a		16.2 a		8.4 a			6.5 b
		Fungi x 10^4							
N_1	9	6.7 a		8.7 ab		12.3 a			5.0 b
N_2	3	6.7 a		10.6 a		8.2 ab			5.6 b
N_3	1	6.7 a		7.4 bc		7.5 b			6.7 b
N_4	1/3	6.7 a		6.1 c		9.6 ab			8.8 a

* Nitrogen applied 12 days after fumigation to bring NH_4 plus NO_3 in soil to 400 ppm N in proportions shown.

a, b, c, Ranges of equivalence. Within each vertical group of four, means accompanied by the same letter are not significantly different at 5 percent.

maximum, or near maximum, numbers were attained by the 33rd. day of incubation (49 days after fumigation).

Prior to the temperature increases on the 51st. day, large numbers of bacteria at the two lower temperatures were associated with low rates of CO_2 loss. Data in table 5 show that large initial increases in numbers of bacteria occurred only in fumigated soil and that these increases were inversely related to temperature. Respiration rates (not shown) were not affected by fumigation (ignoring nitrogen treatment) at any temperature. Thus it appears that decreasing temperature promoted increasingly efficient assimilation of carbon into microbial tissues and that this effect was strikingly expressed on the bacterial components of the recovery population in fumigated soil.

As regards their effects on bacteria, both fumigants behaved similarly under all three temperature regimens throughout the incubation period. There were significant differences, however, in their effects on fungi. Chloropicrin appeared to be more injurious to fungi than dichloropropene. This difference was apparent initially at the two lower temperatures, and, at the end of incubation, under all three temperature regimens.

No clear-cut first-order interactions between temperature and nitrogen treatments were observed (table 6). The tendency noted earlier (table 3) for initial increases

Table 4.--Respiratory activity and microbial numbers as related to temperature.

Temperature on 16th day	Days after fumigation								
	0	35-47	49	51	58-70	75	66-78	80-92	100
*	Respiration rate, mg.C/100g./day								
5°C.(T_1)		1.3 c		‡	4.3 c		4.5 c	3.3 c	
20°C.(T_2)		5.1 b		‡‡	11.2 a		9.6 a	8.7 a	
30°C.(T_3)		10.7 a		‡ ‡	9.3 b		8.1 b	7.2 b	
	Bacteria x 10^6								
5°C.(T_1)	5.8 a		25.2 a	‡		11.5 a			9.8 a
20°C.(T_2)	5.8 a		15.8 b	‡‡		10.0 a			7.7 b
30°C.(T_3)	5.8 a		6.0 c	‡ ‡		7.1 b			6.0 c
	Fungi x 10^4								
5°C.(T_1)	6.7 a		2.5 c	‡		4.4 b			6.3 b
20°C.(T_2)	6.7 a		16.6 a	‡‡		11.6 a			9.8 a
30°C.(T_3)	6.7 a		12.5 b	‡ ‡		15.4 a			11.3 a

* After 3 days at -10° and 8 days at 5°C.

‡ Temperature raised to 20°C.

‡‡ Temperature raised to 30°C.

‡ ‡ Temperature unchanged.

a, b, c Ranges of equivalence. Within each vertical group of three, means accompanied by the same letter are not significantly different at 5 percent.

Table 5.--Microbial numbers as related to fumigation treatment and temperature.

Temperature regimen	Fumigant	Days after fumigation					
		49	75	100	49	75	100
*	**	Bacteria x 10^6			Fungi x 10^4		
T_1	F_1	7.29 e	4.11 d	6.88 cd	3.86 c	3.84 d	9.04 ab
	F_2	51.5 a	17.3 a	11.6 a	0.85 d	4.13 d	3.78 c
	F_3	42.5 ab	21.4 a	9.99 ab	4.90 c	5.39 cd	7.36 b
T_2	F_1	7.88 e	4.67 c	4.41 f	12.9 b	11.1 ab	10.6 ab
	F_2	21.1 cd	9.93 b	10.7 a	11.8 b	8.81 bc	7.26 b
	F_3	23.8 bc	21.6 a	9.61 abc	29.7 a	15.9 ab	12.1 ab
T_3	F_1	2.06 f	6.83 bc	4.81 ef	11.6 b	18.8 a	12.2 ab
	F_2	8.43 e	7.65 b	7.31 bcd	13.0 b	12.3 ab	7.59 b
	F_3	12.2 de	6.89 bc	6.28 de	12.8 b	15.8 ab	15.7 a

* cf. Table 4.

** cf. Table 2.

a, b, ...f Ranges of equivalence. Within a given column, means accompanied by the same letter are not significantly different at 5 percent.

in bacterial numbers to be proportional to nitrate level was most clearly expressed at the lowest temperature. At the two higher temperatures, fungi tended to reach maximum numbers at intermediate levels of nitrate, with significant reductions at the highest level (N_4). A similar tendency for microbial numbers or activity at intermediate levels of nitrate to differ from those at higher or lower levels appears frequently when second order interactions between fumigation, nitrogen treatment and temperature are considered in the following sections.

Interactions of fumigants and nitrogen treatments at low to intermediate temperatures (T_1)

Numerous investigators have observed that peaks in respiratory activity in soils tend to occur 2 to 3 weeks prior to corresponding peaks in microbial numbers (20, 46, 51). For this reason, CO_2 was collected for 2 to 3-week periods preceding each sampling for plate counts during the course of incubation.

Under the T_1 temperature regimen, major differences associated with nitrogen treatment were observed between the two lower initial nitrate levels (N_1 and N_2), on the one hand, and the two higher levels (N_3 and N_4), on the other. The corresponding means are presented graphically in figure 1.

Nothing can be said about changes which may have occurred during the 2-week period after nitrogen was added

Table 6.--Microbial numbers as related to temperature and nitrogen treatment.

Temperature regimen	Nitrogen treatment	Days after fumigation					
		49	75	100	49	75	100
*	**	Bacteria x 10^6			Fungi x 10^4		
T_1	N_1	17.0 abc	13.4 ab	9.60 abc	2.48 de	6.19 cde	4.96 e
	N_2	23.7 abc	11.7 abc	8.55 abcd	3.61 d	3.84 e	5.55 de
	N_3	32.9 a	9.18 abcd	10.4 ab	1.90 e	3.98 de	6.55 cde
	N_4	30.3 ab	11.5 abc	8.64 abcd	2.38 de	3.99 de	8.81 bcde
T_2	N_1	18.3 abc	9.96 abcd	10.5 a	16.7 ab	13.9 ab	7.75 cde
	N_2	13.0 cd	16.1 a	7.15 abcd	19.7 a	13.1 abc	17.2 ab
	N_3	14.8 bcd	10.1 abcd	6.95 bcde	20.0 a	8.10 bcd	6.49 cde
	N_4	17.8 abc	6.21 d	6.69 cde	11.4 bc	12.2 abc	10.5 abcd
T_3	N_1	5.10 e	6.86 cd	6.08 de	15.8 ab	21.5 a	9.53 bcde
	N_2	5.85 e	7.10 cd	5.91 de	17.0 ab	11.0 abc	12.1 abc
	N_3	5.39 e	6.42 d	7.69 abcd	10.6 bc	13.0 abc	6.96 cde
	N_4	7.85 de	8.21 bcd	4.82 e	8.44 c	18.5 a	20.4 a

* cf. Table 4.
** cf. Table 3.

a, b,....e Ranges of equivalence. Within a given column, means accompanied by the same letter are not significantly different at 5 percent.

and the first collection of CO_2 30 days after fumigation. Nonetheless, there is strong evidence that patterns of microbial succession were influenced by both fumigation and the proportions of NH_4 and NO_3 added 16 days after fumigation.

Between the 30th. and 45th. days, marked adjustments in respiration rate occurred. These adjustments were distinctly different at higher nitrate levels (N_3 and N_4) in both fumigated soils than those in unfumigated soil or at lower nitrate levels in fumigated soil. After the temperature change, maximal increases in CO_2 evolution were associated with the N_3 and N_4 treatments, and these occurred earlier in fumigated than in unfumigated soil.

Large increases in numbers of bacteria on the 49th. day occurred only in fumigated soils. These increases were greater with the N_3 and N_4 treatments and reflect the earlier adjustments in CO_2 evolution observed in fumigated soils.

The association of larger numbers of bacteria in the initial recovery population, and of higher rates of loss of CO_2, with higher nitrate additions in fumigated soils suggests that fumigation promoted physiological types capable of utilizing nitrate for growth and for electron exchange. Fungi in the recovery population appeared to be suppressed competitively by these bacterial types on the 49th. day. At the end of incubation, however, maximum recovery of fungi

Figure 1.--Patterns of microbial succession and respiratory activity at low to intermediate temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 51st. day after fumigation. At this time the temperature was raised to 20°C. On the 12th. day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4).

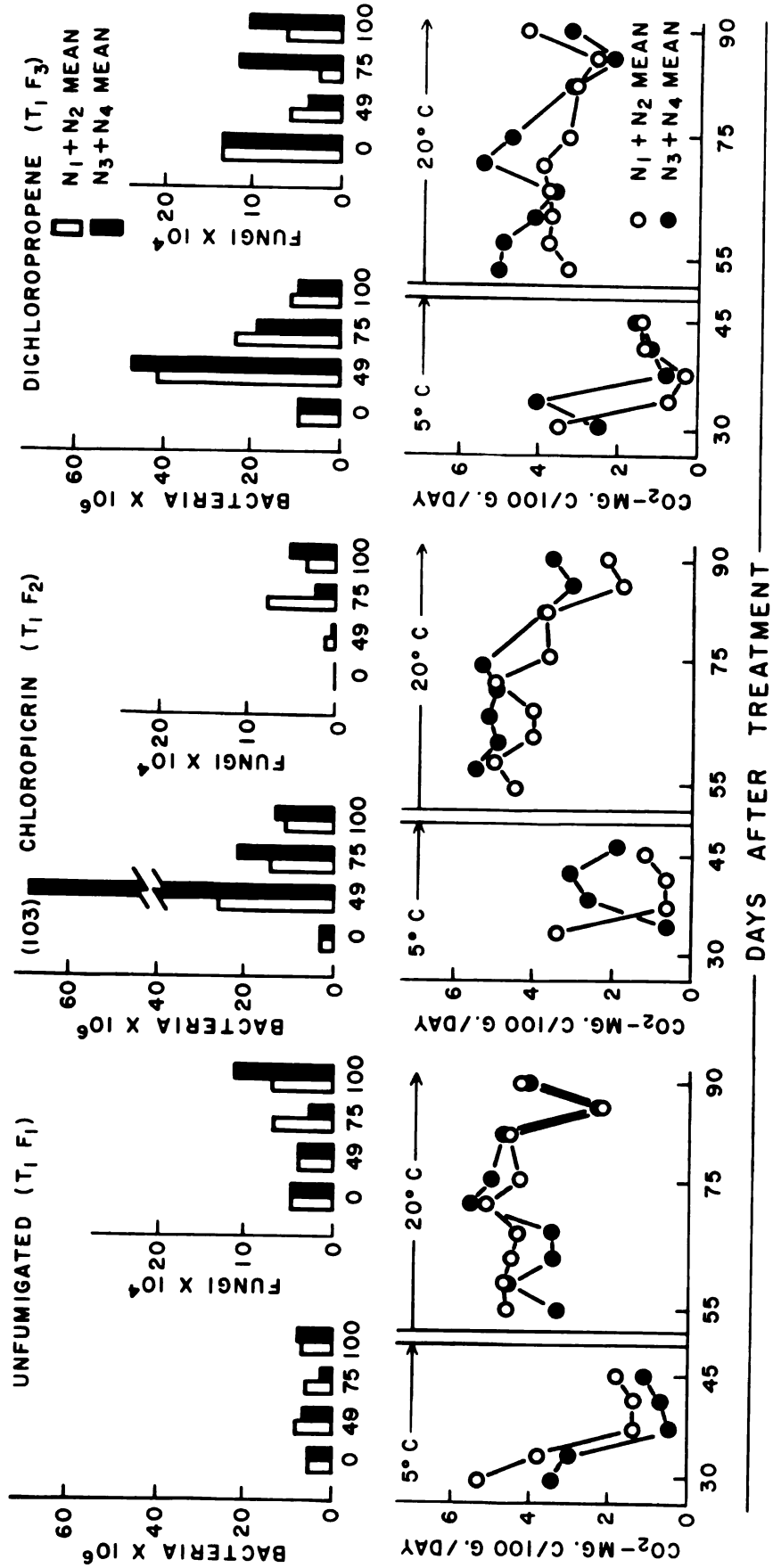
UNFUMIGATED (T1 F1)
CHLOROPICRIN (T1 F2)
DICHLOROPROPENE (T1 F3)
N1+N2 MEAN
N3+N4 MEAN
BACTERIA X 10^6
FUNGI X 10^4
(103)
0 49 75 100
0 20 40 60
0 10 20
5° C
20° C
CO2-MG. C/100 G./DAY
0 2 4 6
30 45 55 75 90
DAYS AFTER TREATMENT

FIGURE 1

occurred with the N_3 and N_4 treatments. This suggests that primary bacterial successions were consummated more rapidly at higher levels of added nitrate.

Interactions of fumigants and nitrogen treatments at intermediate to high temperatures (T_2)

Microbial numbers under the T_2 temperature regimen are shown graphically in figure 2. Again, the data are lacking in short term detail. However, it is apparent that patterns of bacterial succession were markedly different with different N treatments in fumigated soil, whereas without fumigation numbers of bacteria were essentially unrelated to N treatments at all samplings. Prior to the temperature change, numbers of fungi also varied more with N treatment in fumigated than in unfumigated soil.

Relationships in figure 2 between microbial numbers and the ratio of NH_4 to NO_3 applied at the beginning of incubation are neither clear nor direct. It must be recognized that several population successions may have preceded the counts made 49 days after fumigation (33 days after nitrogen additions). The fact that large variations associated with N treatment were still apparent at this time and in the first sampling after the temperature change suggests that recovery populations in fumigated soil had been profoundly altered earlier by additions of NH_4 and NO_3 in varying proportion. Primary populations developing on principally ammonium

Figure 2.--Patterns of microbial succession at intermediate to high temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 16th. day after fumigation. At this time the temperature was raised to 20°C. The temperature was raised again to 30°C. on the 51st. day. On the 12th. day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4).

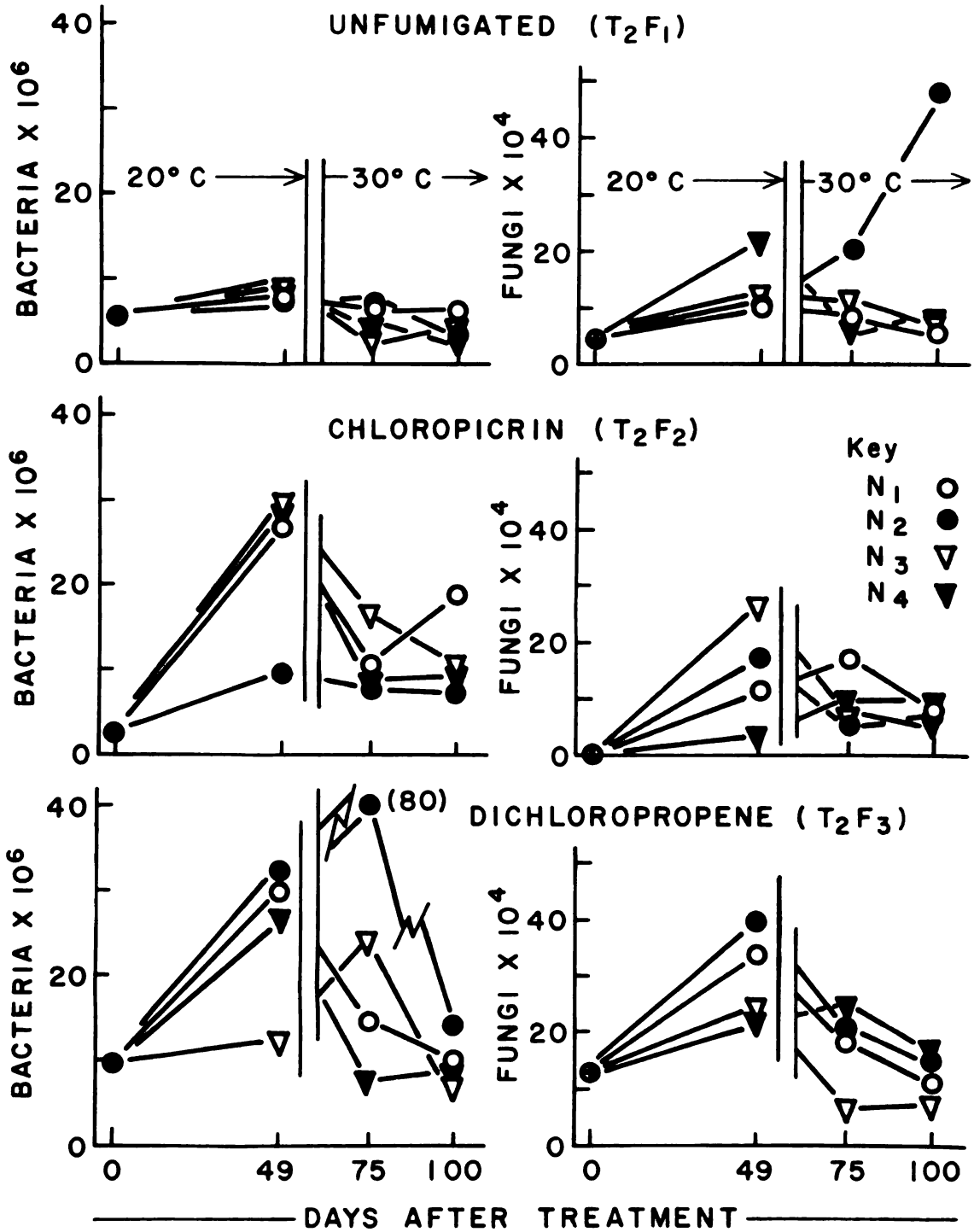
UNFUMIGATED (T_2F_1)
CHLOROPICRIN (T_2F_2)
DICHLOROPROPENE (T_2F_3)
BACTERIA X 10^6
FUNGI X 10^4
20° C
30° C
Key
N_1
N_2
N_3
N_4
(80)
0
20
40
0
49
75
100
DAYS AFTER TREATMENT

FIGURE 2

nutrition (N_1) would likely have been qualitatively as well as quantitatively different from those in the presence of increasing proportions of nitrate.

Respiration rates under the T_2 regimen are shown in figure 3. In unfumigated soil after the temperature change, maximum rates were obtained with the highest additions of NH_4 (N_1) and the lowest rates with the lowest NH_4 additions (N_4). By contrast, respiration rates associated with the N_1 treatment were depressed, - relative to unfumigated soil, - in soil treated with chloropicrin or dichloropropene. Maximum rates with chloropicrin were associated with the N_3 treatment. With dichloropropene, maximum rates were associated with the N_3 and N_4 treatments, although inversions occurred after 75 days. These relationships are consistent with the view that fumigation promoted a type of metabolism with a relatively higher dependence upon NO_3 rather than NH_4.

Interactions of fumigants and nitrogen treatments at constant high temperatures (T_3)

Again under the T_3 temperature regimen, effects of N treatment on bacterial numbers were observed only in fumigated soils (figure 4). With chloropicrin on the 49th. day, there was a strikingly direct relationship between numbers of bacteria and the level of nitrate added on the 16th. day. With dichloropropene, increased numbers of bacteria appeared at this time only at the lowest nitrate level (N_1). However,

numbers of fungi at this time were inversely related to nitrate additions, which suggests that bacterial competition prior to the 49th. day may have been greater at the higher levels of nitrate. With both fumigants, fungi increased markedly after 49 days with the N_3 and/or N_4 treatments. This again suggests that primary bacterial successions were consummated earlier in the presence of higher nitrate additions. In unfumigated soils under both T_2 (figure 2) and T_3 (figure 4) regimens, terminal increases in fungi were associated with the N_2 treatment only, indicating that NH_4/NO_3 ratios may have influenced microbial succession in normal soil populations also.

Interaction effects of fumigation and N treatment on patterns of respiratory activity were even more pronounced under the T_3 regimen (figure 5) than under the T_2 temperature sequences (figure 3). Losses of CO_2 were markedly depressed by fumigation where nitrogen was added principally as NH_4 (N_1). In chloropicrin treated soil, rates prior to the 45th. day were directly related to nitrate additions, as were bacterial numbers on the 49th. day (figure 4). With dichloropropene, maximum respiratory rates were associated with intermediate nitrate additions (N_2 and N_3).

Inversions in relative respiratory activity for N_1 and N_3 occurred after the 75th day (figure 5). Changes in relative numbers of fungi for these two treatments (figure 4) indicate that corresponding major adjustments in the

Figure 3.--Patterns of respiratory activity at intermediate to high temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 16th. day after fumigation. At this time the temperature was raised to 20°C. The temperature was raised again to 30°C. on the 51st. day. On the 12th. day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4).

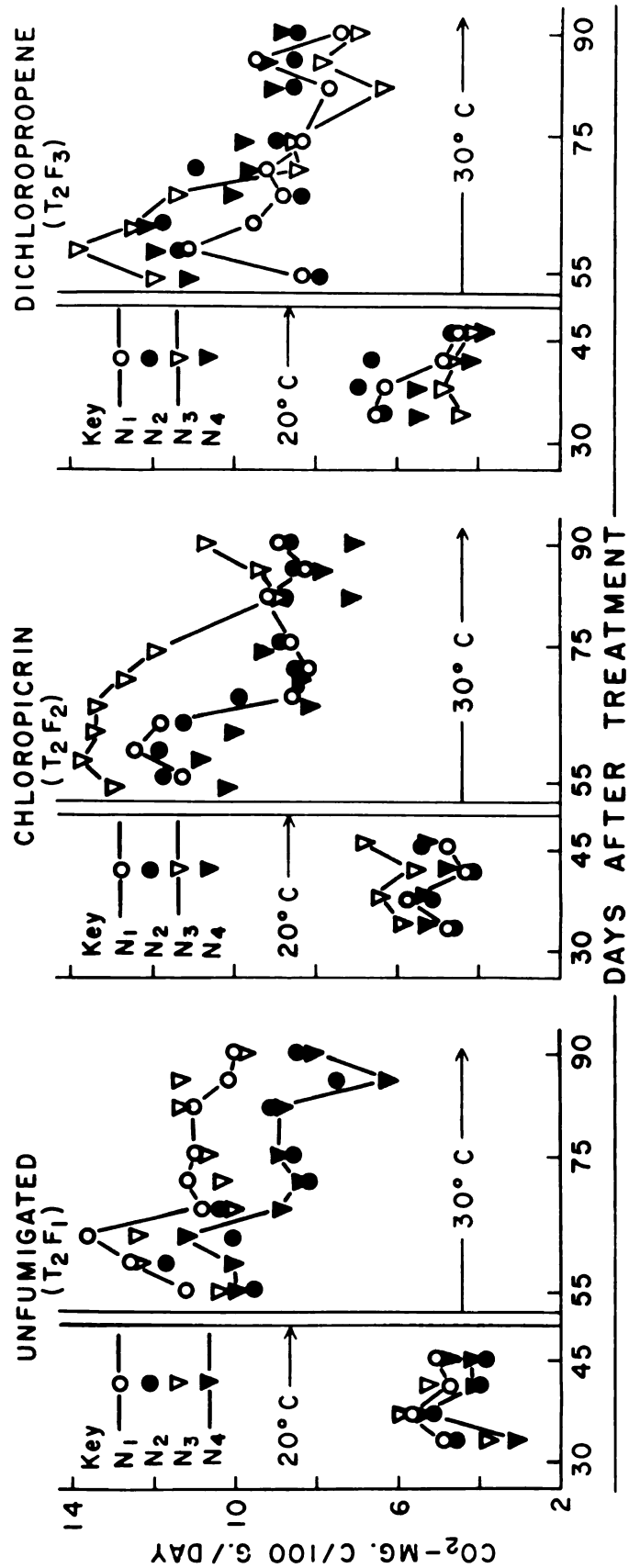
UNFUMIGATED
(T_2F_1)
CHLOROPICRIN
(T_2F_2)
DICHLOROPROPENE
(T_2F_3)
Key
N_1
N_2
N_3
N_4
20° C
30° C
CO_2-MG. C/100 G./DAY
14
10
6
2
30
45
55
75
90
DAYS AFTER TREATMENT

FIGURE 3

Figure 4.--Patterns of microbial succession at high temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 16th. day after fumigation. At this time the temperature was raised to 30°C. for the balance of the incubation period. On the 12th. day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4).

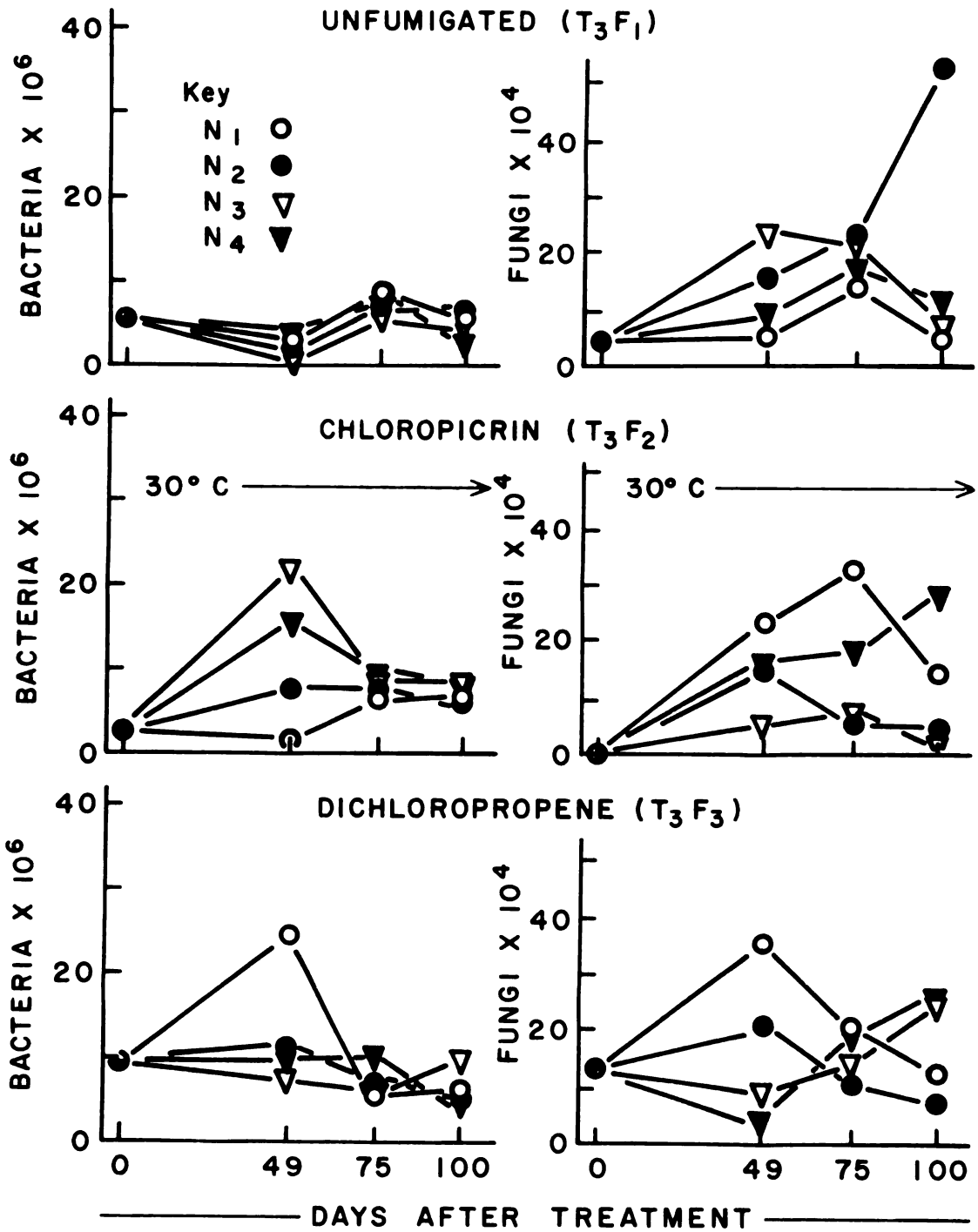
UNFUMIGATED (T_3F_1)
CHLOROPICRIN (T_3F_2)
DICHLOROPROPENE (T_3F_3)
Key
N_1
N_2
N_3
N_4
BACTERIA X 10^6
FUNGI X 10^4
30° C
0
20
40
0
49
75
100
DAYS AFTER TREATMENT

FIGURE 4

Figure 5.--Patterns of respiratory activity at high temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 16th. day after fumigation. At this time the temperature was raised to 30°C. for the balance of the incubation period. On the 12th. day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4).

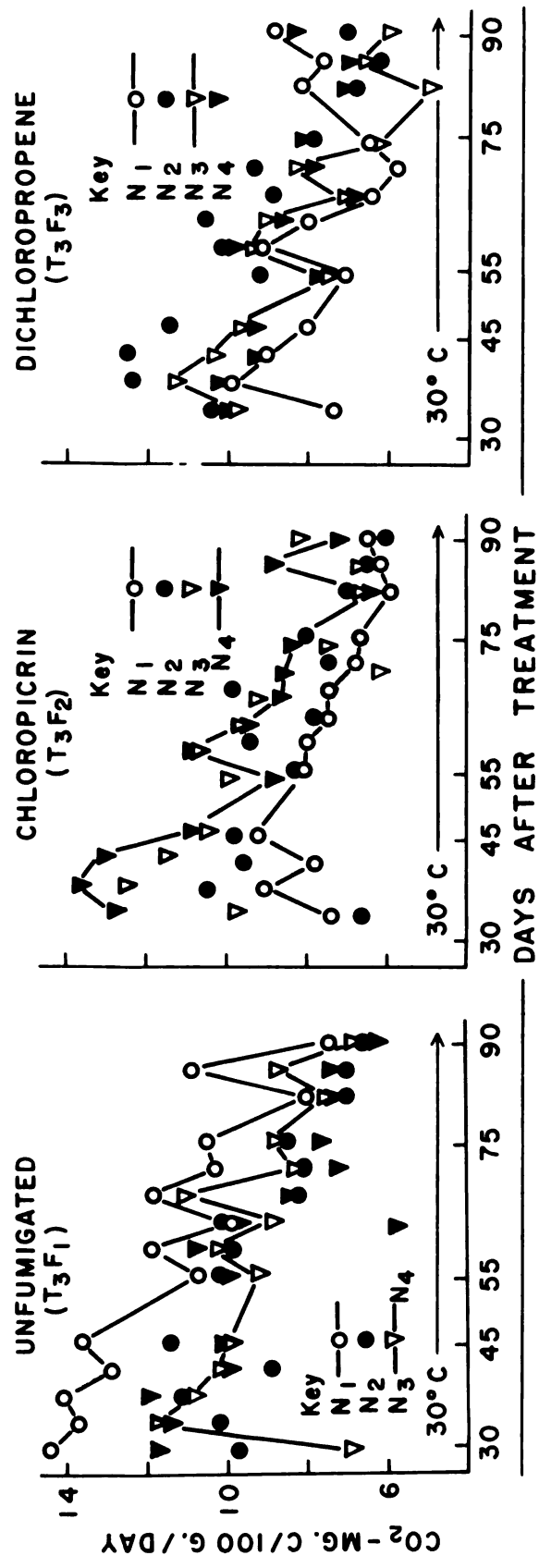
UNFUMIGATED
(T3F1)
CHLOROPICRIN
(T3F2)
DICHLOROPROPENE
(T3F3)
Key
N1
N2
N3
N4
30° C
30
45
55
75
90
14
10
6
CO2 - MG. C/100 G./DAY
DAYS AFTER TREATMENT

FIGURE 5

microbial population were occurring at this time.

Discussion

In evaluating microbial counts in the foregoing sections, it must be recognized that culture plates were incubated aerobically at 22° to 25°C. Obligate anaerobes would not have developed on the plates. Organisms with specific low temperature requirements would also have been discouraged. The bacterial medium supplied minerals, plus nitrogen as asparagine and as nitrate, but no other growth factors. Organisms with more fastidious requirements would not have been counted. It is impossible to say whether fungal numbers represent vegetative forms or viable spores. Nor can anything be said about short term fluctuations in numbers, or about effects on respiratory activity during the first two weeks of incubation.

Nevertheless, the observed second-order interaction effects on both numbers and losses of CO_2 consistently support the inference that recovery populations in fumigated soil were dominated by bacterial types with a marked preference for NO_3 rather than NH_4. This reflects an abnormal type of metabolism for soils. Normal soil populations are characterized by a high degree of preference for NH_4 over NO_3 (3, 21).

Utilization of nitrate by microorganisms, as well as by plants, involves nitrate reductase systems which have

been shown to develop adaptively in a number of bacterial and fungal species when grown on media supplying nitrate (2, 53). Under strict anaerobiosis, nitrate reduction by bacteria leads to gaseous denitrification products, principally N_2 and N_2O. With spore-formers, reduction to NH_4 may also occur. Such dissimilatory or respiratory nitrate reduction is accompanied by assimilation of part of the nitrogen from nitrate into cellular protein. Dissimilatory processes are increasingly inhibited, assimilation of nitrate is enhanced, by increasing oxygen supply. With full aeration, nitrate reduction leads only to protein synthesis.

Fumigation effects a partial pasteurization of the soil. The degree to which the soil fauna and flora are decimated depends upon the conditions of treatment. Spore-formers are most likely to survive initially, although the recovery of certain non-spore-forming bacteria is frequently rapid. Their numbers are greatly reduced initially, but, in the absence of normal competitors and antagonists, certain fast growing types quickly dominate the population, reaching numbers frequently several fold greater than the total population before treatment. Among such fast growers, fluorescent Pseudomonas species are frequently abundant in soils soon after incubation (34).

The ability to reduce nitrate and nitrite is an adaptive characteristic of many bacterial species, including pseudomonads and other fast growing non-spore-formers, as well as

numerous spore-formers (12). The adaptation is promoted by oxygen stress. The soil systems used in the present study involved large aggregates, up to 1/4 inch (6 mm.) in diameter. Moisture initially was high, although short of field capacity. Such a physical matrix would have provided an intimate association of anaerobic micro-habitats conducive to nitrate reduction with fully aerobic environments inhibitory to denitrification and favorable for assimilation of nitrate (9).

Under these conditions of partial anaerobiosis, the adaptation to nitrate utilization would have been promoted by increasing initial nitrate levels in both fumigated and unfumigated soil. However, the appropriate bacterial species would have encountered little competition in fumigated soil from more normal soil microflora. Thus bacterial responses to varying nitrate levels were observed only in fumigated soil, whereas fungal responses were observed in both. In both cases, however, fungal responses may have reflected competitive adjustments to changes in the bacterial flora. The fact that no effects of NH_4/NO_3 ratio on bacterial numbers were observed in unfumigated soil may have been due to failure of species with complex growth factor requirements to grow on the medium used.

Differential responses to initial nitrate level in fumigated soil were most clearly expressed by bacterial numbers at low temperature and by respiratory losses of CO_2 at high temperatures. This was undoubtedly due, in part, to more

efficient assimilation of carbon at low temperatures. However, microbial successions would also have occurred more rapidly with increasing temperature. At the higher temperatures, recovery populations of bacteria comparable to those observed on the 49th. day at low temperatures may have developed and exhausted appropriate energy materials earlier. The earlier recovery of fungi at high temperatures supports this view.

Differences in incubation behavior between the two fumigants reflect differences in initial kill. Initiating recovery populations were both more numerous and more heterogeneous immediately after treatment with dichloropropene than with chloropicrin. These initial differences influenced patterns of microbial succession throughout the incubation period. Notably fungi recovered more slowly in soil treated with chloropicrin, suggesting that this chemical may have been more effective than dichloropropene in killing fungal spores. Relatively large counts for fungi immediately after treatment with dichloropropene were likely due principally to surviving spores.

Nitrogen Transformations

Changes in NH_4 and NO_3 prior to incubation

Early changes in NH_4 and NO_3 prior to controlled temperature incubation are shown by data in table 7. No determinations were made before exposure of soils to fumigants. Immediately after 2 days' exposure to chloropicrin and 2 weeks' exposure to dichloropropene, ammonium levels were higher, nitrate was lower, than in the control. Little change occurred in NH_4 during subsequent leaching and aeration. Leaching was continued for 4 days, at which time no traces of NO_3 were detected in the percolate. Nitrate, to the extent of 33 ppm., had accumulated during the subsequent 24-hour aeration period in control soil only.

Immediately after aeration, soils were frozen for 3 days and then held at 5°C. for 8 days, except for an 8-hour period during which nitrogen additions and moisture adjustments were made. Nitrogen additions were calculated on the basis of NH_4 and NO_3 found after leaching. Calculated amended totals are shown for the 12th. day after fumigation, when nitrogen additions were made. Comparison of these totals with those found 4 days later reveals that marked differential adjustments had occurred during freezing and low temperature storage imposed after leaching and aeration. Net mineralization of organic N had occurred in all soils, leading to accumulations of NO_3 in unfumigated soil and accumulations of both

Table 7.--Early changes in ammonium and nitrate prior to controlled temperature incubation.

N treatment	Fumigant	ppm. NH_4-N after fumigation (0 days)	ppm. NH_4-N after leaching (5 days) *	ppm. NH_4-N Amended to (12 days)	ppm. NH_4-N Found (16 days)	ppm. NO_3-N after fumigation (0 days)	ppm. NO_3-N after leaching (5 days) *	ppm. NO_3-N Amended to (12 days)	ppm. NO_3-N Found (16 days)
N_1	F_1	4	11 b	360	368 b	149	33 a	40	95 d
	F_2	34	48 a	360	405 a	85	0 b	40	110 d
	F_3	46	42 a	360	413 a	89	2 b	40	98 d
N_2	F_1	4	11 b	300	317 c	149	33 a	100	166 bcd
	F_2	34	48 a	300	350 bc	85	0 b	100	146 bcd
	F_3	46	42 a	300	338 bc	89	2 b	100	120 cd
N_3	F_1	4	11 b	200	224 e	149	33 a	200	207 bc
	F_2	34	48 a	200	258 d	85	0 b	200	178 bcd
	F_3	46	42 a	200	252 de	89	2 b	200	214 b
N_4	F_1	4	11 b	100	104 f	149	33 a	300	312 a
	F_2	34	48 a	100	132 f	85	0 b	300	347 a
	F_3	46	42 a	100	125 f	89	2 b	300	346 a

* After leaching 4 days to zero nitrate and aerating 1 day at 20° to 25°C.

a, b, ...f Ranges of equivalence. Within a given column, means accompanied by the same letter are not significantly different at 5 percent.

NH_4 and NO_3 in fumigated soils.

Applying standard errors associated with the determinations on the 16th. day, increases in NH_4 over calculated amended totals in fumigated soils would have been statistically significant, whereas none of the apparent increases in NO_3 would have been. It is of interest, however, that Kirkwood (25) observed similar increases in NO_3 during freezing or subsequent thawing of organic soil samples taken in early spring from field plots fumigated the previous fall and from unfumigated plots.

Main effects of fumigants

Soils were placed under differential temperature regimens on the 16th. day after fumigation. Main effects of fumigants, during subsequent incubation, on NH_4, NO_3 and total mineral N may be seen in table 8.

Conversion of NH_4 to NO_3 was retarded by both fumigants, maximally by chloropicrin.

Between the 16th. and 26th. days, there was a marked reduction in recovery of mineral N in all soils. This was due to the disappearance primarily of NH_4 in control soil and of NO_3 with chloropicrin. With dichloropropene, marked reductions in both NH_4 and NO_3 occurred during this initial 10-day period, and the mineral N total was more sharply reduced. During the balance of incubation, average values for NO_3 remained lower for dichloropropene than in the control,

Table 8.--Ammonium, nitrate and total mineral nitrogen during incubation, as related to fumigation treatment.

Fumigant	Days after fumigation*					
	16	26	41	57	69	94
			ppm. NH_4-N			
None (F_1)	253 b	69 c	28 c	2 c	9 b	2 b
Chloropicrin (F_2)	286 a	273 a	238 a	119 a	104 a	15 a
Dichloropropene (F_3)	282 a	230 b	79 b	72 b	11 b	1 b
			ppm. NO_3-N			
None (F_1)	195 a	325 a	396 a	477 a	522 a	582 a
Chloropicrin (F_2)	195 a	122 b	211 c	362 b	464 b	567 a
Dichloropropene (F_3)	195 a	133 b	325 b	381 b	512 a	543 b
			ppm. mineral N (NH_4+NO_3)			
None (F_1)	448 a	394 a	426 b	480 a	532 b	585 a
Chloropicrin (F_2)	481 a	395 a	449 a	481 a	568 a	583 a
Dichloropropene (F_3)	476 a	363 b	404 c	453 b	523 b	544 b

a, b, c Ranges of equivalence. Within each vertical group of three, means accompanied by the same letter are not significantly different at 5 percent.

* Two days' exposure to chloropicrin, 2 weeks' exposure to dichloropropene.

and mineral N means remained lower than for either control or chloropicrin.

The behavior with dichloropropene might suggest that this chemical may have retarded mineralization of organic N or may have promoted dissimilatory losses of NO_3. This was only apparent in the means, however, and derived from the fact that temporary periods of NO_3 disappearance with dichloropropene occurred at different times with different combinations of temperature and nitrogen treatment. Mineral N totals were significantly depressed at these times relative to control and chloropicrin treated soil but recovered in later samplings. It appeared likely that similar recoveries would have followed significant depressions encountered in the last sampling.

Main effects of nitrogen treatments

When effects of temperature and fumigation were averaged out, significant differences in NH_4 and NO_3 were related essentially to differences in amounts added (table 9). Declines in total mineral N during the initial 10-day period tended to be greater and subsequent recoveries less rapid at the lower levels of NO_3 addition. Totals at the end of incubation were essentially indentical for all N treatments.

Relationships with temperature

The rate of conversion of NH_4 to NO_3 increased with increasing temperature (table 10). Total mineral N declined

Table 9.--Ammonium, nitrate and total mineral nitrogen during incubation, as related to nitrogen treatment.

Nitrogen treatment	NH_4/NO_3	Days after fumigation treatment					
		16	26	41	57	69	94
	*			ppm. NH_4-N			
N_1	9	395 a	271 a	166 a	84 a	56 a	8 a
N_2	3	335 b	234 b	137 b	82 a	47 b	13 a
N_3	1	245 c	162 c	95 c	57 b	36 c	2 b
N_4	1/3	120 d	97 d	63 c	37 c	26 d	2 b
				ppm. NO_3-N			
N_1	9	101 c	105 d	258 d	384 b	471 b	555 a
N_2	3	144 c	146 c	284 c	385 b	504 a	558 a
N_3	1	199 b	232 b	322 b	404 b	504 a	575 a
N_4	1/3	335 a	290 a	380 a	453 a	519 a	570 a
				ppm. mineral N (NH_4+NO_3)			
N_1	9	496 a	376 a	424 ab	468 ab	528 a	562 a
N_2	3	478 a	380 a	421 ab	466 ab	550 a	570 a
N_3	1	444 a	394 a	417 b	461 b	540 a	576 a
N_4	1/3	455 a	387 a	443 a	490 a	545 a	572 a

* Nitrogen applied 12 days after fumigation to bring NH_4 plus NO_3 in soil to 400 ppm. N in proportions shown.

a, b, c, d Ranges of equivalence. Within each vertical group of four, means accompanied by the same letter are not significantly different at 5 percent.

Table 10.--Ammonium, nitrate and total mineral nitrogen during incubation, as related to temperature.

Temperature imposed on 16th day	Days after fumigation treatment						
	16	26	41	51	57	69	94
*	ppm. NH_4-N						
5°C. (T_1)	274 a	227 a	183 a	↓	159 a	105 a	14 a
20°C. (T_2)	274 a	170 b	91 b	↓↓	30 b	11 b	2 b
30°C. (T_3)	274 a	176 b	73 c	↕	5 c	8 b	2 b
	ppm. NO_3-N						
5°C. (T_1)	195 a	154 c	194 c	↓	250 c	378 c	481 c
20°C. (T_2)	195 a	194 b	338 b	↓↓	440 b	537 b	592 b
30°C. (T_3)	195 a	232 a	400 a	↕	592 a	583 a	619 a
	ppm. mineral N (NH_4+ NO_3)						
5°C. (T_1)	469 a	381 b	377 c	↓	409 c	483 c	496 c
20°C. (T_2)	469 a	364 b	429 b	↓↓	471 b	548 b	595 b
30°C. (T_3)	469 a	407 a	473 a	↕	534 a	529 a	621 a

* After 3 days at -10° and 7 days at 5°C.

↓ Temperature raised to 20°C.
↓↓ Temperature raised to 30°C.
↕ Temperature unchanged.

a, b, c Ranges of equivalence. Within each vertical group of three, means accompanied by the same letter are not significantly different at 5 percent.

sharply during the first 10 days at the two lower temperatures, less sharply at 30°C. Subsequent release was rapid at the two higher temperatures, whereas mineral N remained depressed through the 41st. day at 5°C. The extent and duration of mineral N disappearance were directly related to numbers of bacteria observed on the 49th. day (cf. table 4). This indicates that temporary reductions in mineral N at the beginning of incubation were due primarily to immobilization in microbial tissues.

Net release of mineral N from soil organic matter, as observed in the last sampling, increased with increasing average temperatures experienced during incubation.

Interactions of fumigants, nitrogen treatments and temperature

Numerous first and second-order interactions between the three treatment variables in their effects on NO_3, NH_4 and total mineral N were expressed with statistical significance during the course of incubation. Mean NO_3 values for duplicate jars of each ultimate treatment are presented graphically in figures 6,7, and 8. The totals of NH_4 plus NO_3 shown in these figures represent averages for the three fumigation treatments, except where significant differences between them were encountered within a given combination of temperature and nitrogen treatment.

The most striking feature of the data in these figures is the fact that early declines in mineral N were accompanied by a disappearance of NO_3 in fumigated soils, whereas in control soils, NH_4 disappeared and NO_3 accumulated at rates which were increasingly rapid with increasing temperature. This confirms the earlier inferences from microbial numbers and respiratory activities (pages 50-53) that the heterotrophic microflora in fumigated soils were characterized by an abnormal dependence upon NO_3.

Discussion

The patterns of delayed NO_3 accumulation observed in figures 6, 7, and 8 for fumigated soils are not characteristic for lag periods associated with slow recovery in numbers of nitrifiers. Rather, they represent a masking of early nitrifying activity by reason of the fact that associated heterotrophic bacterial populations were concurrently consuming NO_3. The larger these bacterial populations were, or the longer they persisted in the soil, the longer was NO_3 accumulation delayed (figures 1, 2, 4).

There is little to indicate whether or for how long nitrifiers were specifically inhibited by fumigation. With dichloropropene, NO_3 accumulated at rates equal to or greater than in controls as soon as net disappearance of mineral N ceased. This indicates that full recovery of nitrifying

Figure 6.--Nitrogen transformations at low to intermediate temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 51st. day after fumigation. At this time the temperature was raised to 20°C. On the 12th day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4). Insets show effects of leaching unfumigated soil (F_1) and soil treated with chloropicrin (F_2) or dichloropropene (F_3).

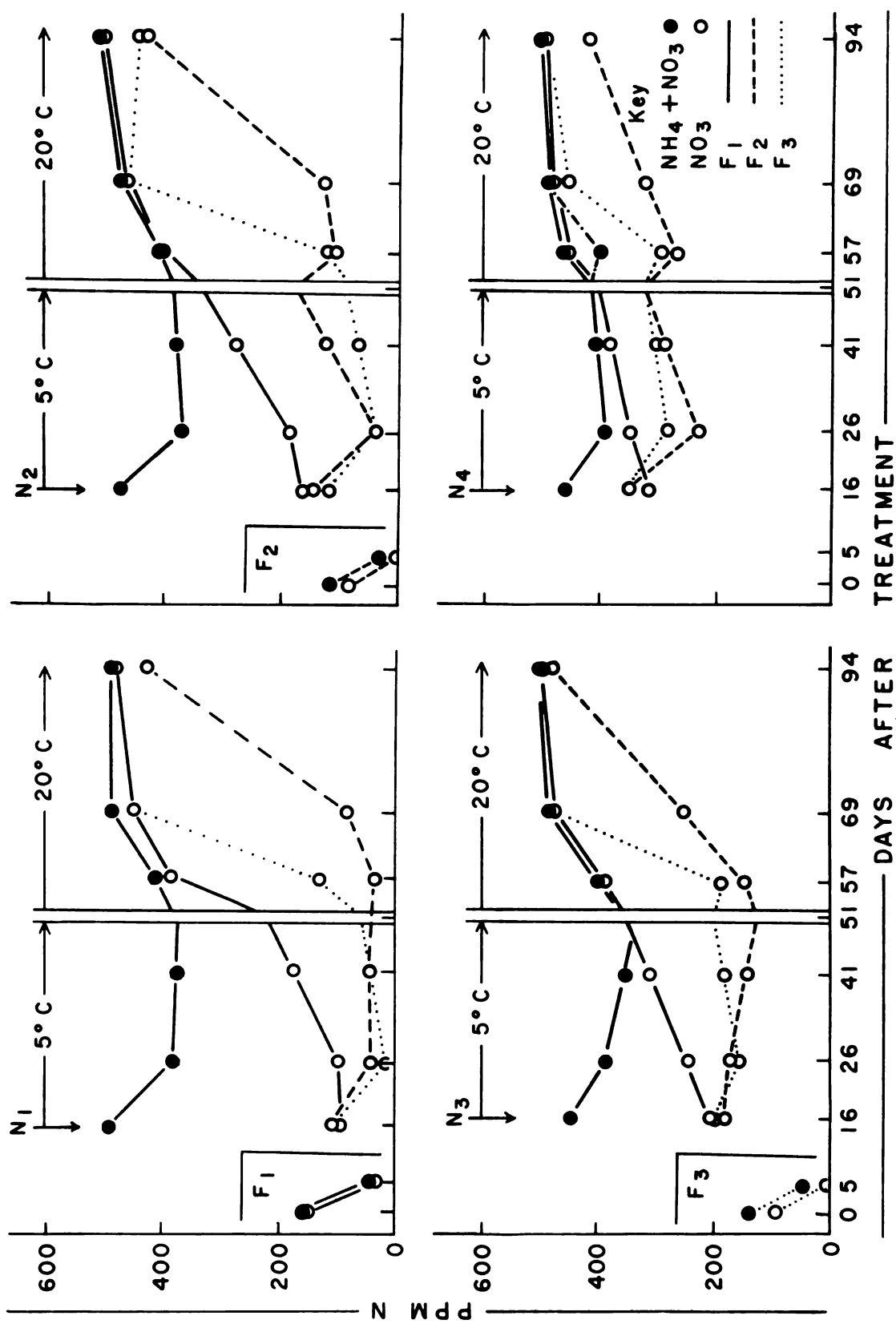
ppm N
600
400
200
0
N1
5° C
20° C
F1
N2
F2
N3
F3
N4
Key
NH4+NO3
NO3
F1
F2
F3
0 5 16 26 41 51 57 69 94
DAYS AFTER TREATMENT

FIGURE 6

Figure 7.--Nitrogen transformations at intermediate to high temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 16th. day after fumigation. At this time the temperature was raised to 20°C. The temperature was raised again to 30°C. on the 51st. day. On the 12th. day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4). Insets show effects of leaching unfumigated soil (F_1) and soil treated with chloropicrin (F_2) or dichloropropene (F_3).

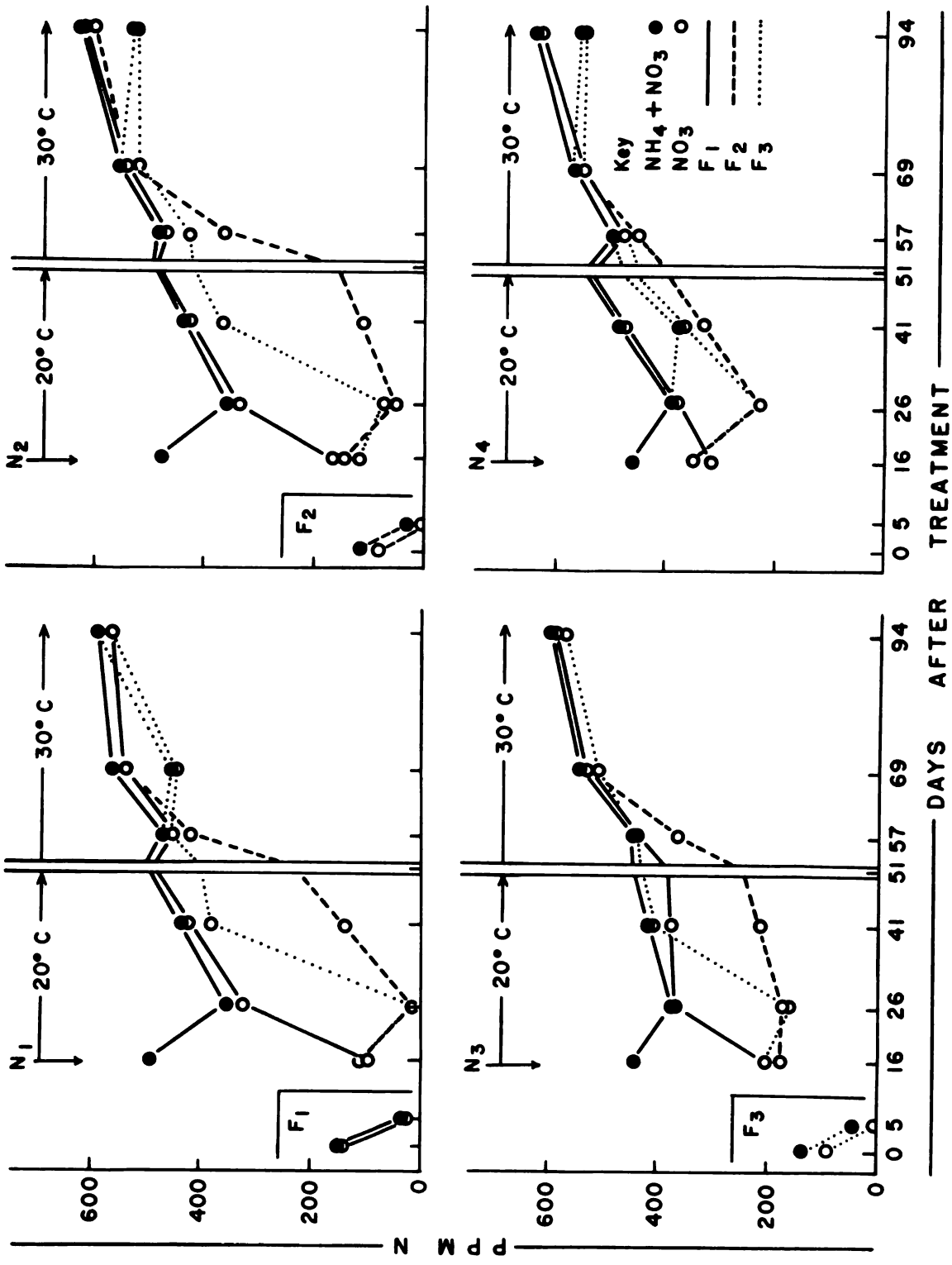
N1
20° C
30° C
F1
N2
F2
N3
F3
N4
Key
NH4 + NO3
NO3
F1
F2
F3
PPM N
0
200
400
600
0 5 16 26 41 51 57 69 94
DAYS AFTER TREATMENT

FIGURE 7

Figure 8.--Nitrogen transformations at high temperatures.

Houghton muck was exposed 2 days to chloropicrin or 2 weeks to dichloropropene, after which control and treated soils were exhaustively leached, aerated, frozen for 3 days and then maintained at 5°C. through the 16th. day after fumigation. At this time the temperature was raised to 30°C. for the balance of the incubation period. On the 12th day after fumigation, all soils were amended to 400 ppm. N with NH_4 and NO_3 in ratios of 9:1 (N_1), 3:1 (N_2), 1:1 (N_3) and 1:3 (N_4). Insets show effects of leaching unfumigated soil (F_1) and soil treated with chloropicrin (F_2) or dichloropropene (F_3).

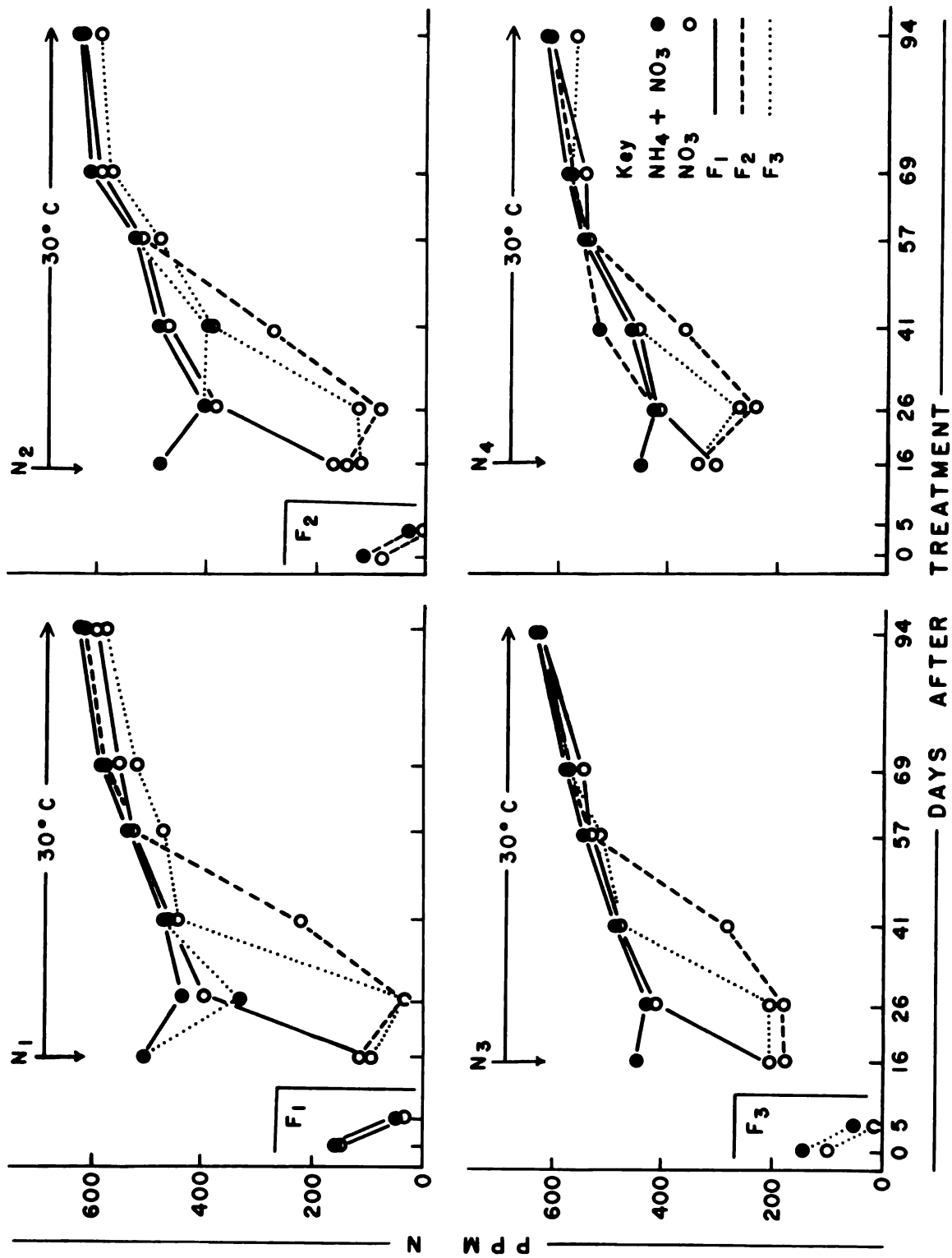
N1
30° C
F1
N2
30° C
F2
N3
30° C
F3
N4
30° C
Key
NH4 + NO3 ●
NO3 ○
F1
F2
F3
ppm N
0
200
400
600
0 5 16 26 41 57 69 94
DAYS AFTER TREATMENT

FIGURE 8

populations occurred prior to the time when net accumulation of NO_3 was observed.

Accumulation of NO_3 was delayed longer and maximum rates were less with chloropicrin than with dichloropropene, notably at lower temperatures. This might suggest that nitrifiers were specifically suppressed more by chloropicrin. However, there is evidence from bacterial counts on the 49th day at 5°C (figure 1) and at 30°C. (figure 4) that NO_3 dependence persisted longer in soils treated with chloropicrin than with dichloropropene. This would suggest that nitrification was masked by concurrent NO_3 utilization for longer periods in chloropicrin treated soil. The evidence of bacterial numbers at 20°C. (Fig. 2) is equivocal but not essentially damaging, since there is no indication that numbers observed on the 49th. day represented comparable successional populations in soils treated with the two chemicals.

Increases in NO_3 occurred in both fumigated and unfumigated soils during pre-incubation equilibration at 5°C. (table 7). This suggests that specific inhibition of nitrifiers ceased as soon as fumigant chemicals were physically removed from the soil by leaching and aeration. Disappearance and retarded accumulation of NO_3 became apparent only after an adaptation for using NO_3 had developed in dominant elements of the heterotrophic population.

Discontinuities in accumulation of NO_3 and total mineral N were associated with the mid-incubation temperature changes under the T_1 and T_2 regimens (figures 6 and 7). Microbial activity was strikingly enhanced by these abrupt rises in temperature (figures 1 and 3). Marked shifts in microbial balance also occurred (figures 1 and 2). It would appear that abrupt increases in temperature enhanced microbial consumption of mineral N in all soils. In fumigated soils, NO_3 was the principal form consumed. As a result, abrupt mid-incubation increases in temperature tended to extend the delay in NO_3 accumulation for both chemicals, particularly where the temperature had been held at $5^{o}C.$ up to this point (figure 6).

In general, it appeared that NO_3 utilization by the microflora of fumigated soils was primarily assimilatory. Disappearance of NO_3 was accompanied by only temporary differential decreases in total mineral N. Subsequent release reestablished mineral N totals equivalent to those in unfumigated controls

Apparent exceptions to this involved disappearance of NO_3 in the last sampling of soil treated with dichloropropene in the T_2 room (figure 7). Similar reductions in mineral N, significantly greater for dichloropropene than for chloropicrin, were observed earlier during incubation under both the T_2 and the T_3 temperature regimens (figures 7 and 8). The time when

these occurred was related to both temperature and nitrogen treatment, but in all cases they were followed by full recovery of mineral N, within the limits of experimental error. It is likely that mineral N apparently lost on the 94th. day with dichloropropene at the N_2 and N_4 levels in figure 7 would have been recovered again in a later sampling.

No attempt was made to make a full accounting for mineral and organic N. It is possible that dissimilatory losses of nitrate as N_2 and N_2O may have occurred. If such losses occurred, however, they were not differentially promoted by fumigation.

GENERAL DISCUSSION

It was rather clearly established that heterotrophic bacteria capable of adapting to NO_3 utilization are among the first to recover in fumigated soil. The adaptation is likely promoted by anaerobic conditions in soils high in moisture. Complete water-logging is unnecessary, since appropriately anaerobic micro-environments can develop in well aggregated soils, even when macropores are well supplied with oxygen. The intimate association of anerobic interior environments within aggregates and contact meniscuses with fully aerobic surface environments in moisture films adjacent to air-filled macropores may promote assimilatory utilization of NO_3 by reason of the known inhibitory effect of oxygen on dissimilatory nitrate reduction (12, 53).

It was shown at low temperatures that the size of primary recovery populations was dependent upon the level of NO_3 added and upon differences in size of the populations surviving treatment with chloropicrin or with dichloropropene. Larger numbers of NO_3 dependent bacteria developed and persisted longer where initial kill was greater and competing species were eliminated more completely with chloropicrin.

Respiratory loss of CO_2 was increased, numbers of bacteria observed in the first sampling were reduced, by early increases in temperature. The efficiency of carbon assimilation may have been reduced, but earlier depletion of

energy materials was most likely responsible for greatly reduced numbers of bacteria at higher temperatures.

At the higher temperatures, primary recovery populations in fumigated soil had probably been replaced by successional populations by the time the first counts were made 33 days after the first temperature increases were imposed. This conclusion is supported by the earlier recovery in numbers of fungi at higher temperatures. Nevertheless, the nitrate dependence of initiating populations was reflected in CO_2 production and by bacterial and fungal numbers at this time, as well as by adjustments in microbial balance and respiratory activity associated with temperature increases imposed 51 days after fumigation (35 days after nitrogen additions).

Temporary disappearances of mineral N during the first 5 weeks of incubation were due primarily to disappearance of NH_4 in unfumigated soil and primarily to disappearance of NO_3 in soil fumigated with chloropicrin. Disappearance of both NH_4 and NO_3 in soil treated with dichloropropene reflected the fact that a more heterogeneous microflora survived treatment, as shown by substantially larger numbers of bacteria and fungi immediately after fumigation then with chloropicrin.

Apparent accumulations of NO_3 during low temperature storage for 10 days prior to incubation lead to the tentative

conclusion that specific inhibition of nitrification ceased as soon as fumigant chemicals were physically removed from the soil by leaching and aeration. Disappearance and delayed accumulation of NO_3 became apparent only after the adaptation for using NO_3 had developed in dominant elements of the heterotrophic population.

This adaptation to using NO_3 may have begun to develop during low temperature storage, but it reached its maximum expression during the first 10 days of incubation (the third week following fumigation treatment). The adaptation may have developed earlier with the N_3 treatment (NH_4:NO_3 = 1:1), since preincubation accumulations of NO_3 were not observed (table 7), and disappearance of NO_3 during the first 10 days of incubation was negligible (figures 6, 7, 8). There is no readily apparent explanation for this unique behavior with the N_3 treatment. It may have derived from differences in pH buffering effects of phosphate salt mixtures added with different N treatments.

If allowance is made for NO_3 that may have disappeared prior to incubation in the N_3 treatment, initial decreases in NO_3 were surprisingly similar for all combinations of nitrogen treatment and temperature and for both fumigants. This would be expected, since utilization of NO_3 would be a function of the availability of suitable energy substrates. Differences in soluble organic substrates resulting from

fumigation would have been eliminated by exhaustive leaching. Release of soluble materials during subsequent freezing and thawing would reasonably have been similar in fumigated and unfumigated soil. Temporary decreases in mineral N in control soils were generally equivalent to those in fumigated soils, indicating that the utilization of mineral nitrogen was supported by equivalent supplies of energy carbon in both cases.

It is not known to what extent nitrogen was utilized for assimilation or whether dissimilatory reduction of NO_3 to gaseous products may have also contributed to disappearance of mineral N. If dissimilatory losses did occur, they were essentially the same in controls and in fumigated soils. Differential deficits for dichloropropene were encountered frequently, but these were temporary. The temporary character of mineral N losses in figures 6, 7 and 8 makes it appear that they were due primarily to assimilation and immobilization of nitrogen in microbial tissues.

On the basis of this assumption, net immobilization during the early stages of incubation declined with increasing temperature. This would be expected, since concurrent mineralization of organic matter was also enhanced, as evidenced by the fact that losses of CO_2 increased as temperature was increased.

Net immobilization of N also declined with increasing level of NO_3 addition, notably at the two higher temperature regimens (figure 7 and 8). Respiratory activity and mineralization of organic matter at these higher temperatures were enhanced by increasing NO_3 in fumigated soil, but the reverse was true in control soils (figures 3 and 5). The apparently similar equilibria established between mineralization and immobilization under these opposite situations appear fortuitous. It is possible that these apparent effects of NO_3 addition on net immobilization may have been related to early pH effects, - the salt mixtures for N_1 and N_2 treatments would have tended to raise the pH of the soil, those for N_3 and N_4 would have tended to lower it.

In fumigated soils, more intense and longer periods of net immobilization at low temperatures were associated with greatly increased numbers of bacteria. Differences of similar magnitude in bacterial numbers were not observed in unfumigated soil. This may have been due to failure of types with fastidious growth requirements to develop on the medium used, or viable climactic peaks may have occurred earlier in unfumigated soil and had disappeared by the time of the first count. Nitrate utilization in fumigated soils would have been an adaptive characteristic, and it would be expected that the responsible organisms would reach peak numbers later.

With both fumigants, it appeared that delays in NO_3 accumulation were related directly to numbers and persistence of NO_3 utilizing bacteria. Contrary to the original hypothesis, however, initial delays in NO_3 accumulation were drastically reduced by increases in temperature imposed the 16th. day after fumigation. This appeared to be due to the fact that the extent of NO_3 assimilation was restricted by available energy substrates at all temperatures, whereas the activity of nitrifiers was greatly enhanced at 20° and 30°C. After additional energy materials, in the form of metabolic intermediates and cellular debris, had accumulated during continued incubation at 5° and 20°C, abrupt increases in temperature 51 days after fumigation did result in enhanced utilization of NO_3 in fumigated soil.

Where the temperature at this time was raised from 5° to 20°C. (figure 6), utilization of NO_3 markedly extended the delay in NO_3 accumulation. This behavior is consistent with the original hypothesis and with observations in the field during the spring and early summer months following fall fumigation.

SUMMARY

Three lots of organic soil, untreated and fumigated with chloropicrin or dichloropropene, were subjected to exhaustive leaching, freezing and a period of low temperature storage (5°C.) to simulate environmental conditions encountered during the winter and early spring months in the field after fall fumigation. During the period of low temperature storage, the soils were amended with ammonium and nitrate in varying proportions, and moisture was adjusted to the highest level compatible with retention of stable aggregation (somewhat less than field capacity). They were then subjected to incubation under conditions of rising temperature.

In fumigated soils, an adaptation for utilizing nitrate developed during or just after low temperature storage and was maximally expressed during the third week after fumigation. It appeared that delayed nitrate accumulation in fumigated soils was due to utilization of nitrate by heterotrophic bacteria rather than to extended inhibition of the nitrifiers themselves. Nitrate utilization appeared to be assimilatory rather than dissimilatory and led to no permanent differential losses of mineral nitrogen as compared with unfumigated soil.

Nitrate assimilation was essentially the same at all temperatures and appeared to be limited by the same sources of available carbon as were involved in the assimilation of ammonium in unfumigated controls. Nitrifying activity, on the

other hand, increased sharply with increases in temperature. As a result, delays in observed accumulation of nitrate were greatly reduced when the temperature was raised to 20° or 30°C. on the 16th day after fumigation.

Where the preliminary storage temperature of 5°C. was continued into the incubation period, nitrate accumulated slowly in control soils, but at greatly reduced rates or not at all in fumigated soils. When the temperature was raised from 5° to 20°C. 51 days after fumigation, nitrate accumulation was additionally delayed by enhanced nitrate utilization. This behavior is similar to that observed during periods of rising temperature in spring and early summer in the field following fall fumigation.

Longer delays in nitrate accumulation with chloropicrin than with dichloropropene were associated with larger numbers and longer persistence of nitrate dependent heterotrophic bacteria. These differences between the two chemicals were greatly reduced at 30°C.

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