

# EFFECT OF ALTERNATE AEROBIC AND ANAEROBIC CONDITIONS ON REDOX POTENTIAL, ORGANIC MATTER DECOMPOSITION AND NITROGEN LOSS IN A FLOODED SOIL

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**Summary**—The effect of several cycles of varying length of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and loss of added and native nitrogen was investigated under laboratory conditions in flooded soil incubated for 128 days. Redox potential decreased rapidly when air was replaced with argon for the short-time cycles, but decreased more slowly where the aerobic period was long enough to permit build-up of nitrate. The minimum redox potential reached during the anaerobic period was generally lower for the longer cycles, but in all cases was low enough for denitrification to occur. Rate of decomposition of organic matter was faster in the treatments with a greater number of alternate aerobic and anaerobic periods. Total N (native and applied) losses as high as 24.3 per cent occurred in the treatment with the maximum number of cycles and with alternate aerobic and anaerobic periods of 2 and 2 days. Increasing the durations of the aerobic-anaerobic periods decreased the loss of N. A maximum loss of 63.0 per cent of applied  $^{15}\text{NH}_4\text{-N}$  resulted from the shortest (2 and 2 day) aerobic and anaerobic incubation. For soil undergoing frequent changes in aeration status the only labelled N that remained at the end of incubation was found in the organic fraction. Loss of N may have been even greater if labelled inorganic N had not been immobilized by microorganisms decomposing the added rice straw. The greater loss of N resulting from the 2 and 2 day aerobic-anaerobic incubation shows that, in soils where the redox potential falls low enough for denitrification to occur, increasing the frequency of changing from aerobic to anaerobic conditions will increase the loss of N.

## INTRODUCTION

It is known that alternate aerobic and anaerobic conditions affect a number of important soil processes. Several studies have shown that the rate and products of decomposition of organic matter are different in flooded and nonflooded soils (Tenny and Waksman, 1930; Alexander, 1961). Decomposition is faster under continuous aerobic conditions than under continuous anaerobic conditions, although inorganic N is usually released earlier to the soil solution under anaerobic conditions. Many workers, namely, Gooding and McCalla, 1945; Stephenson, 1956; Birch, 1960; and others have shown that decomposition of organic matter is stimulated by alternately wetting and drying the soil.

Inorganic N transformations are also influenced by alternate aerobic and anaerobic conditions. Severe N loss has been shown to occur in soils subjected to periods of alternate drained (aerobic) and flooded (anaerobic) conditions (Wijler and Delwiche, 1954; Russell, 1961; Patrick and Wyatt, 1964; MacRae, Ancajas and Salandanan, 1967). Under certain conditions organic N is converted to ammonium N (this process occurs in both aerobic and anaerobic soils), under aerobic conditions the ammonium N is oxidized to nitrate and under anaerobic conditions the resulting nitrate denitri-

fied. Loss of N through sequential nitrification and denitrification is especially high in soils planted to lowland rice, where water management practices sometimes require frequent draining and reflooding.

Russell and Richards (1917) were among the first to recognize the importance of alternate aerobic and anaerobic conditions in determining loss of N. Their studies showed large losses of N from farmyard manure under alternate wetting and drying conditions, but little loss when the material was maintained under either aerobic or anaerobic conditions. Wijler and Delwiche (1954) noted that alternating aerobic and anaerobic conditions should result in greater total N loss from the soil than would be found under continuous anaerobic conditions. Patrick and Wyatt (1964) observed large losses of N (up to 20 per cent of total N or 200  $\mu\text{g/g}$ ) as a result of repeated cycles of flooding and drying to field moisture. Tusneem and Patrick (1971) showed that ammonium N was highly unstable under alternate flooded and moist conditions of several weeks' duration, since only trace amount of applied labelled ammonium N was recovered at the end of a 120-day incubation period.

Most of the studies conducted so far show that alternate wetting and drying increases the loss of N from the soil, but these studies do not provide information

Table 1. Showing the changes in total nitrogen as a result of alternate aerobic and anaerobic treatments

Length of aerobic period (CO <sub>2</sub> -free air) (Days)	Length of anaerobic period (Ar) (Days)	Number of complete cycles during incubation period	Total nitrogen* ( $\mu\text{g/g}$ )		
			At the end of experiment	Net loss	Loss (%)
2	2	32	704	226	24.3
4	4	16	717	213	22.9
8	8	8	730	200	21.5
16	16	4	744	186	20.0
32	32	2	762	168	18.0
64	64	1	810	120	12.9
128	—	Completely aerobic	866	64	6.9
—	128	Completely anaerobic	925	5	0.5

\* At the beginning of the experiment soils in all treatments contained 930  $\mu\text{g N/g}$  of soil.

on the redox status of the soil under this changing environment or the minimum periods of aerobic and anaerobic conditions necessary to cause alternate nitrification and denitrification. The present study was designed to show the effect of frequency and length of alternating aerobic and anaerobic periods on loss of both native and added N and on soil organic matter decomposition. Changes in the redox potential of the soil under these alternate aerobic and anaerobic conditions were also measured.

#### MATERIALS AND METHODS

A Crowley silt loam, sampled from the Rice Experiment Station, Crowley, Louisiana, was used in this study. The soil had a carbon (C) content of 0.702 per cent, a total N content of 805  $\mu\text{g/g}$  and a pH of 5.6. The soil was thoroughly mixed and ground to pass through 0.4 mm mesh. An energy source of 0.5 per cent finely

ground rice straw (48 per cent carbon) and 100  $\mu\text{g/g}$  N in the form of  $(\text{NH}_4)_2\text{SO}_4$  containing 10.1458 at % excess  $^{15}\text{N}$  were thoroughly mixed with the soil. Aerobic and anaerobic conditions were accomplished by bubbling either CO<sub>2</sub>-free air or Ar through the incubation flasks. The various treatments used are shown in Table 1.

#### Incubation procedure

Duplicate flasks for each treatment were set up as shown in Fig. 1. One hundred and fifty grams of soil was weighed into each of the two incubation flasks along with 300 ml distilled water to give a soil to water ratio of 1:2 and then incubated at 30°C under constant stirring with a magnetic stirrer for a period of 128 days. A platinum electrode was permanently inserted through a rubber stopper to measure the redox potential of the soil suspension. The flasks were connected by means of glass and rubber tubing for continuous

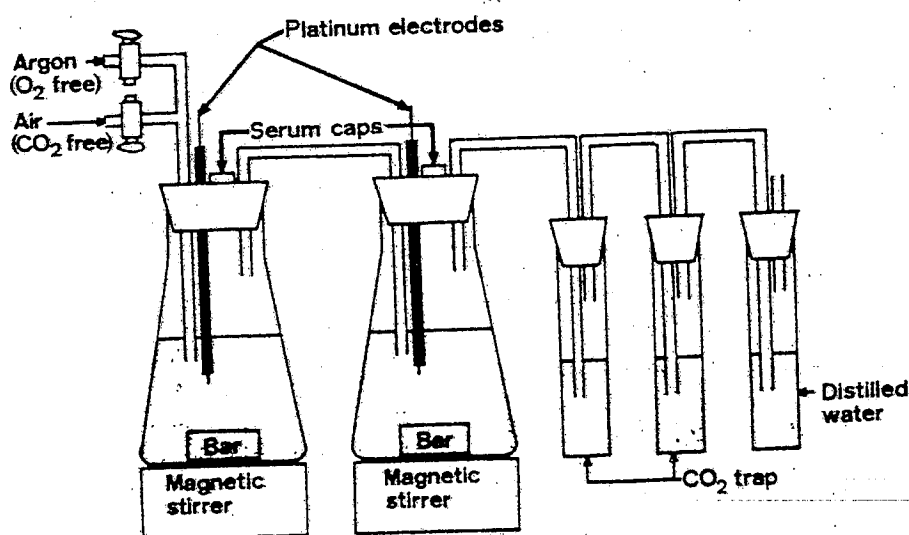


Fig. 1. Diagram of the apparatus used for studying the effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil.

flow of CO<sub>2</sub>-free air or Ar for creating aerobic or anaerobic conditions. The CO<sub>2</sub> evolved during microbial respiration was trapped in a 0.5 N KOH solution.

#### *Redox potential measurements*

Redox potential measurements were made by connecting the Pt electrode to a pH meter, using a saturated calomel half cell as reference electrode (Patrick and Wyatt, 1964). A complete cell was formed by inserting a salt bridge (glass tube) through a serum cap in the rubber stopper of the flask. Redox potential measurements were made daily during the incubation period of 128 days. The pH of soil suspension at the end of the 128-day incubation period was also measured.

#### *Analytical methods*

The CO<sub>2</sub> produced during decomposition of organic matter was measured (Stotzky, 1965). Total C remaining in the soil at the end of incubation period was measured by the dry combustion method (Allison, Bollen and Moodie, 1965). Inorganic and organic N fractions were analyzed by the steam distillation and the modified Kjeldhal methods, respectively, as given by Bremner (1965a, 1965b). The titrated samples containing N at a concentration of approximately 1 mg N per ml were analyzed for <sup>15</sup>N using a Dupont Model 21-614 isotope ratio mass spectrometer (Bremner, 1965c).

## RESULTS AND DISCUSSION

### *Redox potential of the soil during alternate aerobic and anaerobic conditions*

The redox potential of the soil responded rapidly to changes in the aeration status of the soil (Fig. 2(a-g)). The maximum redox potential under aerobic conditions for all treatments was approximately +600 mV. Displacement of air by Ar caused a rapid decrease in redox potential. The level to which the redox potential fell was approximately +200 mV for the treatment in which the gas was alternated every 2 days and reached lower values when the anaerobic period was extended. For the 4 and 4 day and 8 and 8 day aerobic-anaerobic periods the first decrease in redox potential was greater than subsequent decreases. In the continuously aerobic treatment the potential rose rapidly to approximately +600 mV and remained at this value throughout the 128-day incubation period. For the continuously anaerobic treatment the potential decreased rapidly to approximately -300 mV and remained constant for the entire incubation period. When the length of the aerobic-anaerobic periods was extended beyond 8 and 8 days the potential did not decrease very rapidly when Ar was bubbled through the samples. This slower initiation of reducing conditions was likely due to the presence of nitrate which had accumulated as a result of ammonium oxidation during the relatively long aerobic period. Nitrate has been shown to prevent a rapid decline in redox potential. In the soil that was main-

ment (Fig. 2(f)), the redox potential pattern after introduction of Ar was typical of that observed in a flooded soil containing both O<sub>2</sub> and nitrate (Engler, Patrick and Antic, 1974, in preparation). Rapid depletion of the O<sub>2</sub> as a result of displacement by Ar and consumption by the soil caused an initial decrease in redox potential from +600 mV to +200 mV at which value the potential was stabilized for several weeks. Stabilization of the potential at approximately +200 mV was very likely due to the accumulated nitrate and when this nitrate was denitrified after approximately 100 days the redox potential rapidly fell to approximately -300 mV.

The redox potential values at the end of the incubation period for the various treatments are shown in Fig. 3(a). Since the soil was anaerobic during the last half cycle the potentials are generally reducing, except for the treatment that went through rapid aerobic-anaerobic changes and for the treatment that was maintained under continuously aerobic conditions. There appeared to be a close relationship between the redox potential at the end of incubation and the length of the aerobic-anaerobic periods with a long final anaerobic half cycle being associated with a low redox potential.

The final pH of the soil was influenced by the treatment (Fig. 3(b)). For the soil carried through the various aerobic-anaerobic cycles the final pH was higher for the longer cycles, increasing from pH 6.5 for 2 and 2 day to 7.4 for the 64 and 64 day treatment. The controlling factor determining pH in these treatments was very likely redox potential during the final incubation period since a close inverse relationship between redox potential and pH was observed. For the continuous aerobic and continuous anaerobic treatments the same inverse relationship between redox potential and pH was evident. The soil continuously aerated had a lower final pH than did the original soil, probably resulting from the accumulation of nitrate and sulfate during the long aerobic period. Completely anaerobic conditions resulted in a pH of 7.8, a value somewhat higher than is usually encountered when acid soils are flooded. It should be noted that continuous passage of Ar through the suspension prevented precipitation of carbonate compounds which function to stabilize pH near the neutral point. This suggests that the pH value of flooded soils is sensitive to loss of CO<sub>2</sub>. The pH of most reduced soils equilibrated with CO<sub>2</sub> at 1 atm. is 6.1 (Ponnamperuma, Castro and Valencia, 1969).

### *Decomposition of organic matter as a result of alternate aerobic and anaerobic conditions*

The aeration status of the soil had a marked effect on organic matter breakdown. Carbon dioxide evolution for the various treatments is shown in Fig. 4(A-H). There was little difference in CO<sub>2</sub> evolution patterns for aerobic-anaerobic cycles ranging from 2 and 2 32 and 32 days. Carbon dioxide evolution was decreased considerably, however, when the cycle was increased to 64 and 64 days. Aerobic conditions for the

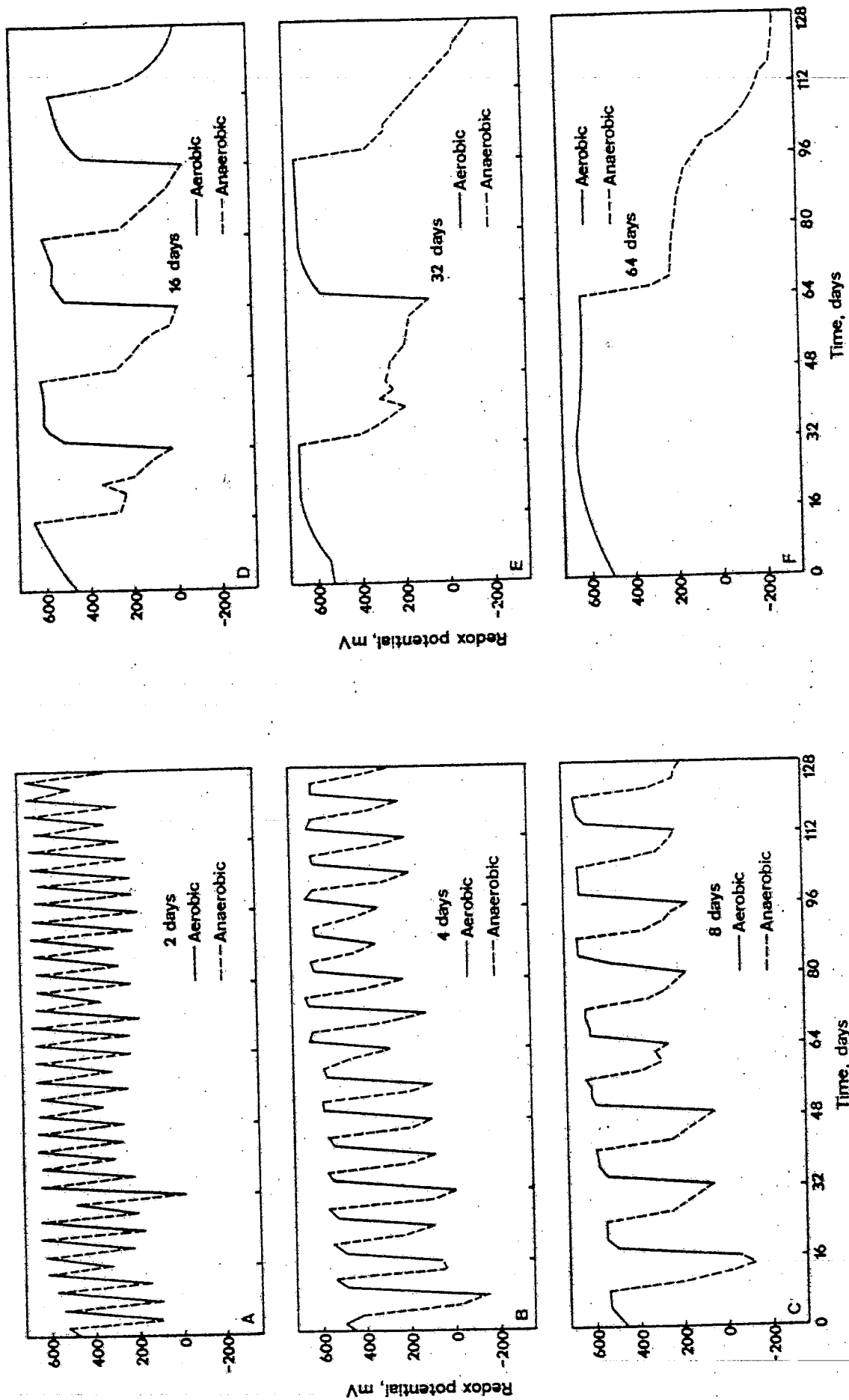


Fig. 2. (A-F).

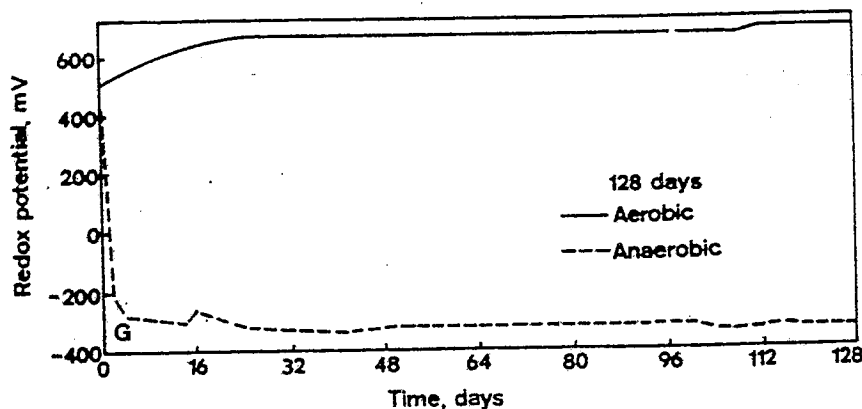


Fig. 2. (A, B, C, D, E, F and G). Changes in redox potential of the soil as affected by alternate aerobic and anaerobic conditions.

entire incubation resulted in approximately the same loss of CO<sub>2</sub> as was observed for most of the aerobic-anaerobic treatments. For the soil kept under continuous anaerobic conditions CO<sub>2</sub> evolution was decreased to approximately half the values obtained for the other treatments. Organic matter breakdown is known to be slower under anaerobic conditions than under aerobic conditions (Acharya, 1935; Alexander, 1961; Tenny and Waksman, 1930). Since C can also be lost from anaerobic soil as CH<sub>4</sub>, which was not measured in this experiment, total C loss was calculated from total C content at the beginning and end of the experiment. Carbon loss as determined from dry combustion analysis of organic C at the beginning and end

of the experiment showed remarkable agreement with the values obtained by CO<sub>2</sub> evolution (Fig. 5(a)), indicating little C loss as CH<sub>4</sub>. The only treatments that showed much discrepancy in C loss between the two methods was the 8 and 8 day aerobic-anaerobic periods and the continuously anaerobic treatment. The slightly greater loss of C for the continuously anaerobic treatment may have been due to CH<sub>4</sub> formation.

*Loss of added and native N as a result of alternate aerobic and anaerobic conditions*

The loss of N as a result of the various aerobic-anaerobic cycles is shown in Table 1 and Fig. 5(b). The greater loss of N took place in the treatments that alternated between aerobic and anaerobic conditions every 2 days. Approximately one-fourth of the total N (24.3 per cent) was lost during 32 of these cycles. Increasing the durations of the aerobic-anaerobic periods decreased the loss of N with approximately 13 per cent loss for the 64 and 64 day treatment. Almost no N was lost in the continuously anaerobic treatment while 7 per cent (64 μg/g) was lost in the continuously aerobic treatment. N is sometimes lost from apparently well aerated soils because of the presence of anaerobic microzones where denitrification can take place, but the continuous stirring and bubbling of air through the soil suspension should have prevented such anaerobic microzones from being established. This small loss of N may have resulted from chemical decomposition of nitrite. Some workers have shown that N losses during the nitrification process are the consequence of chemical decomposition of nitrite under acid soil conditions (Tyler and Broadbent, 1960; Smith and Clark, 1960; Cady and Bartholemew, 1963). No decomposition of nitrite was observed by Greenwood (1962) and Meek and Mackenzie (1965) in alkaline soils. Nitrate formed during the nitrification process might suffer some loss under the low pH conditions of the continuously aerated treatment. In all of the aerobic-anaerobic cycles the redox potential fluctuated between values that were oxidizing enough to support nitrification and reducing enough (below approximately +340 mV) to support denitrification

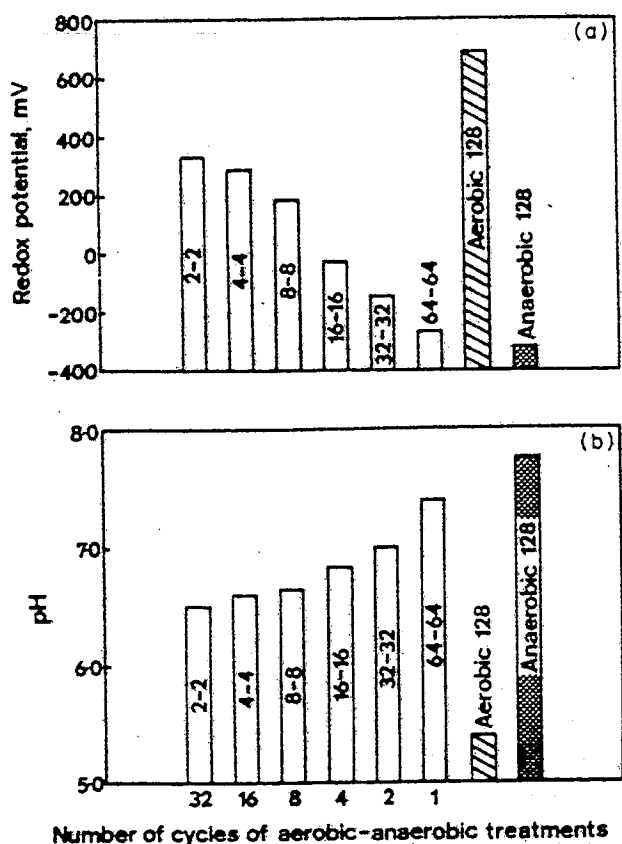


Fig. 3. (a and b). Redox potential and pH of the soil at the

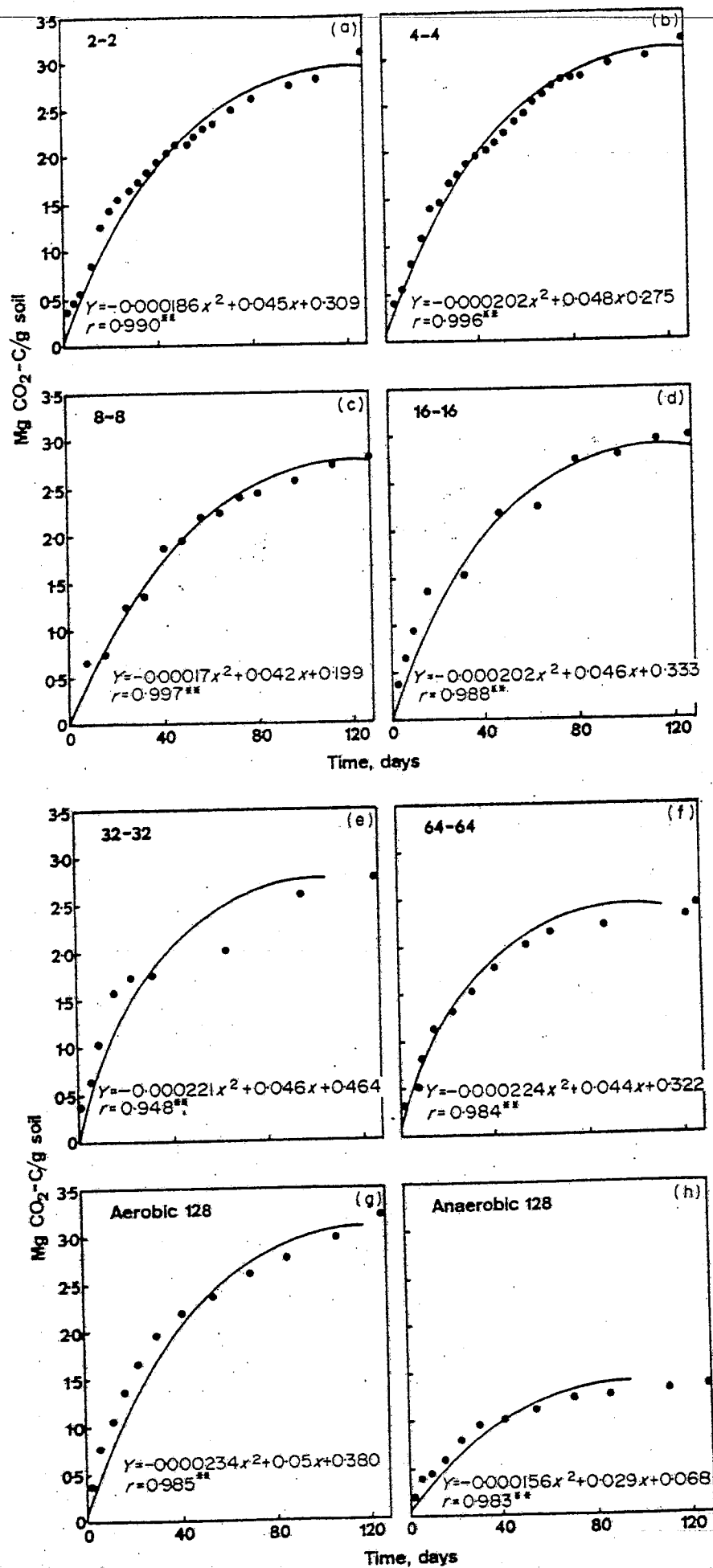


Fig. 4. (a, b, c, d, e, f, g and h). Rate of decomposition of organic matter as influenced by alternate aerobic and anaerobic conditions.

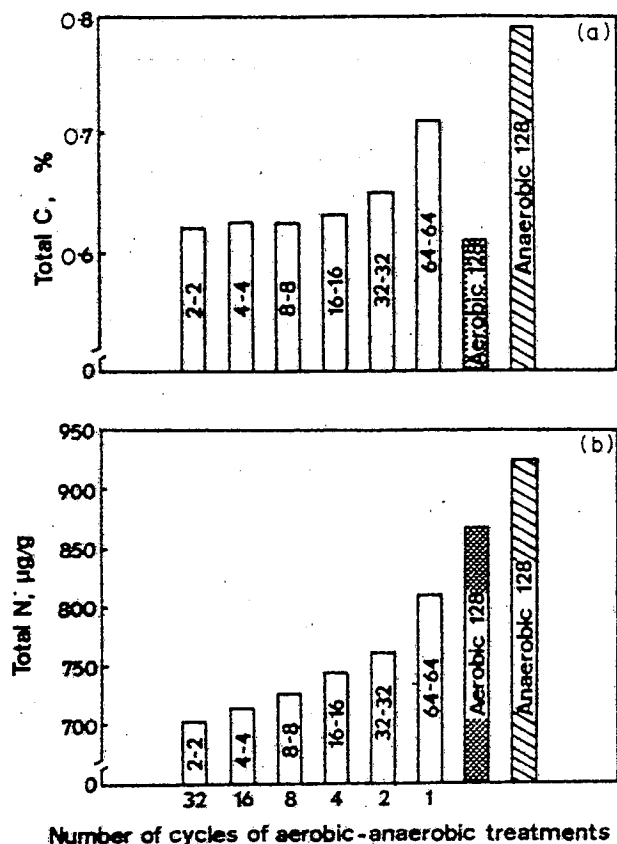


Fig. 5. (a and b). Total carbon and total nitrogen content of the soil at the end of 128-day incubation period as influenced by various treatments.

bic-anaerobic cycle was chosen because it was thought that the duration of the anaerobic half cycle would be too short for the redox potential to fall below the critical value necessary for nitrate reduction. The significant loss of N from the soil subjected to the 2 and 2 day aerobic-anaerobic periods indicates, however, that a 2 day period was long enough for nitrification to occur when air was bubbled through the suspension. A 2 day period was also long enough to support denitrification when Ar was bubbled through. Loss of N during short aerobic and anaerobic periods may be of more significance in nonflooded soils where temporary anaerobic conditions can occur than in flooded soils.

The amount of added labelled N remaining in the soil following incubation for 128 days for the various aerobic-anaerobic treatments is shown in Fig. 6(a). Less than half of the added labelled N (37 per cent in the case of the 2 and 2 day cycle) was recovered in all of the treatments where the soil underwent alternate aerobic and anaerobic incubation. Almost all of the N that was recovered was present in the organic fraction. This was especially true for the treatments with short aerobic-anaerobic periods; no inorganic N was detected in the 2 and 2, 4 and 4 and 8 and 8 day treatments. It is likely that N loss would have been greater if an appreciable part of the added labelled N had not been incorporated into microbial tissue, from which

All of the added labelled N was recovered in the completely anaerobic treatment, either as organic N or as ammonium N. The completely aerobic treatment showed a loss of 18 per cent of the added 100 µg/g labelled ammonium N. As pointed out above, this loss could have resulted from denitrification in a system being continuously stirred in the presence of O<sub>2</sub>, or as is also likely, from chemical breakdown of nitrite during the nitrification process. All of the inorganic N recovered in the continuously aerobic treatment was in the nitrate form.

Inorganic N (labelled and unlabelled) at the end of the experiment for the various treatments is shown in Fig. 6(b). Little inorganic N, either labelled or unlabelled, was recovered at the end of incubation for treatments with aerobic-anaerobic cycles shorter than 32 and 32 days. For all of the alternating aerobic-anaerobic treatments except the 2 and 2 day treatment, all of the inorganic N was present as NH<sub>4</sub><sup>+</sup>. A trace of nitrate was present for the 2 and 2 day treatment. It should be remembered that the last half cycle was under anaerobic conditions. Completely aerobic or completely anaerobic conditions resulted in accumulation of appreciable inorganic N. This N was present as NO<sub>3</sub><sup>-</sup> in the aerobic treatment and as NH<sub>4</sub><sup>+</sup> in the anaerobic treatment. For both these treatments and for the alternate aerobic and anaerobic treatments

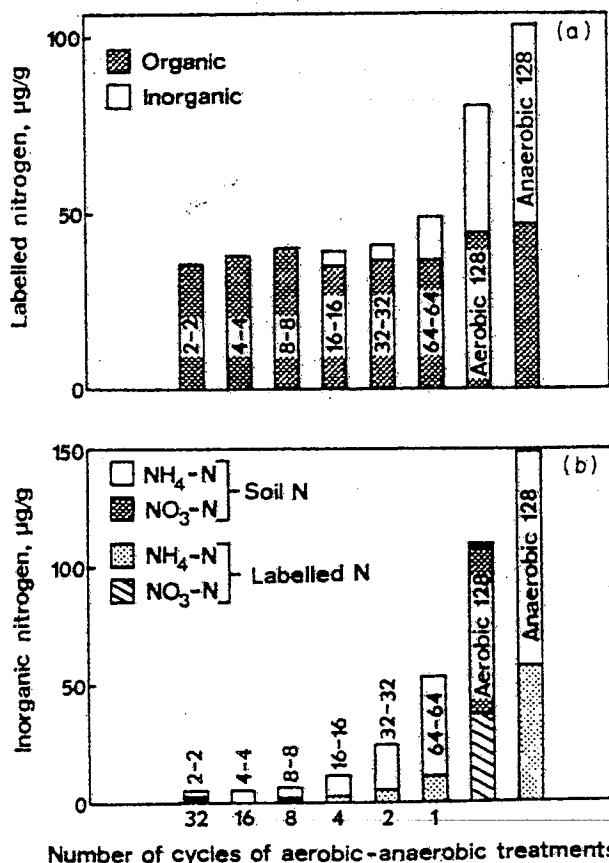


Fig. 6. (a and b). Amount of inorganic nitrogen and labelled nitrogen remaining in the soil following incubation for 128 days under alternate aerobic and anaerobic

which had inorganic N present at the end of incubation, approximately one-third of the inorganic N was derived from the labelled ammonium N and two-thirds from reactive soil N.

This study shows that increasing the frequency of changing from aerobic to anaerobic soil conditions to 2 days aerobic and 2 days anaerobic increases the loss of N. This loss was especially severe for added ammonium N, with 63 per cent of added labelled ammonium N being lost for the 2 and 2 day treatment during a 128-day incubation. More N would possibly have been lost except for immobilization of part of the added ammonium N by added rice straw.

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