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ALUMINUM STRESS EFFECTS ON MICROBIAL ACTIVITY IN SOIL ECOSYSTEMS

By

MATTHEW REY VILA

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

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DOCTOR OF PHILOSOPHY

Department of Crop and Soil Science

1984

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ABSTRACT

ALUMINUM STRESS EFFECTS ON MICROBIAL ACTIVITY IN SOIL ECOSYSTEMS

BY

MATTHEW REY VILA

The assessment of aluminum (Al) stress effects on microbial activity (CO_2 evolution rate) was conducted for soil ecosystems and artificial soil mixtures. The possible biological interactions of Al and Al in the presence of ferric iron (Fe^{+3}) were examined. Also, the activity of several soil enzymes were assayed for comparison between Al stressed and non-Al stressed soils.

The results from these studies suggest that in the presence of Fe^{+3}, Al stress is intensified. This investigation also demonstrated an increase in biomass associated Al in the presence of Fe^{+3}. The significance of this finding is discussed relative to Al stressed Oxisols and soils in general with the occurence of soluble Fe in these enviroments. The results of the enzyme activity studies reflect a possible phosphate limited environment for the Al stressed soils.

DEDICATION

This dissertation is dedicated to my wife Susanne

who's love, patience, and help made

my duration of this work an enjoyable one.

ACKNOWLEDGEMENTS

I would like to thank my major Professor, Dr. Vernon W. Meints for his support and the freedom with which he allowed me to explore soil science. Also, appreciation is expressed for his allowing me to experience soil science first hand by working in the Soil Testing Laboratory at Michigan State University during my tenure there.

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INTRODUCTION

The world wide occurrence of aluminum (Al) stressed acid soils warrants the study of Al stress effects on biological systems. These soils are most frequently found in the tropics and subtropics, and generally are classified as oxisols. The temperature and humidity of these regions are ideal for crop production. The common occurence of high exchangeable Al in soils of these regions often limits production. High concentrations of soluble Al and iron (Fe) species are characteristic of these soils along with kaolinitic clays and low pH.

Currently, the specific effects of Al on agricultural and ecological systems are not well understood. A general understanding of soil microbial activity under Al stress would help explain the problems associated with rhizosphere associations between plants and their respective microorganisms. This study was conducted to assess the effects of Al stress on microbial activity in acid aluminum soils.

A survey of the current literature revealed a subtle suggestion that Fe might be implicated in the intensification of Al stress effects on microbial activity. In the literature, the involvement of Fe^{+3} in Al stress response was not addressed directly. Contradictory views as to whether microbial activity in acid soils is even subject to Al inhibition are found in the literature. This study addressed the hypothesis that soluble Fe^{+3} will intensify Al stress effects on microbial activity.

LITERATURE REVIEW

Aluminum stress effects on crop production related to acid soils have been extensively studied (Mattson and Hester, 1933; Chernov, 1947; Harward and Coleman, 1954; McLean et. al., 1965; Evans and Kamprath, 1970; Hoyt and Turner, 1975; Thomas, 1975; Reid, 1976). However, only recently have the effects of Al stress on the soil microflora been investigated. While several investigations into the effects of Al stress on microbial activity have been conducted, results in the literature have been inconclusive as to the specific effects of Al on microbial metabolism (Keyser and Munn, 1979a ,and Keyser and Munn, 1979b). To understand the biological effects induced by Al stress, knowledge of the chemical state of Al in the medium used for experimentation is most important to the determination of bioactive forms of Al.

In mineral soils, hydrogen (H) and Al are the two cations primarily responsible for soil acidity. Chernov (1947) made an early association between trivalent cations (i.e. Al and Fe^{+3}) and acid soils. Chernov realized the instability of proposed H saturated soils and suggested that Al and Fe saturate exchange sites in acid soils. Jenny (1961) reported that the weak acid properties of acid clays in fact result from a complete or partial saturation of exchange sites by various hydrolyzed Al species. Another characteristic of Al controlled acidity is that H saturated clays respond more like strong acids than Al saturated soils which behave more like weak acids (Bohn, et. al., 1979).

Trivalent Al occurs in soil in a variety of forms. These forms of soil Al include various degrees of hydroxylated monomeric and polymeric species (Bohn, et.al., 1979). In acid soil where pH ranges from 5.0 to 3.0, monomeric Al may occur as hydrated forms of Al^{+3}, $AlOH^{+2}$, $Al(OH)_2^{+}$ and $Al(OH)_3^{o}$ in solution. Gibbsite or gibbsite-like minerals are suggested to be the major forms of Al which precipitate when Al excedes its solubility product (Bohn, et.al., 1979; and Linsay, 1979). Polymeric forms of Al occur between hydroxylated species forming large units with a general formulation of $(Al(OH)_x(H_2O)_{(6-x)}^{+(3-x)})n$ where n is the number of Al ions per unit polymer. Polymerization is promoted by colliod surfaces present in solution and suggests proximity enhancement. Recent studies suggest that monomeric as well as polymeric species may be involved as sources of exchangable Al (Turner, 1967; Vieth, 1978). Previously, it was held that only monomeric species had the overall mobility to be exchangeable. Exchangeable Al is the form most often associated with the phytotoxic effects of Al stressed soils (Barnhisel and Bertsch, 1982; and Reeves and Sumner, 1970). A question still remains as to whether polymeric exchangeable Al polymerizes prior to or after exchange extraction (Barnhisel and Bertsch, 1982). Hargrove and Thomas (1981) have examined plant growth in soils amended with Al-citrate, Al-EDTA, Al-fulvate, $Al(OH)_2Cl$ and no Al. There were no differences among Al-citrate, Al-EDTA, Al-fulvate, and no Al relative to plant growth. However, soils amended with $Al(OH)_2Cl$ demonstrated severe phytotoxic effects. Hargrove and Thomas(1981) also demonstrated a strong negative relationship between exchangeable soil Al and plant growth.

Plants grown in acid soils often appear to exhibit a variety of nutrient problems. In acid soils with high Al percent saturation values, calcium (Ca) as well as magnesium (Mg) are usually displaced by Al at exchange sites. Potassium (K) levels are also depressed in Al stressed soils. Molybdenum (Mo) availability is decreased under low pH conditions and often provides for Mo deficiency problems for nitrogen-fixing legumes (Bohn, et.al., 1979; and Jackson, et.al., 1963).

A variety of micronutrients become increasingly soluble, often to phytotoxic levels, in acid soils (Brady, 1974). Iron and manganese (Mn) toxicities often occur under highly solublizing acid conditions. Zinc (Zn) toxicity also may occur in acid soils, although this is a rare phenomena (Vitosh, et. al., 1981). Under acid-Al soil conditions, phosphorous (P) added to soils may become rapidly unavailable due to fixation and precipitation with Al compounds present (Hsu and Rennie, 1962; Hsu, 1965; Parfitt, 1977; and Sims and Ellis, 1982).

All of the effects on nutrient availability just described confuse the issue of Al toxicity problems in acid soils. These effects often occur in association with acid-Al stressed soils. The assessment of the direct effects of Al on organisms living in the soils has been unsucessful. Complications arising from the various nutritional problems mentioned make observation of the direct relationships between Al and soil organisms difficult to resolve.

A review of the literature indicates Al stress conditions are inhibitory but not usually lethal to microbial populations (Keyser and Munns, 1979a; Cooper and Morgan, 1979a; and Munns and Keyser, 1981).

Hartel and Alexander (1983) claim that Al in acid soils is of no general consequence to the activity and the survival of cowpea Rhizobia strains in soils. Munn and Keyser (1981) concluded that under prolonged Al stress spontaneous mutation toward Al tolerant strains did not occur. These authors also demonstrated by synchronous culture methods that cell division is greatly delayed but that Al was not generally lethal to Rhizobia cell survival.

Studies examining Ca deficiencies in Rhizobia have also been conducted. Amendments of Ca to Al stressed cultures of Rhizobia strains demonstrated no relief from the Al toxicity experienced by the cultures (Keyser and Munns, 1979a). Keyser and Munns (1979b) also examined the effects of Mn toxicity under Al stress conditions. They found no enhancement of the toxic effects of Al stress on Rhizobia strains in the presence of soluble Mn. The effect of Al stress and low P availability relative to Rhizobia strains has been examined. While a low P concentration did limit growth in Rhizobia strains, the effects of Al and acidity were found to be much more severe (Keyser and Munns, 1979b). The effect that allophane clay has on the growth of Escherica coli has been examined. In a study by Cooper and Morgan (1979b), it was demonstrated that allophane at pH 5.0 did not exhibit Al stress responses when amended to E. coli cultures. However, soluble Al added at 0.2 umol Al/mL demonstrated a significant reduction in cell respiration and cell division. Zwarun and Thomas (1973) demonstrated that exchangable Al alone had little effect on microbial activity, but that soluble Al did reduce viability for cultures of Pseudomonas stutzeri . Furthermore, Zwarun and Thomas (1971) found

no effects on a Bacillus sp. exposed to Al-saturated clays with only exchangable Al available. From a review of the literature, it appears that in bacterial cultures where exchangable Al is the source of Al, only a minimal effect, if any, is noticeable. Primary effects come from additional amendments of soluble Al (Zwarun and Thomas, 1971,1973; and Cooper and Morgan 1981b).

Cooper and Morgan (1981b) suggested that in clay systems the H^{+} given off by microbial growth is absorbed by the clay. These investigators noted that when pH was monitored in simultaneous treatments, decreases in pH due to growth of E.coli were reduced in the presence of allophane, while the metabolic rates were the same with or without allophane. A slight enhancement was noted in the metabolic rate as the amount of allophane was increased (Cooper and Morgan, 1979a, and 1979b). This enhancement is in agreement with the observations of Stotzky and Rem (1966) concerning microbial interactions with clays.

A review of the literature revealed no references to the specific physiological effects of Al on microorganisms. However, indirect references to one area of microbial physiology were made in several articles. These subtle comments point to an involvement of soluble Fe^{+3} in the intensification of Al stress on soil microorganisms. In an extensive review of Fe transport, Arceneaux and Byers (1976), cite an experiment by Davis and Byers (1971) in which Al was used as an inhibitor for a permease-like Fe uptake mechanism. In this experiment, Al was thought to coprecipitate Fe^{+3}, originally $FeCl_3$, making it unavailable to the transport-permease system. The organisms used were Bacillus megaterium mutants which lacked the siderochr-

ome chelates to supercede the permease system. When exogenous siderochrome for that organism was amended to the system, Fe^{+3} transport resumed immune to the presence of Al at $4x10^{-5}$ M concentration (Davis and Byer, 1971). Arceneaux and Byers (1976) cite examples which demonstrate that microorganisms which are able to take up one kind of microbial siderochrome can usually utilize a variety of Fe-chelates produced by other microorganisms. Such microbial produced chelates include citric acid, a variety of catechols, and hydroxamic acid polymers. In Escherica coli , Bacillus megaterium , Aerobacter aerogenes , and Bacillus subtilis , it has been demonstrated that high Fe^{+3} concentrations (10^{-6} -10^{-7} M) repress synthesis of the enzyme system which inturn synthesizes siderochrome chelate. Under high Fe concentration, membrane bound carriers transport Fe^{+3} into the cell (Downer, et.al.,1970).

Under Al stress, Rhizobium japonicum demonstrated some relief from Al-stress when Fe(III)-EDTA replaced an equilvalent concentration of Fe^{+3} as $FeCl_3$ (Keyser and Munns, 1979b). There is no immediate explanation for this effect except that EDTA might be chelating soluble Al. This explanation is doubted by the investigators, and it is not supported by a relatively low stability constant for an Al-EDTA complex at pH 4.5, and a high stability constant for Fe(III)-EDTA (Sillen and Martell, 1974; and Mortvedt, et.al., 1974). Finally, Cooper and Morgan (1979a, 1979b) noted in experiments with Al-stressed E. coli that one treatment at an intermediate Al concentration demonstrated a greater stress response than treatments at higher Al concentrations. In these treatments, E. coli were subjected to alloph-

ane clay and soluble Al. The investigators stated that Fe^{+3} released by allophane occurred in the intermediate Al stress experiment. They suggested that the Fe^{+3} caused a precipitation of bacterial cells which resulted in the greater than expected stress response (Cooper and Morgan, 1979a, and 1979b).

The chemical properties of Al and Fe^{+3} under acid conditions are very similar. In general, Fe^{+3} and Al exhibit similar solubility and hydration characteristics. It has been suggested that Fe^{+3} under acid conditions can precipitate microorganisms in solution (Tenny and Stumm, 1965). Cooper and Morgan (1979a) found flocculation of E. coli in the presence of allophane clay and/or Al, but they did not attribute the Al complexing phenomena to a reduction in microbial activity. At the pH of 4.5, Fe^{+3} and Al have similar binding affinities for soil organic matter (Bloom, 1981). However, under situations where the specific association of Fe^{+3} or Al with organic matter is by chelation; Fe^{+3} out-competes Al for the chelate. This observation is supported by Fe^{+3} high stability constant for compounds like EDTA when compared with Al (Log K=25.0 for Fe^{+3}, 16.1 for Al) (Bohn et. al.,1979). This difference is attributed to the ability of transition elements like Fe^{+3} to orient their electronic configurations to optomize the ligands general configuration. Aluminum being much more rigid in its electronic structure is not as competitive (Bohn et. al., 1979). This redistribution of electronic structure for Fe and not for Al may be the reason for the observations by Davis and Byers (1971) that bacterial siderochromes selectively chelated Fe(III) out of a solution containing $4x10^{-5}$ M Al. In both classes of microbial

chelates, hexadentate cages of six oxygens hold Fe securely (Silver, 1978).

The membrane bound carriers for Fe would appear to be a reasonable site for physiological inhibition by Al. No literature is available relative to Al uptake by Fe^{+3} membrane assimilation mechanisms. Evidence suggests that membrane carriers for inorganic ion species may be relatively non-specific for ions sharing certain similar properties. Most likely, these carriers are optimized toward a specific ion. However, similar to enzyme systems, these carriers might interact with other non-optimal ions sharing similar ionic properties. These interactions with less optimal ion species are not unlike enzymatic transformations of substrate analogs which differ only slightly from their nominal substrates for a given enzymatic reaction. Evidence for a similar process in ionic solute assimilation can be found in the Mg^{+2} transport system. The Mg^{+2} transport system has been shown to be optimal for Mg^{+2}, but competetively inhibited by a variety of divalent cations. These competetive divalent cations include Co^{+2}, Cu^{+2}, and Ni^{+2} (Silver, 1978). To date, no highly specific uptake mechanism exists to explain how these trace elements are assimilated by microorganisms. Silver (1978) suggested that these cations are taken up in a sufficient quantity through competetive assimilation through the Mg^{+2} transport system. Excessive extracellular Mn^{+2} or Co^{+2} have been shown to enter E. coli creating cytotoxic levels through the Mg transport system (Silver, 1978).

Ionic properties such as ionic radius, ion charge, calculated activities, and ionic potentials are so similar that it is not

surprizing that competetive uptake occurs for these divalent trace metal ions in the Mg^{+2} transport system (Silver, 1978; and Bohn et. al., 1979). It seems reasonable that a similar competition between soluble Fe^{+3} and Al^{+3} might occur for the membrane bound Fe^{+3} carrier as the ionic properties of Fe^{+3} and Al^{+3} are quite similar (Bohn et. al., 1979).

MATERIALS AND METHODS

SOIL RESPIRATION STUDY:

The respiration studies were conducted utilizing the alkali absorption method for carbon dioxide. The general methods for the carbon dioxide assay were adapted along the guidelines set forth by Van Cleve, et. al., (1979). Ten grams of soil were dispensed into a 50 mL erlynmeyer flask, the appropriate substrates added, the CO_2 cache put in place, and the flask stoppered (See Fig. 1).

The CO_2 catche consisted of a 2.0ml plastic cup filled with 1.0ml of 0.4 M NaOH solution. The catche was attached to a 30 gauge wire which was held pinched between the stopper and the flask. At the end of an experiment, the 1.0 mL of NaOH was removed from the flask and added to 5.0 mL of 10% $BaCl_2$ solution. This solution was titrated with 0.1 N HCl (standardized with T.H.A.M.). The carbon dioxide evolved is reported as nmol C/g soil/h. The formula to obtain the carbon dioxide evolution rate is as follows:

$$\text{nmol C/g Soil/h} = ((B-A) \times N \times 1000)/\text{g Soil/Total time(h)}$$

B = ml of acid titrated to blank

A = ml of acid titrated to active sample

N = the normality of the acid

The artificial soil was prepared by saturating a montmorillon-

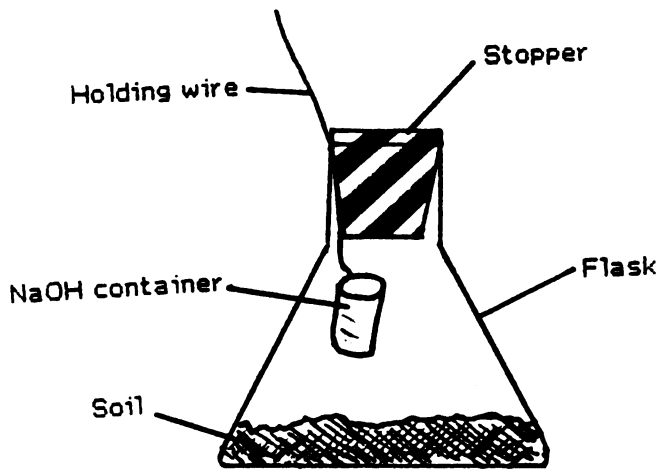

Fig. 1. Soil respiration flask.

ite clay (commercial grade "vol-clay") and leaching it with a 1.0 M $AlCl_3$ solution. The clay was washed with distilled water until no free Cl^- was detectable with $AgSO_4$, and then mixed with sand to achieve a 5% clay mixture by weight. This system was found to contain 4.44 umoles of exchangeable Al per gram of mix. The sand-clay mixture exhibited a pH of 4.6 ± 0.2, while the pure sand gave a pH of 4.7 ± 0.1. Throughout this study, the pH of these artificial soils did not vary more than 0.2 pH units. Measurements for pH were made before and after each experiment. All respiration experiments were conducted at 25 ± 0.5 C. Ten grams (air dried wt.) of artificial soil were added to a flask, and brought to 30% moisture content through the innoculation with microbial cell slurry's and substrate amendments. All experiments were run for 18 hours. For natural soils, 10 g (air dried wt.) were added to the incubation flasks, and were treated in a manner similar to the artificial soils. Amendments to the artificial soils included 1.0 mL of microbial cell slurry, 1.0 mL of carbon substrate solution, and 1.0ml of additional amendments or sterile distilled water. For the natural soils, the same amendments were used except the cell slurry was replaced by 1.0 mL of sterile distilled water 24 hours prior to the start of the experiment. The natural soils consisted of Al stressed and non-stressed soils.

The carbon substrate solutions used for the artificial soils consisted of a 1:1 mixture of glucose (Mallinckrodt) and yeast extract (Difco, Lot-652609). This solution was analyzed for percent C and adjusted to give a final concentration of 4, 8, 12, 16, and 20 umol sub-

strate C/g soil when 1.0 mL of the substrate solution was added to 10 g of soil. For the natural systems, only glucose at 4, 8, 12, 16, and 20 umol substrate C/g soil was used. Cell slurries for the artificial soils were prepared by centrifuging broth cultures, washing the pellet in distilled water, centrifuging and washing the pellet again, and re-suspending the pellet in distilled water. Percent carbon determinations were made on the cell slurry, and the slurry was diluted to yield 200 ug microbial C/g soil when 1.0 mL of the suspension was added to 10 g of soil. The Fe amemdments were made prior to the addition of the carbon substrate for both artificial and natural soils. Iron was amended to the soils at 0.01, 0.1, 1.0, and 1.3 umol Fe /g of soil as $FeCl_3$.

The respiration experiments using Fe(III) chelates included EDTA, NTA, and citric acid as the complexing agents. Each of the Fe^{+3} chelates (Fe(III)EDTA, Fe(III)NTA, and Fe(III)Citrate) were brought to three concentrations in solution, 10^{-2} M, 10^{-4} M, and 10^{-6} M. These iron solutions were amended to the artificial soil system at 1.0 mL/10 g of soil. Utilization of the chelated forms of Fe^{+3} allowed for control of soluable Fe^{+3}.

GROWTH STUDIES:

The effects of different Fe^{+3} and Al treatments on the growth rates of Bacillus megaterium (B-12) and Rhizobium spp. (I-110) were conducted turbimetrically on a Bosch and Lomb Spectronic-20 spectrophotometer using optical side-arm culture flasks. Growth rates were

monitored for 48 hours and maintained at approximately 25 C. The control flask consisted of a sodium acetate buffer, 0.02 M , pH 4.6; and glucose-yeast extract (1:1), at 8.0 umol C/mL. The subsequent treatments included the control media above plus Al (3.8×10^{-6} M) or Fe (1.0×10^{-4} M Fe(III)Citrate) giving an Fe^{+3} molar activity of approximately 10^{-9} M .

The treatments included the control, acetate buffer plus glucose-yeast extract (GY), GY with Fe^{+3} amended (GY+Fe) and GY with an initial Al amendment (GY+Al). Two other treatments included were GY+Fe with Al amended after 18 hours of growth (GY+Fe > Al) and GY+Al with Fe^{+3} amended after 18 hours of growth (GY+Al > Fe).

SOIL DESCRIPTIONS:

The soils used in this study included the following low Al soils: IB, (Owosso) a fine-loamy, mixed, mesic, Typic Hapludalf; IC, (Capac) a fine-loamy, mixed, mesic, Aeric Ochraqualf; AS1A, (Barry) a fine-loamy, mixed, mesic, Typic Argiaquoll; FS1A, (Boyer) a coarse-loamy, mixed, mesic, Typic Hapudalf; and CK-19, (Brookston) a fine-loamy, mixed, mesic, Typic Haplaquoll. The Al stressed soil was represented by the group IA - VA, (Kalamazoo sandy loam) a fine-loamy, mixed, mesic, Typic Hapludalf.

BACTERIA AND CULTURE METHODS:

Bacillus megaterium (B-12) was obtained from the culture collection of the Dept. of Microbiology and Public Health at Michigan State University. Stock cultures of Bacillus megaterium (B-12) were

kept on nutrient agar slants (Difco). The slow growing Rhizobium spp. (I-110) was obtained from the laboratory of Dr. Frank Dazzo, Dept. of Microbiology and Public Health, Michigan State University. The Rhizobium strain was maintained on mannitol-yeast extract agar slants with mannitol, 10 g/L; yeast extract (Difco), 1.0 g/L; $K_2HPO_4 \cdot 3H_2O$, 0.65 g/L; $MgSO_4 \cdot 7H\ O$, 0.2 g/L; NaCl, 0.1 g/L; and special agar (Nobel, Difco), 15 g/L. Broth culture media for cell slurry production for both B. megaterium (B-12) and Rhizobium spp. (I-110) contained 5.0 g/L glucose and 5.0 g/L yeast extract (Difco) incubated at 25 C, for 24 hours for B. megaterium (B-12), and 48 to 72 hours for the Rhizobium spp. (I-110).

BIOMASS ESTIMATES:

The cell slurry biomass estimate, carbon content, was determined by transfering 1.0 mL of the washed cell suspension to a container holding 10ml of 0.5 N $Na_2Cr_2O_7$. To this solution, 10 mL of concentrated H_2SO_4 was added. The mixture was allowed to digest for 30 minutes, and then was read on a Bousch and Lomb Spect-20 spectrophotometer at 645nm. Glucose solutions of known carbon content were used for calibration.

Natural soil biomass estimates were conducted according to the respiration method of Anderson and Domsch (1978). In this method, natural soils were amended with 0.5, 2.7, 5.5, 8.3, and 11.1 umol glucose/g soil. The soils were monitored for CO_2 evolution as described above. Incubation was conducted for 2 hours at 22 $\pm$ 0.5 C.

ENZYME ACTIVITY ASSAYS:

Pyrophosphatase:

The procedure of Tabatabai (1982) for the assay of pyrophosphate activity was modified. The procedure used here differed in that no buffer was added, and the incubation temperature was maintained at 25 ± 0.5 C. Activity was reported as umol P/g soil/h.

Phosphatase:

The procedure of Tabatabai (1982) was modified. The modifications included substituting distilled water for the Modified Universal Buffer, and incubation at 25 ± 0.5 C. para-nitrophenol phosphate was the substrate used for this assay. Enzyme activity was reported as umol nitrophenol/g soil/h.

Sulfatase:

The procedure of Tabatabai (1982) was used and modified. Modifications included the substitution of distilled water for the acetate buffer, and incubation at 25 ± 0.5 C. The substrate utilized in this assay was p-nitrophenol sulfate. Activity was reported as umol nitrophenol/g soil/h.

Dehydrogenase:

One gram of soil (air dry weight) was transfered to a 30 mL

test tube. One milliliter of substrate solution (yielding triphenyl tetrazolium chloride (TTC) at 90 umol TTC/g soil) was added to the soil and incubated 24 h. at 25 C. Extraction with 10ml 95% methanol was conducted by mixing the methanol and soil, then pouring the suspension into a funnel with No.42 filter paper (Whatman), and finally washing the sample on the filter paper with an additional 10 mL of 95% methanol. The extracted triphenyl formazan was then analyzed colorimetrically on a Bousch and Lomb Spect-20 at 545nm. Soils with no TTC added were incubated and extracted for subtaction of background.

ALUMINUM ASSIMILATION STUDY:

Bacillus megaterium (B-12) and Rhizobium spp. (I-110) were grown in culture media containing 1.0 g glucose and 1.0 g of yeast extract (Difco) in 100ml of water. They were incubated at 25 C for 24 and 48 hours, respectively. The broth cultures were centrifuged and washed 3 times with 0.02 M acetate buffer at pH 4.6. The third washing was decanted and the cell pellet resuspended in 30 mL of acetate buffer. There were five treatments for both B. megaterium (B-12) and Rhizobium spp. (I-110). The treatments included a control with no Al and four Al treatments. The four Al treatments were all brought to 1.11 umol Al/mL in solution. The first two of the four treatments were divided into azide and non-azide treatments. The next two treatments included 0.01 umol Fe/mL ($FeCl_3$) with azide and non-azide subtreatments. The azide had a final concentration of 3.0×10^{-3} M . Biomass C was determined by wet oxidation as described earlier.

After incubation, the cell suspentions were centrifuged and washed 3 times with 0.02 M acetate buffer with a pH of 4.6. After the third centrifugation, the supernatant was decanted, the pellets resuspended in 10 mL 30% H_2O_2, and digested for one hour. Next, 5.0 mL of concentrated HCl were added to the suspentions giving a final molarity of approximately 5.0 M HCl. The samples were allowed to digest for 48 hrs. They were assayed for aluminum on a SMI (Beckman) DC plasma emission spectrophotometer at 308.2 nm.

SOIL CHEMISTRY ASSAYS:

Assays for pH, P, K, Fe, Mn, Zn, NO_3, and Organic C were done in accordance with the methods put forth by Danke (1980) in Recommended Chemical Soil Test Procedures for the North Central Region Calcium and Mg were assayed from the same extract (ammonium acetate) that was obtained for K.

Soil pH was determined by the water method, with the soil to water ratio 1:1 by soil dry weight (McLean, 1980). Potassium, Ca, and Mg were extracted from 2.5 g of air dry soil with 20 mL of 1.0 N ammonium acetate buffer at pH 7.0 (Carson, 1980). The extract was analyzed on a Technicon Autoanalyser II employing flame emission (propane) for K and Ca, and a colorimetric assay for Mg. Phosphorous was assayed using the Bray-P1-Ascorbic acid method for orthophosphate (Knudsen, 1980).

The micronutrients Fe, Mn, and Zn were extracted from 2.0 g of air dried soil with 20 mL of 0.1 N HCl (Whitney, 1980). The extracts

were assayed by atomic absorption on a Perkin-Elmer 290 atomic absorption unit.

Nitrate was assayed with an Orion nitrate ion selective probe. Twenty grams of air dry soil were extracted with 50 mL saturated $CaSO_4$. The slurry was then measured directly with the nitrate electrode (Carson, 1980).

Exchangeable Al was extracted with 1.0 N KCl (5.0 g/50 mL) according to the procedure of Barnhisel and Bertsch (1982). Aluminum was assayed by the aluminon colorimetric method.

Soil organic carbon was determined by chromic acid digestion. In this method, 1.0 g of soil was transfered to a 30 mL test tube. Ten millimeters of 0.5 M $Na_2Cr_2O_7$ were next added to the test tube, and then 10 mL conc. H_2SO_4 was added causiously. The samples were digested for 24 hours. Five milliliters of the digest were decanted and diluted with 10 mL of distilled water. After mixing, the sample was read at 645 nm on a Bousch & Lomb Spect-20. Soils of known organic C content were used as standards.

RESULTS AND DISCUSSION

Artificial Soil Study:

An artificial soil system was developed to examine the effect(s) of Fe^{+3} on bacteria-aluminum interactions. First, the effects of different exchangeable Al concentrations on microbial activity were examined in a sand-clay (95% sand, 5% clay) system. The results for the microorganisms Bacillus megaterium (B-12) and Rhizobium spp. (I-110) can be seen in fig. 2a and 2b respectively. These figures illustrate the effects of exchangeable Al on cell respiration in the form of carbon dioxide evolution rates versus substrate carbon concentration. With the organism B. megaterium (B-12), the effect of exchangeable Al on the carbon dioxide evolution rate was dramatic as exchangeable Al was increased from 0 to 2.22 umol exchangeable Al/g soil. B.megaterium (B-12) demonstrated a decrease in both the maximal velocity and the initial velocity. Carbon dioxide evolution for B.megaterium (B-12) was reduced by 90 to 100 percent when the exchangeable Al concentration was 2.22 umol exchangeable Al/g Soil (Fig. 2a).

With Rhizobium spp. (I-110), carbon dioxide evolution rates at 2.22 umol exchangeable Al/g soil were only reduced to 80 percent of that obtained with no exchangable Al present. The major effect on Rhizobium spp. (I-110) carbon dioxide evolution kinetics was the slight reduction of the maximal velocities (Fig. 2b).

The hyperbolic character of the data presented in figures 2a and 2b suggested that the best assessment of this microbial activity would come from Monod growth kinetics analysis (Spain, 1982). Table 1 gives the parameters derived from the data in figures 2a and 2b using the

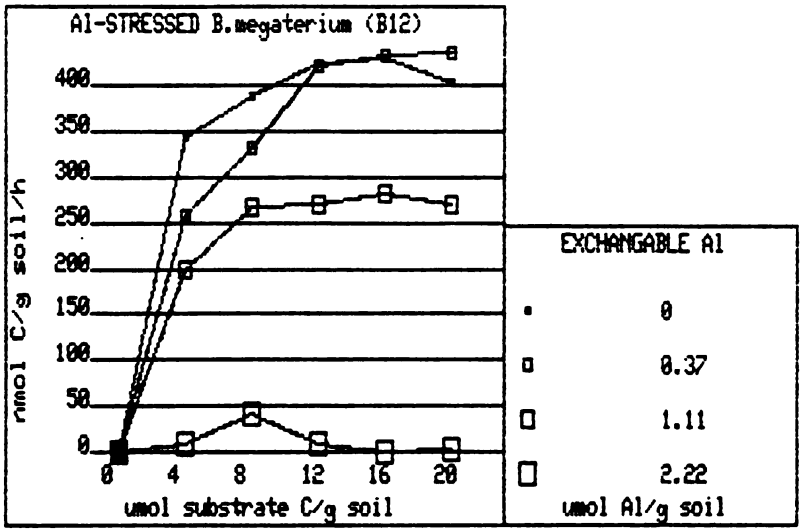

Fig. 2a. Aluminum stressed Bacillus megaterium (B-12) with glucose-yeast extract (1:1) as C substrate.

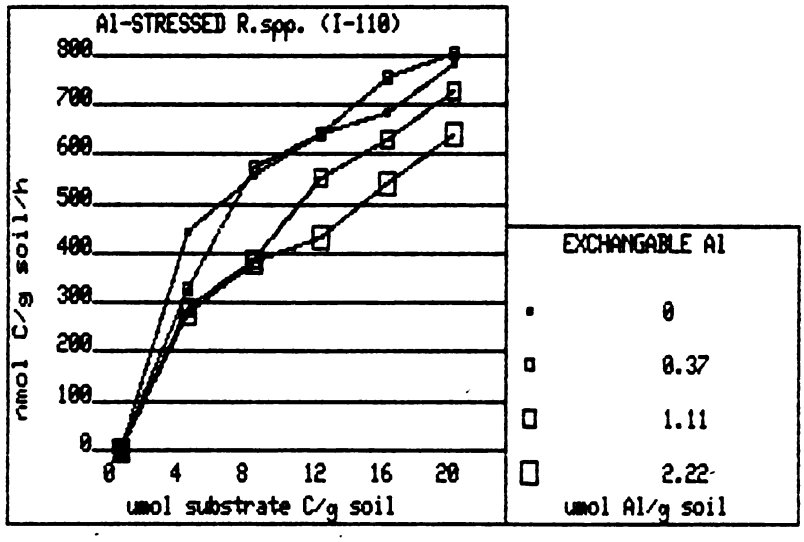

Fig.2b. Aluminum stressed Rhizobium spp. (I-110) with glucose-yeast extract (1:1) as C substrate.

Table 1. Monod model parameters for carbon dioxide evolution from artificial soils under aluminum stress.

	Bacillus megaterium (B-12)			
umol Al/g soil	0	0.37	1.11	2.22
u (nmol C/g soil/h)	473	537	323	51.9
K (umol C/g soil)	1.58	4.40	2.17	27.3
X (Biomass, ug C/mL)	200	200	200	200

	Rhizobium spp. (I-110)			
umol exch. Al/g soil	0	0.37	1.11	2.22
u (nmol C/g soil/h)	958	1226	1000	937
K (umol C/g soil)	5.50	10.8	15.3	11.6
X (Biomass, ug C/mL)	200	200	200	200

Monod model equation:

$$dX/dt = uX = u \cdot (S/(K+S)) \cdot X$$

S = umol substrate C/mL

Monod growth model. Upon amendment of Fe^{+3} to Al stressed sand-clay artificial soil systems, both B. megaterium (B-12) and Rhizobium spp. (I-110) demonstrated an intensified Al stress response (Fig. 3a and 3b).

The similarity in chemical properties of Al and Fe^{+3} at pH 4.6 suggests that microorganisms might accumulate Al through the Fe^{+3} uptake mechanisms.

An important reason for examining the antagonistic effects of Fe on Al stress lays in the soil chemistry of Al stressed environments. Aluminum stressed soils exist primarily in the tropic and subtropic environments of the world. These soils are primarily oxisols in classification. They are typified as low pH, highly weathered soils containing kaolinitic clays and relatively high concentrations of Al and Fe oxides. Ecologically, the oxisol just described might represent a similar antagonistic environment as exhibited in the experiments shown in Fig. 3a and 3b.

In pure culture studies, several authors (De Carvalho, et.al., 1981; and Hartel and Alexander, 1983) have suggested that slow growing Rhizobia species in the presence of soluble Al demonstrates no major inhibitory response. The data presented in figure 2b for Rhizobium spp. (I-110) support these views. Aluminum on its own demonstrated little inhibitory effect on Rhizobium spp. (I-110).

From an ecological viewpoint, soluble Fe^{+3} could be expected to be present in acid soils. Figures 3a and 3b illustrate the devastating effect of soluble Fe^{+3} under Al stress conditions.

When Fe^{+3} is insoluble or bound, bacteria can utilize a series of biologically produced chelating compounds to assimilate Fe

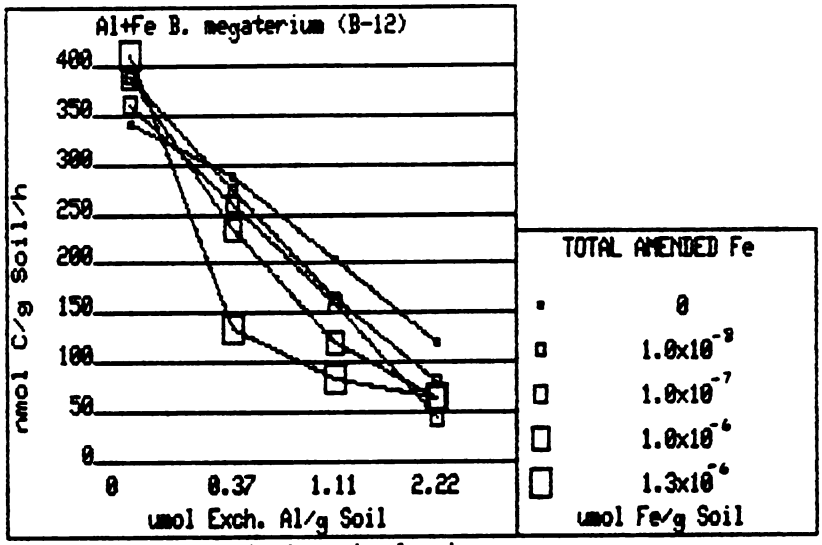

Fig. 3a. Iron induced aluminum stressed B. megaterium (B-12) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil.

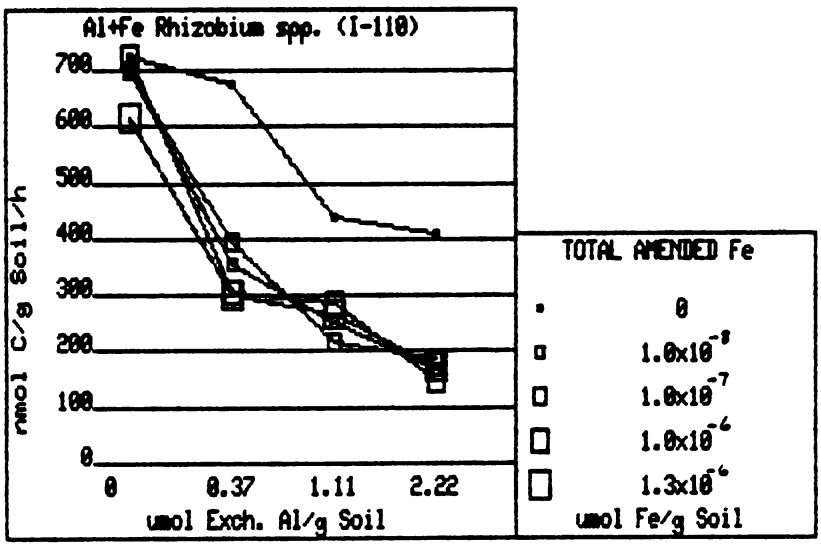

Fig. 3b. Iron induced aluminum stressed Rhizobium spp. (I-110) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil.

. Since these are truely chelating compounds, it is suspected that their affinity for Al is low because of Al's rigid electronic configuration, unlike the flexible configurations of Fe^{+3} for ligand binding. Davis and Byers (1971) demonstrated the ability of Fe^{+3} chelating agents produced by B. megaterium to chelate Fe^{+3} coprecipated by 10 uM Al, with no apparent interference from Al. Emery (1974) has shown that certain siderochromes (biological Fe(III) chelating agents) which bind Cu(II) are not transported across microbial membranes. Selective binding of Cr(III) by Fe siderochromes have been demonstrated. Leong (1971) showed that Salmonella typhimurium assimilated Cr(III) chelated by bacterial siderochromes. The only other siderochrome system found which was interfered with by other metals was ferrichrome. Ferrichrome is only produced by fungi, but has been found to transport Al across the cell membranes of Ustilago spaerogena (Emery, 1974). All Fe chelate transport systems have been found to be repressible by high Fe^{+3} concentrations (Downer, et. al., 1970; Silver, 1978).

Frost and Roseberg (1975) have demonstrated a low affinity membrane carrier for uncomplexed Fe^{+3} . The carrier is not dependent on metabolic energy and is suspected to be a facillitating transport permease for Fe^{+3} . This permease is not repressible by high Fe^{+3} concentrations, and is presumed inducible by its substrate as are most permeases (Silver, 1978).

The investigator suggests that the differences seen in the initial kinetics between B.megaterium (B-12) and Rhizobium spp. (I-110) under Fe induced Al stress lay in the morphological differences between the two microorganisms. Bacillus megaterium (B-12) is a gram

(+) microorganism with the outer cell surface (cell wall and cell membrane) directly exposed to the local environment. Rhizobium spp. (I-110) is a gram (-) microorganism with the cell-proper shrouded behind the outer membrane. Therefore, one could postulate a more direct and dynamic effect by antagonistic inorganic ions for gram (+) microorganisms. This concept is indeed supported by the data presented in Fig. 2a, 2b, 3a, and 3b. The most likely involvement of the outer membrane under Al stress is to function as a limiting diffusional barrier against Al associating with the cell proper for gram (-) microorganisms.

Under Al stress, Rhizobium spp. (I-110) cells seemed relatively immune to antagonistic effects by Al. The maximum CO_2 evolution rate decreased only slightly for Rhizobium spp. (I-110) (Fig. 2b). With the occurrence of Fe induced Al stress, the outer membrane protection may have been compromised (Fig. 3b). The investigator suggests that under Al stress, Al enters the periplasmic space, that a secondary diffusional barrier might arise. The periplasmic space may become saturated with loosely bound Al. A variety of phospholipids and proteins lining the periplasmic space could provide ample binding sites. Once saturated with Al the diffusion gradient for Al across the outer membrane could collapse offering a weak protective effect. Aluminum binding in the periplasmic space could even slightly concentrate Al to the extent of reversing the diffusion potential for Al across the outer membrane. Suzuki, et. al. (1976) demonstrated that a gram (-) mutant, Escherichia coli , occurring with structural changes in an outer membrane lipoprotein was more inhibited by a variety of metal ions than was the wild type. Their study suggested that the E.coli mut-

ants outer membrane no longer functioned as a impermeable barrier to the ions under examination. This mutant demonstrated a marked inhibitory response to the metal ions used in the study. This article lends support to the concept of the outer membrane of gram (-) microorganisms forming a protective barrier against general metal toxicities. Once this barrier is defeated the gram (-) organism is likely subject to the same general effects of metal toxicities as are gram (+) organisms.

Duxbury and Bicknell (1983) have demonstrated that in soils subjected to a variety of toxic metals, the gram (-) organisms exhibited a greater tolerence to metal toxicity or stress than did the gram (+) organisms. This article lends support to the mechanism postulated for explaining the less dynamic response of Rhizobium spp. (I-110) to Al stress. This investigator suggests that when Fe is not present, the outer membrane of Rhizobium spp. (I-110) acts as a formidable barrier to Al under Al stress conditions. If increasing soluble Fe^{+3} induces the Fe^{+3} transport system, the permeability barrier of the outer membrane of Rhizobium spp. (I-110) may collapse due to the import and the binding of Al due to Fe^{+3} transport sites or import of other Al bound compounds.

Figures 3a and 3b illustrate the effects of $FeCl_3$ amendments on Al stressed microbial activity for the test organisms. The range of $FeCl_3$ concentrations used was 0 to 10^{-6} M . The average calculated range of molar activities for those Fe^{+3} amendments was 10^{-10} to 10^{-11}, depending on whether Fe^{+3} was assumed to be in equilibrium with amorphous $Fe(OH)_3^o$ or soil $Fe(OH)_3$ as defined by Lindsay (1979). When no free Fe^{+3} was present, a fairly linear decreasing response was exhibit-

ed by B. megaterium (B-12) to increasing exchangeable Al concentrations (Fig. 3a). As the Fe concentration was increased, the Al stress response of B. megaterium (B-12) demonstrated a greater exponential character. There were discernable differences among treatments.

The same general trends were seen with Rhizobium spp. (I-110) as were seen with B. megaterium (B-12) (Fig. 3b). When no Fe^{+3} was present, the Al stress response curve took on a reverse "S" shape. The various Al stressed Fe^{+3} treatments exhibited a decreasing exponential character with no discernable differences among the Fe^{+3} treatments (Fig. 3b).

To further explore the effects of Fe^{+3} on Al stressed microbial activity, chelated forms of Fe^{+3} were amended to the Al stressed systems. The forms studied included Fe(III)-EDTA, Fe(III)-NTA, and Fe(III)-Citrate. The use of chelated forms of Fe^{+3} allowed greater control of soluble Fe^{+3} in solution. For each chelate, treatments of 10^{-2}, 10^{-4}, and 10^{-6} M total Fe(III)-chelate were added for a given treatment. The activity of Fe^{+3} then varied from chelate to chelate and from one total concentration to another.

Aluminum stressed B. megaterium (B-12) demonstrated a geat difference in activity between the Al only control and Al plus Fe(III)-EDTA treatments (Fig. 3c). While there was a degree of variation among treatments, there were no discernable trends among the Fe(III)-EDTA treatments. Again, the Al only control for B. megaterium (B-12) responded with generally a linear decreasing response to increasing Al (Fig. 3c). The activity response to the Al plus Fe(III)-EDTA treatments was not clearly exponential in character. However, it did decrease rapidly at low Al concentrations and generally leveled off at

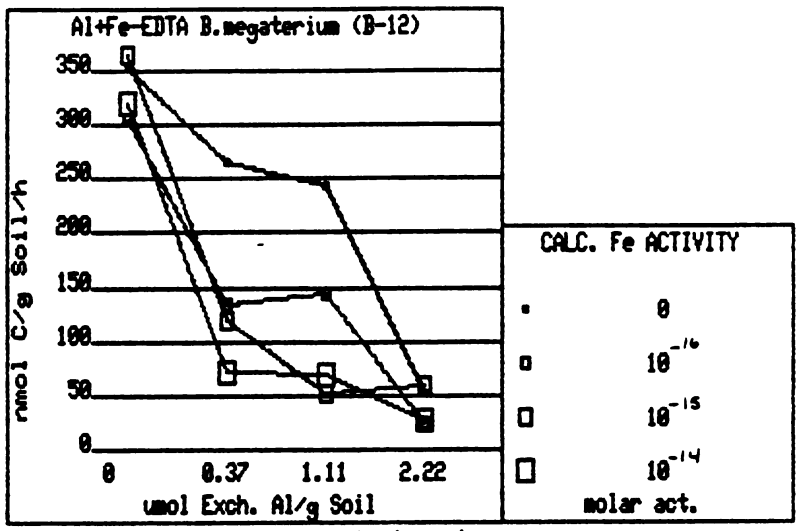

Fig. 3c. Fe(III)EDTA induced aluminum stressed B. megaterium (B-12) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil, at pH 4.6.

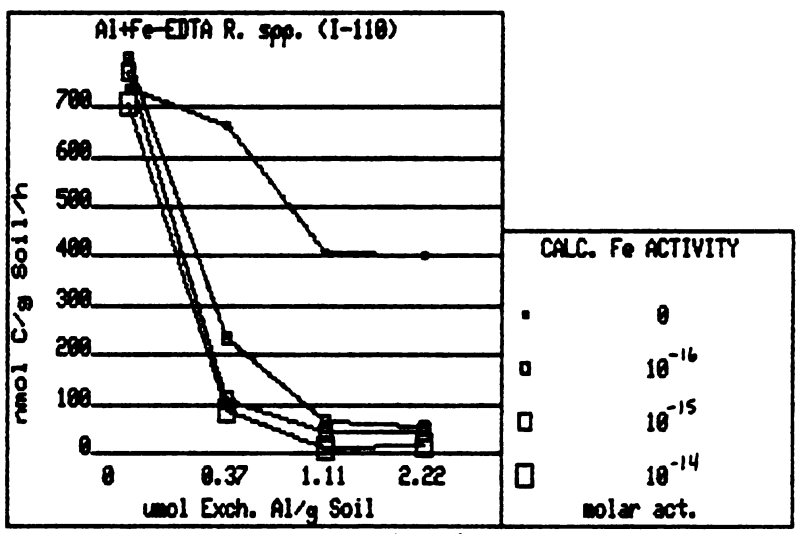

Fig. 3d. Fe(III)EDTA induced aluminum stressed Rhizobium spp. (I-110) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil, at pH 4.6.

1.11 and 2.22 mol Exch. Al/g soil.

With Rhizobium spp. (I-110) the Al only control exhibited a reversed "S" shape character. The Al plus Fe-EDTA treatments demonstrated a strong exponential character in the decreasing inhibitory response. Iron(III)-EDTA should have given the lowest molar activity treatment of Fe^{+3} to which the test organisms were exposed. The stability constant for the Fe(III)-EDTA complex was 25 (Log K).

The next treatment involved the amendment of Fe(III)-NTA to the Al stressed artificial soil system. The stability constant for the Fe(III)-NTA complex was approximately 17 (Log K). Bacillus megaterium (B-12) exhibited a general linear decrease in activity in response to increasing exchangeable Al concentrations (Fig 3e). For the Fe(III)-NTA treatment, an exponential decrease in activity as described for Fe(III)-EDTA treatments was observed (Fig. 3e). The Fe(III)-NTA plus Al treatments demonstrated depressed activity in comparison with the Al only control (Fig. 3e).

The data in figure 3f exhibited an exponential decrease in activity for the Al plus Fe(III)-NTA treatments for Rhizobium spp. (I-110). The decrease was similar to that described in figures 3b and 3d.

Finally, the test organisms were treated with Fe(III)-Citrate under Al stress conditions (Fig 3g and 3h).
The Fe(III)-Citrate treatments yielded curves of similar character as that obtained for the other Fe chelate treatments.

Generally, B. megaterium (B-12) exhibited a linear decrease in carbon dioxide evolution with respect to increasing exchangeable Al. Carbon dioxide evolution was further depressed in an exponential fashion in the presence of soluble Fe^{+3} (Fig. 3a, 3c, 3e, and 3g). Iron in

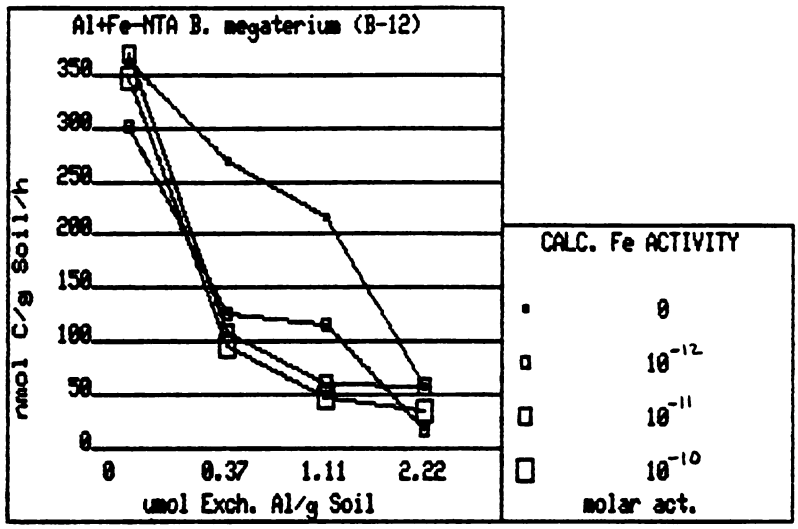

Fig. 3e. Fe(III)NTA induced aluminum stressed B. megaterium (B-12) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil, at pH 4.6.

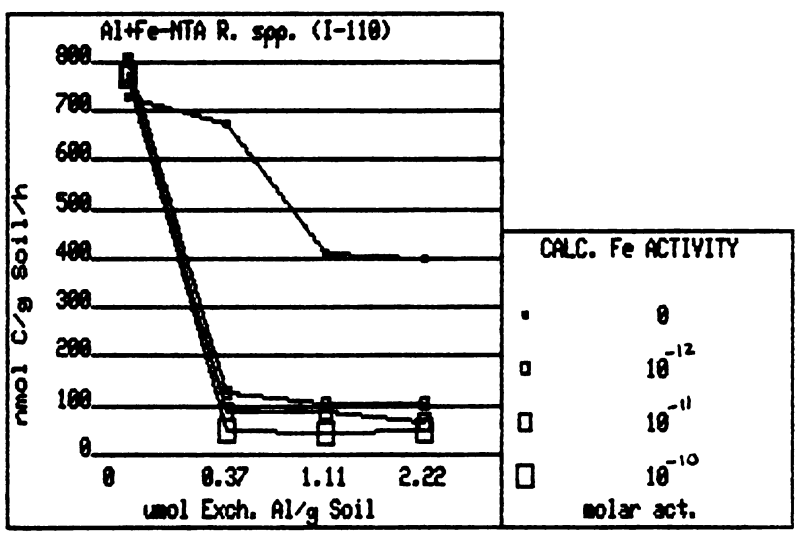

Fig. 3f. Fe(III)NTA induced aluminum stressed Rhizobium spp. (I-110) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil, at pH 4.6.

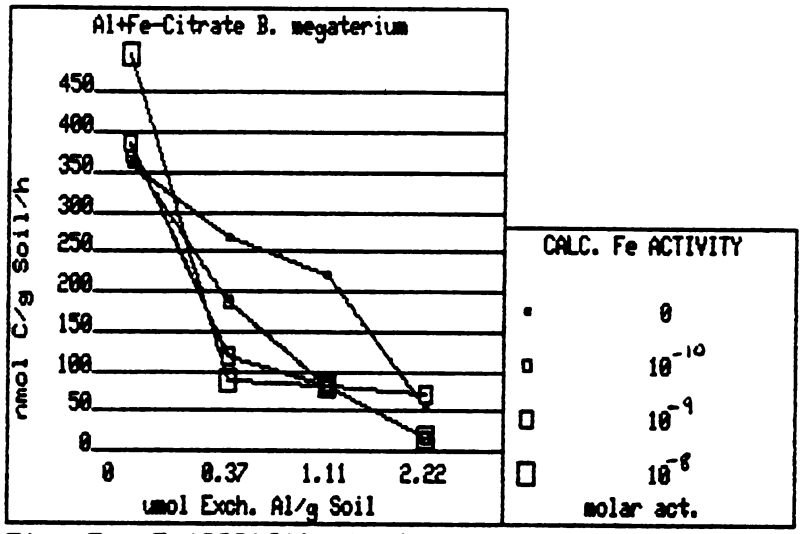

Fig. 3g. Fe(III)Citrate induced aluminum stressed B. megaterium (B-12) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil, at pH 4.6.

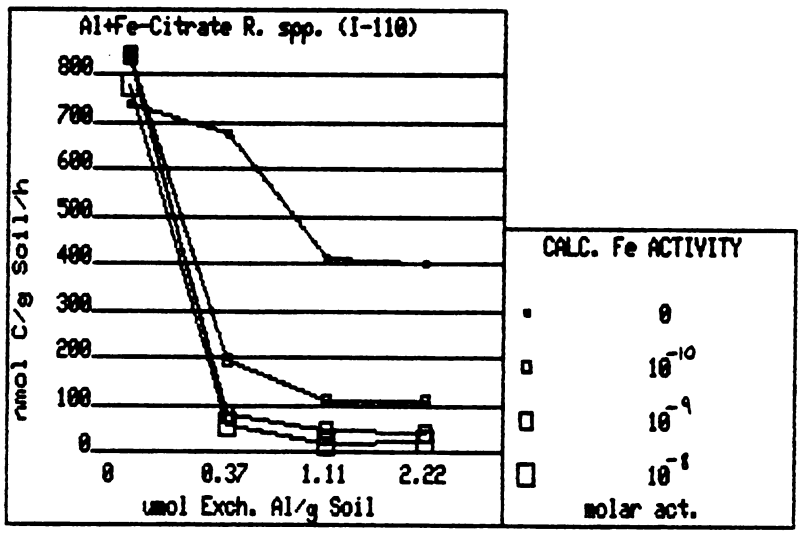

Fig. 3h. Fe(III)Citrate induced aluminum stressed Rhizobium spp. (I-110) with glucose-yeast extract (1:1) as the C substrate at 8 umol C/g soil, at pH 4.6.

the absence of Al generally exhibited a slightly enhancing effect or no effect towards microbial activity.

With only Al present, Rhizobium spp. (I-110) typically gave a decreasing carbon dioxide evolution response to increasing Al in the soil system. This decrease took the form of the reverse "S" curve described earlier. With soluble Fe^{+3} present, Al stressed Rhizobium spp. (I-110) exhibited a strong exponential decrease in CO_2 rate response to the increasing Al concentration (Fig. 3b, 3d, 3f, and 3h).

To further substantiate the Fe-Al microbial interaction, an experiment with both microorganisms was conducted to establish Al assimilation. Both B. megaterium (B-12) and Rhizobium spp. (I-110) were incubated in media in separate experiments with treatments of no Al (control), 1.11 umol Al/mL, Al only, and 1.11 umol Al/mL plus 0.01 umol Fe^{+3} /mL. With both organisms, Fe^{+3} was found to increase the amount of Al associated with the bacterial biomass (Fig. 4a and 4b). It is not possible to conclusively prove with this data that Al was assimilated into the cells, and not associated by means of surface interactions. However, if the association of Al with these cells were due to surface interactions only, one might expect a dilution effect or no change in Al concentration associated with the biomass when compared to the Al-only and azide controls.

Because there was a significant increase in associated biomass Al when compared to the azide controls, the investigator suggests that the increase is due to an uptake of Al associated with Fe^{+3} assimilation. This conclusion is supported indirectly by the observations of Keyser and Munns (1979b) with Fe(III)-EDTA described earlier, and by the observations of Cooper and Morgan (1979a and 1979b) when they not-

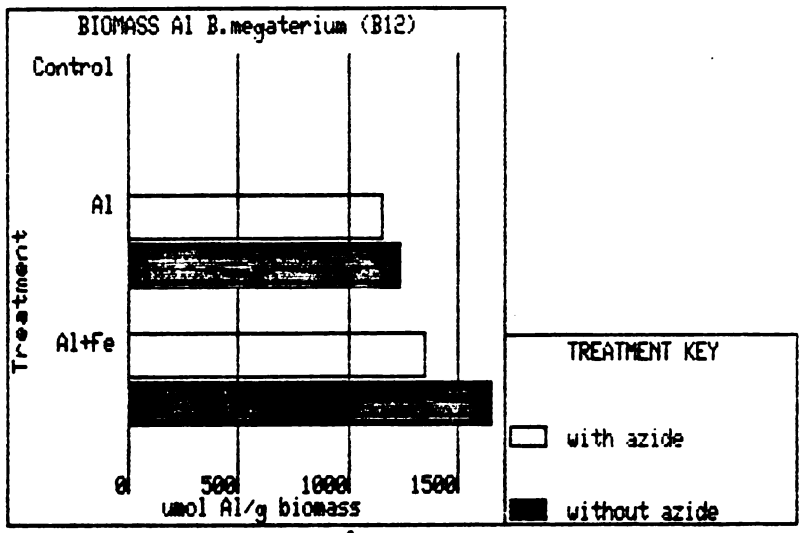

Fig. 4a. Aluminum-biomass association experiment for B. megaterium (B-12). The concentration of aluminum was was 1.11 umol Al/mL, and iron was 0.018 umol Fe(III)/mL.

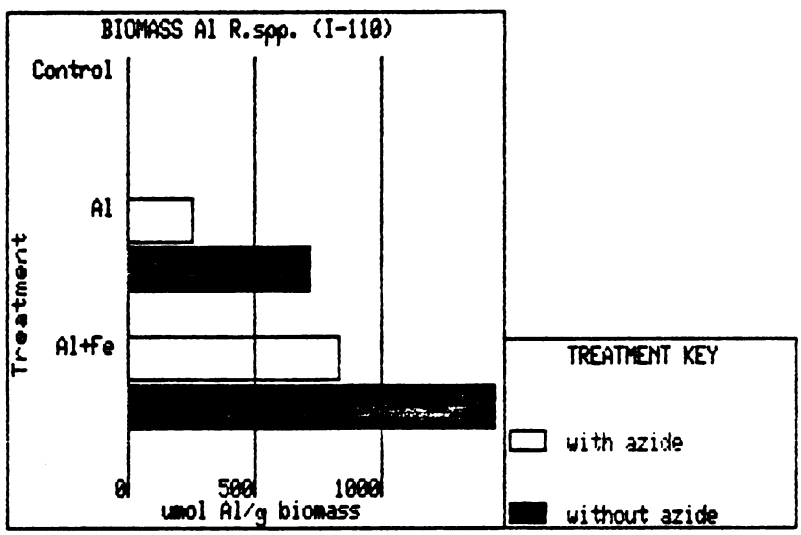

Fig 4b. Aluminum-biomass association experiment for R. spp. (I-110). The concentration of aluminum was 1.11 umol Al/mL, and iron was 0.018 umol Fe(III)/mL.

ed increased aluminum stress in the presence of amorphous Fe .

To futher assess the effects of Al and Al plus Fe on microbial growth, turbidity studies were conducted. There were five treatments for each of the test organisms. The first treatment was the control consisting of glucose and yeast extract (GY). The second treatment included GY and Fe(III)Citrate, with a calculated Fe^{+3} activity of 1.0 x 10^{-9} M . The third treatment included GY and Al at 3.8 x 10^{-6} M . The fourth treatment was the same as the second except that at 18 hours Al was added giving a final Al concentration of 3.8 x 10^{-6} M . The fifth and final treatment was the same as the third except that at 18 hours, Fe(III)-Citrate was added giving a calculated Fe^{+3} activity of 1.0 x 10^{-9} M . All culture media were buffered with acetate buffer at pH 4.6 and incubated at 25 C for 48 h. Figure 5a illustrates the growth study results for B. megaterium (B-12) with the five treatments just described. Optical densities for the GY and GY + Fe reached their maximums by 30 h. Treatment GY + Fe > Al, with Al added at 18 h, reached maximum optical density by 42 h. Treatment GY + Al for B. megaterium (B-12) reached its submaximum at approximately 40 h. Treatment GY + Al > Fe , with Fe-Citrate added at 18 h exhibited the greatest inhibitory response of any of the treatments for B. megaterium (B-12) in these experiments (Fig. a). The same treatments for Rhizobium spp. (I-110) yielded a similar distribution of growth curves (Fig. 5b). The most interesting aspect of figures 5a and 5b are that the cells grown in the presence of 3.8 x 10^{-6} M Al and subsequently amended with Fe(III)Citrate exhibited the most intense inhibitory responses. In the presence of GY and Fe(III)Citrate, both organisms demonstrate an enhanced growth response relative to GY only.

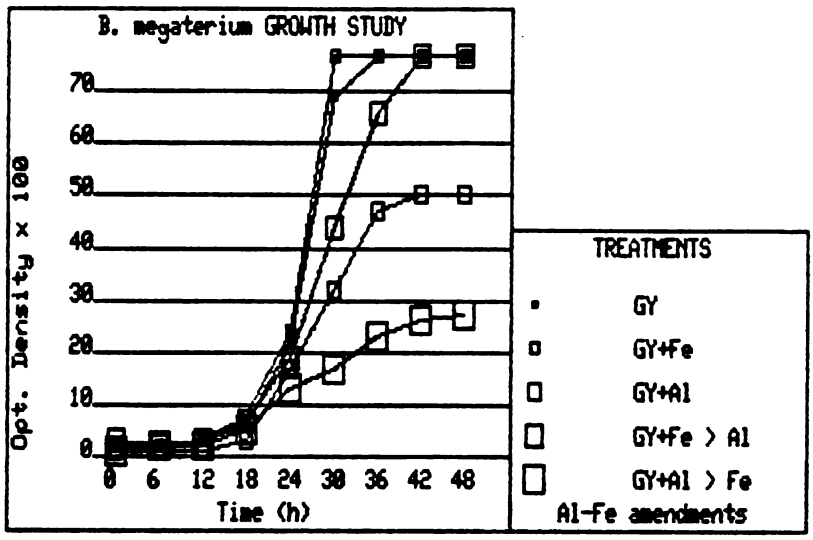

Fig. 5a. Fe(III)Citrate induced aluminum stress time study for B. megaterium (B-12). Glucose-yeast extract (GY), Fe source Fe(III)Citrate (Fe), and Al source $AlCl_3$ (Al).

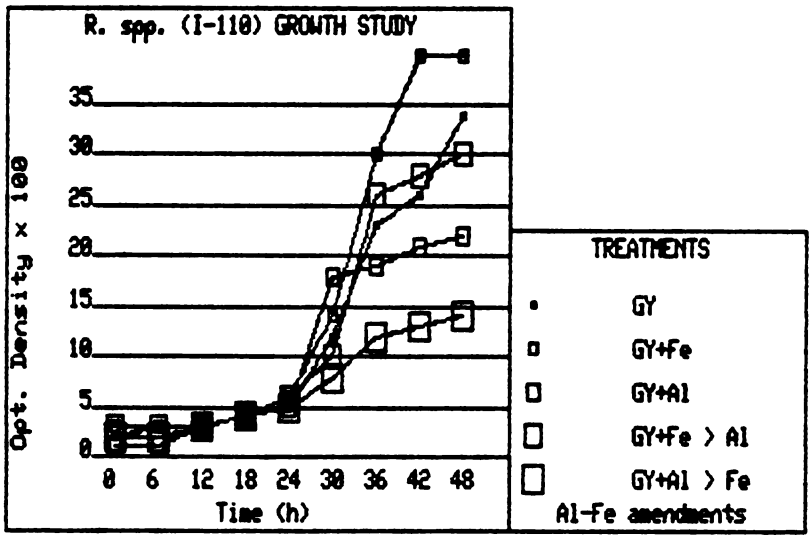

Fig. 5b. Fe(III)Citrate induced aluminum stress time study for Rhizobium spp. (I-110). Glucose-yeast extract (GY), Fe source Fe(III)Citrate (Fe), and Al source $AlCl_3$ (Al).

The low rate of growth for the GY+Al>Fe treatment suggests an interaction between involving Al and Fe(III)Citrate which intensifies Al stress interactions with these microorganisms.

There would seem to be three possible explanations for the Fe induced Al stress response. One explanation might include surface interactions with the microorganisms. Certainly, carboxyl and hydroxyl groups on cell surfaces provide ample binding sites for Al. What is hard to reconcile is the difference in the results between the GY+Fe>Al and GY+Al>Fe treatments (Fig. 5a and 5b). If the Fe induced Al stress response was due to precipitation or exchange phenomena, the difference in the net response of the last two treatments would not be expected. The use of chelated Fe^{+3} should preclude any major precipitation phenomena. One argument in favor of surface exchange phenomena might be related to the specific order of amendments. If Al is added first (GY+Al>Fe), then subsequent addition of Fe might displace Al from exchange sites causing hydrolysis of water and production of H^{+}, lowering the pH. However, the systems under study were both buffered for pH and in equilibrium with Fe(III)Citrate. Between the buffering capacity of the acetate buffer and free citrate taking up H^{+}, pH changes should have been negligible.

The second explanation lays in the possibility of the formation and uptake of Al-Citrate. Undoubtly, formation of Al-Citrate will occur due to the equilibrium product of free citrate from the presence of Fe(III)Citrate. In the case of B. megaterium (B-12), it is well documented that this organism can assimilate and utilize Fe(III)Citrate for both the Fe and citrate components (Byers and Arceneaux, 1976). If Al-Citrate were to compete or be assimilated by other

means, a toxic accumulation of Al might occur intracellularly.

The third explanation is similar to the second. The effects of Fe^{+3} have in general been demonstrated to enhance microbial growth rates in this study in the absence of Al. The implicit increase in general metabolic rates in the presence of Fe may increase the cellular import of one or several nutrients which in the presence of aluminum bind Al. Again, accumulation of intracellular Al could occur to toxic levels if such compounds were imported. Further complicating this explanation, this indirect effect could manifest itself in a non-linear fashion.

Figure 5c demonstrates the relationship between accumulative CO_2 evolution and time for treatment GY for both test organisms.

In summary, it has been demonstrated that under Al stress conditions, B.megaterium (B-12) appears to demonstrate primarily a non-competitive inhibitory response. Rhizobium spp. (I-110) exhibited only the slightest indication of non-competitive inhibition under Al stress. With Fe induced Al stress, B.megaterium (B-12) and Rhizobium spp. (I-110) both demonstrated intensified inhibitory responses.

These results suggest that the assessment of Al stress on microbial activity must include analysis of factors which might appear secondary to the stress effect. In this case, past ignorance of environmental factors such as the presence of Fe^{+3} in the natural environment have likely led to false assumptions about Al stress effects on microbial systems. Particularly, in oxisols which compose the majority of known acid Al soils, the effect of soluble Fe^{+3} likely to be present can not be overlooked in light of the data presented here. Several papers recently published (De Carvalho, et.al., 1981; Hartel and

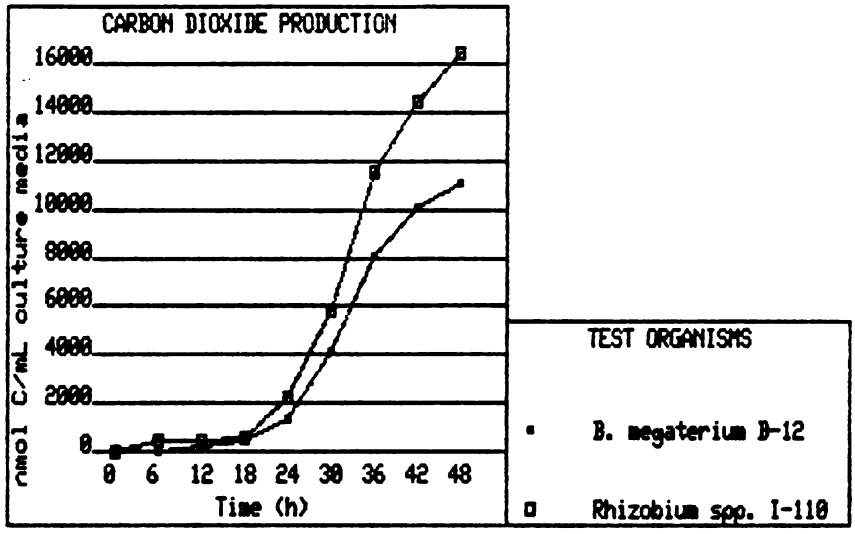

Fig. 5c. Carbon dioxide production for both test organisms shown in figures 5a and 5b. Carbon dioxide production monitored were for the GY treatments only. This data can be used to relate CO_2 production to biomass production in figures 5a and 5b.

Alexander, 1983) have suggested that Al stress is vertually nonexistant for Rhizobium species. However, neither group of investigators took into account the effects Fe^{+3} on Al stress.

NATURAL SOIL STUDIES:

To examine the plausibility of Fe^{+3} induced Al stress occurring in nature, several natural soils including an Al stressed soil were studied. The major portion of the study involved a comparison of the carbon dioxide evolution characteristics in a fashion similar the simulated soils discussed earlier. These studies included examination of the soils amended with only a carbon source (control), and the same soils amended with the same carbon source plus Fe^{+3}. The carbon source amended to the soils included 4, 8, 12, 16, and 20 umol substrate C/g soil. The substrate C source consisted of glucose. Iron amendments included 0.01, 0.1, 1.0, and 1.3 umol Fe^{+3}/g soil treatments of Fe^{+3} as $FeCl_3$. The general chemical and biological-biochemical characteristics of the soils collected for this study are given in Table 2. Table 3 gives the Monod growth parameters derived from data in figures 6a and 6b for the soils collected for this study.

Five of the soils sampled from around the state of Michigan were considered non-Al stressed. The general criteria used to define an Al stressed soil included a soil pH < 5.5 (soil to water, 1:1), and > 0.37 umol exchangeable Al per gram of soil. The Al stressed soils used in this study were located at the Kellogg Biological Station operated by Michigan State University, Hickory Corners, Michigan. The Al stressed soil samples were collected along a 200 m transect, acquiring 30 subsamples at each station. There were 5 stations, 40 m

Table 2. Soil chemistry and biological assays

	Aluminum stressed soils					Low aluminum soils				
	umol/g soil									
ASSAY	IA	IIA	IIIA	IVA	VA	IB	IC	ASIA	FSIA	CK19
pH	5.20	4.70	4.70	4.50	4.60	6.20	7.50	5.90	7.10	7.50
K	2.38	3.49	3.87	3.10	2.97	0.85	1.10	4.36	0.33	2.26
Ca	6.50	4.85	8.15	4.85	8.15	28.1	28.1	5.45	9.63	74.7
Mg	0.25	0.33	1.08	0.58	2.75	7.88	7.88	1.67	3.67	8.92
Fe	0.39	0.39	0.30	0.39	0.39	0.48	0.66	0.80	0.39	0.21
Mn	0.31	0.29	0.31	0.31	0.15	0.20	0.24	0.55	0.40	1.29
Zn	0.03	0.03	0.05	0.03	0.02	0.02	0.03	0.02	0.03	0.05
Al	1.19	2.85	2.22	2.96	5.44	0.11	0.04	0.15	0.07	0.00
PO_4	1.48	1.65	0.68	0.68	0.52	0.81	1.39	2.03	0.61	1.58
ORGC	841	783	867	841	700	783	841	992	816	1725
BMC	26.8	23.7	23.7	13.9	18.0	26.3	15.9	27.3	18.0	36.5
BMFeC	20.6	6.80	24.7	14.9	5.20	27.3	19.6	30.8	25.6	39.1
BMMgC	32.4	14.4	23.7	15.4	15.9	13.9	7.80	10.3	25.8	59.2
PP	2.30	2.50	2.70	0.90	1.00	1.50	2.70	3.20	4.10	4.10
ϕ-P	12.1	9.90	8.10	9.40	8.10	18.8	14.8	0.00	18.8	9.40
ϕ-S	8.10	8.60	9.40	9.40	10.7	8.10	7.25	0.00	12.9	10.8
DH	5.72	0.75	0.75	0.00	0.00	23.8	19.2	37.1	21.1	55.2

ORGC-(umol soil organic C/g soil)
BMC-(umol biomass C/g soil)
BMFeC-Fe(III) amended soil biomass C (umol biomass C/g soil)
BMMgC-Mg amended soil biomass C (umol biomass C/g soil)
PP-Pyrophosphatase activity (umol P/g soil/h)
ϕ-P -Phosphatase activity (umol p-nitrophen./g soil/h)
ϕ-S -Sulfatase activity (umol p-nitrophen./g soil/h).
DH-Dehydrogenase activity (umol TPF/g soil/h)

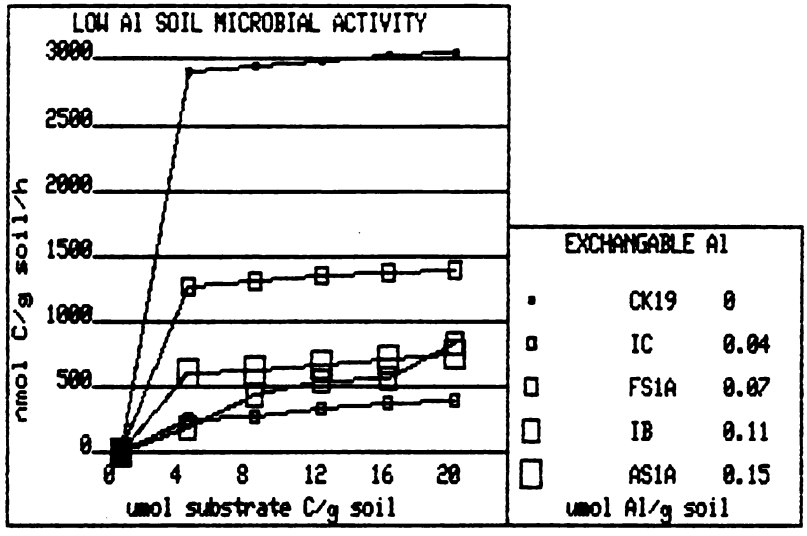

Fig. 6a. Carbon dioxide evolution rates from low aluminum soils. Carbon substrate used was glucose.

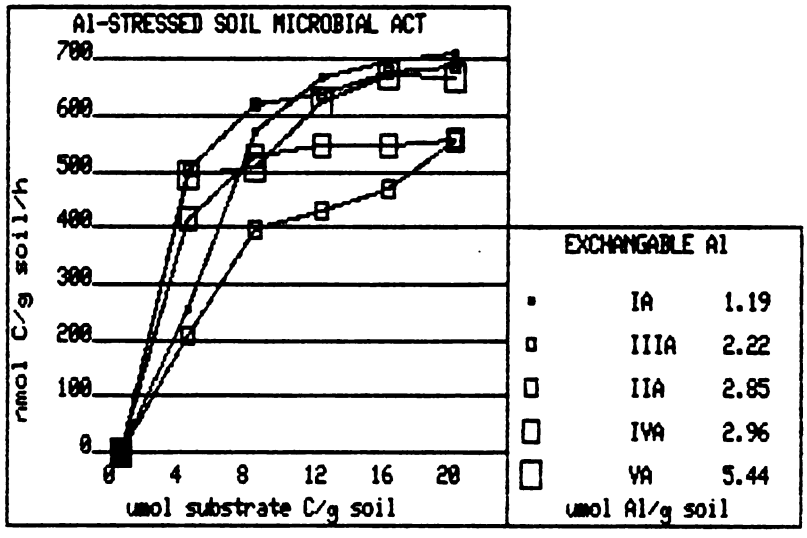

Fig. 6b. Carbon dioxide evolution rates from aluminum stressed soils. Carbon substrate used was glucose.

Table 3. Monod model parameters for carbon dioxide evolution from natural soils.

	Low Aluminum Stress Soils				
umol Al/g soil	0	0.04	0.07	0.11	0.15
u (nmol C/g soil/h)	3077	490	1433	736	800
K (umol C/g soil)	0.31	5.05	6.74	5.01	1.95
X (Biomass, ug C/mL)	438	191	216	315	327
	Aluminum Stressed Soils				
umol exch. Al/g soil	1.19	2.22	2.85	2.96	5.44
u (nmol C/g soil/h)	1277	755	845	602	939
K (umol C/g soil)	14.0	1.90	11.1	1.46	7.00
X (Biomass, ug C/mL)	321	284	284	167	216

Monod model equation:

$$dX/dt = uX = u \cdot (S/(K+S)) \cdot X$$

S = umol substrate C/mL

apart along the transect.

The carbon dioxide evolution activities for the non-Al stressed soils exhibited maximum velocities ranging from 490 to 3077 nmol C/g Soil/h (Fig. 6a) with the previously mentioned carbon amendments. Carbon dioxide evolution rates for the Al stressed soil gave a maximum velocity range of 602 to 1277 nmol C/g Soil/h (Fig. 6b). Aside from the differences in magnitude, the low-Al stressed soils demonstrated as a group a more rapid increase to the maximal velocity than did the Al stressed soil series (Fig. 6a and 6b).

Amendments of Fe^{+3} and C substrate to the low-Al stressed soils were seen to slighty increase the carbon dioxide evolution rates (Fig. 7a). When similar Fe^{+3} and C substrate amendments were made to the Al stressed soil series, activities not unlike that demonstrated by B.megaterium (B-12) (Fig. 3a), were observed (Fig. 7b).

Biomass estimates were assayed by the method of Anderson and Domsch (1978) for both group of soils. The biomass estimates were conducted with glucose-only, glucose-Fe^{+3}, and glucose-Mg.

Figures 8a and 8b show the results for the glucose only amendments plotted against the exchangable Al concentrations for both groups of soils. No trend can be seen in the low-Al stressed soils relating exchangeable Al to biomass carbon (Fig. 8a). For the Al stressed soil group, a generally decreasing trend for biomass carbon against increasing exchangeable Al can be seen (Fig. 8b).

When the same biomass estimates are made in the presence of Fe , significant changes in the biomass estimates occur relative to the glucose only estimates. The low-Al stressed soils all exhibited increases in biomass carbon compared to the glucose only estimates

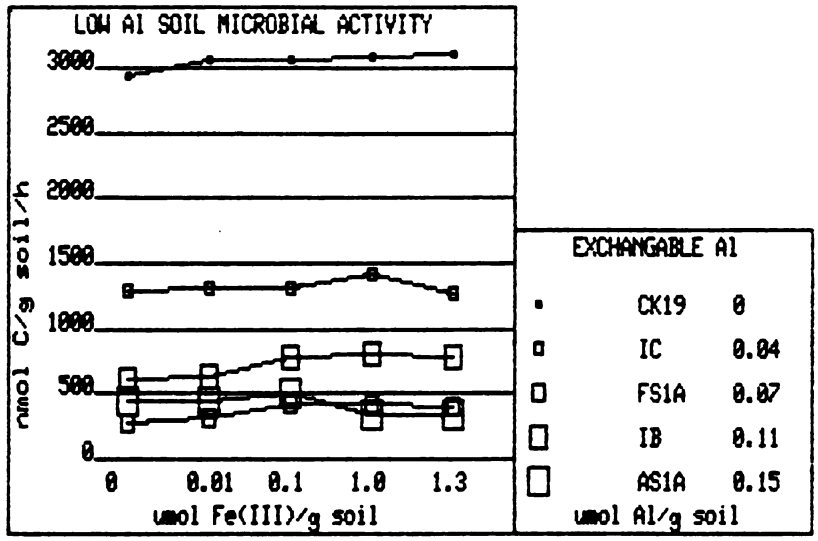

Fig. 7a. Carbon dioxide evolution rates from Fe(III) amended low aluminum soils. Carbon substrate used was glucose at 8 umol C/g soil.

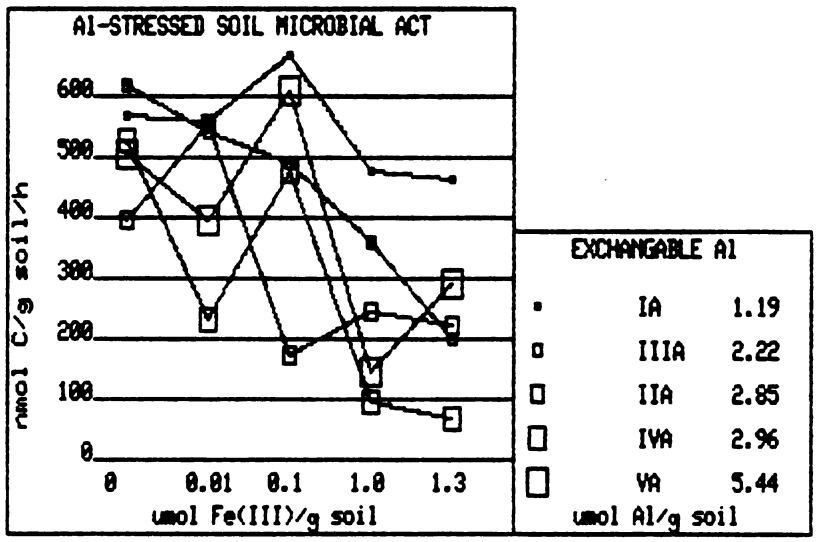

Fig. 7b. Carbon dioxide evolution rates from Fe(III) amended aluminum stressed soils. Carbon substrate used was glucose at 8 umol C/g soil.

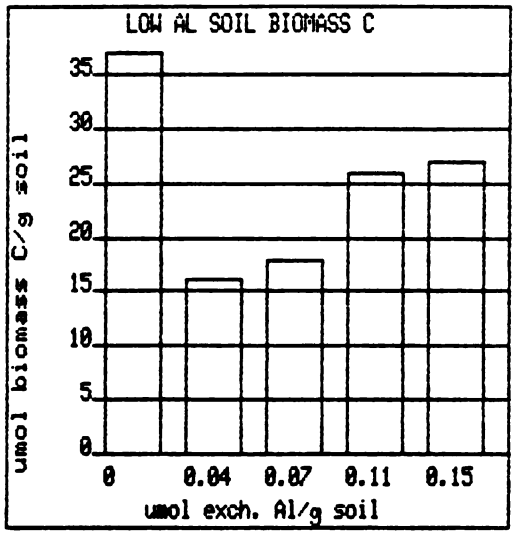

Fig. 8a. The relationship between soil biomass C and exchangeable soil Al for the low aluminum soils.

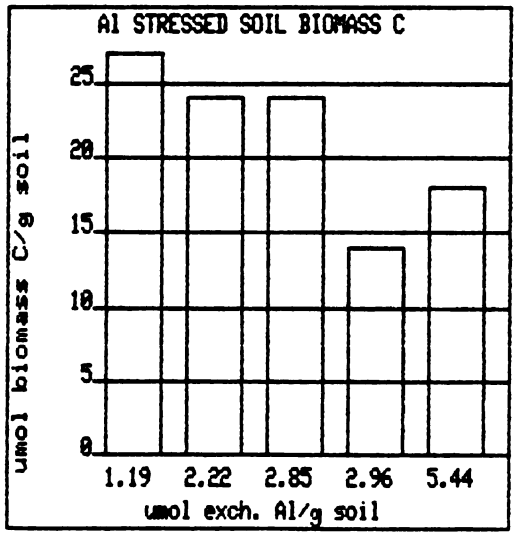

Fig. 8b. The relationship between soil biomass C and exchangeable soil Al for the aluminum stessed soils.

(Fig. 9a). With the Al stressed soils plus Fe amendment, all soils except IIIA demonstrated decreases in biomass carbon relative to the glucose only estimates (Fig. 8b and 9b). Significant differences were determined for the glucose and glucose-Fe biomass estimates using the F test. The F test was conducted at ($P < 0.05$) and ($P < 0.01$) levels of confidence.

The depressed biomass carbon estimates for the Al stressed soils amended with Fe^{+3} (Fig. 9b) would appear to support the previous hypothesis regarding iron induced Al stress.

A third treatment involving Mg amendments to the two groups of soils were conducted (Fig. 10a and 10b). Past investigators (Keyser and Munns, 1979a; Keyser and Munns, 1979b) have examined the effects of PO_4, Mn^{+2}, and Ca amendments on microbial activity under Al stress. These investigators found no significant releif from Al stress by these amendments. Examination of Al stressed soils often demonstrate low available Mg concentrations. In the low Al stress soils, random changes in active biomass C were observed (Fig. 8a and 10a). For the Al stressed soil amended with Mg, only the sample with 2.85 umol exchangeable Al/g Soil exhibited a significant decrease in biomass C, while the other demonstrated no significant difference from the glucose only treatments (Fig. 8b and 10b).

Hargrove and Thomas (1981) have demonstrated that increasing soil organic matter can relieve the phytotoxic effects of Al in Al stressed soils. They showed that increasing soil organic matter from 1 to 2 percent could increase the dry weight yield of barley by 10 to 15 percent. Figures 11a and 11b demonstrate the relationship between biomass C and soil organic matter for the two groups of soils. In

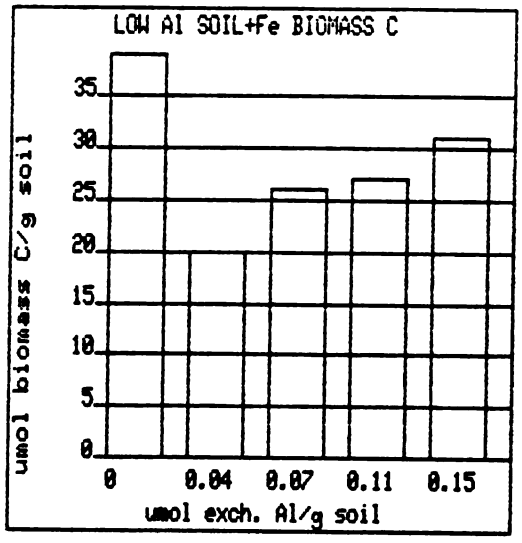

Fig. 9a. The relationship between biomass C and exchangeable Al for the Fe(III) amended low aluminum soils.

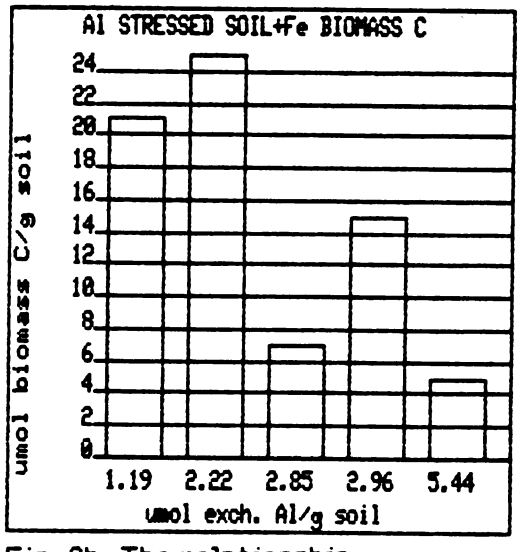

Fig. 9b. The relationship between biomass C and exchangeable Al for the Fe(III) amended aluminum stressed soils.

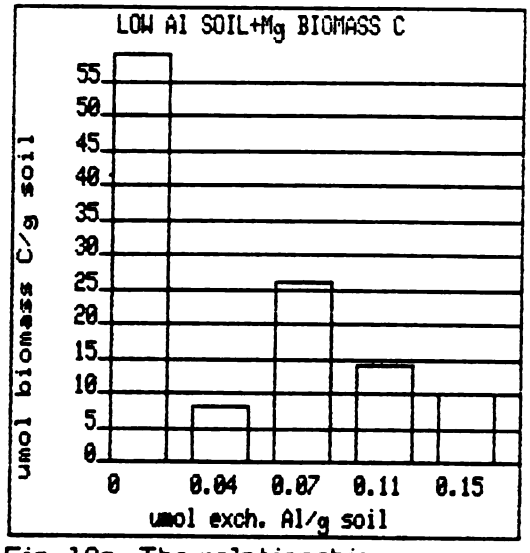

Fig. 10a. The relationship between biomass C and exchangeable Al for the Mg amended low aluminum soils.

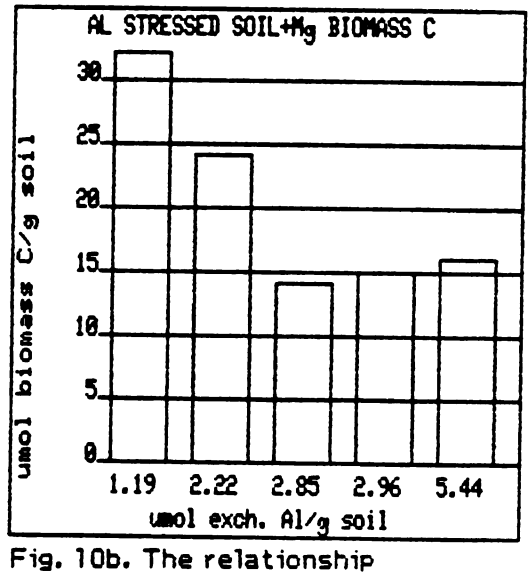

Fig. 10b. The relationship between biomass C and exchangeable Al for the Mg amended aluminum stressed soils.

contrast to Hargrove and Thomas (1981) the data in Figures 11a and 11b exhibit no apparent trend relating biomass C and soil organic matter for these soils.

The amendment of the two groups of soils with Fe^{+3} demonstrated two interesting trends. The low Al stress soils all exhibited slight increases in active biomass C over the glucose only amendments (Fig. 11a and 12a). This increase was generally expected. However, Fe^{+3} amendments to the Al stressed soil group were associated with a significant ($P < 0.05$) positive correlation (0.879) between biomass C and soil organic matter (Fig. 12b), varying greatly from the glucose only control (Fig. 11b).

The association between biomass C and soil organic matter in soils amended with Fe^{+3} suggests a participation by organic matter in reducing the effects of Fe induced Al stress on microbial activity. Most likely, the effect of soil organic matter in reducing Fe induced Al stress is the reduction of Fe^{+3} activity through various ionic associations. Assuming Fe^{+3} binding by soil organic matter, it seems fair to suggest that the data in Fig. 12b support the Fe induced Al stress model described earlier. A significant correlation between exchangeable Al and soil organic matter was not found for either group of soils. However, a negative correlation of (-)0.875 was found between exchangeable Al and soil organic matter for the Al stressed soils.

The Mg amended treatments exhibited no significant correlation between biomass C and soil organic matter (Fig. 13a and 13b). In the Al stressed soil, the active biomass C did give a significant ($P < 0.05$) correlation with soil pH ($r=0.899$).

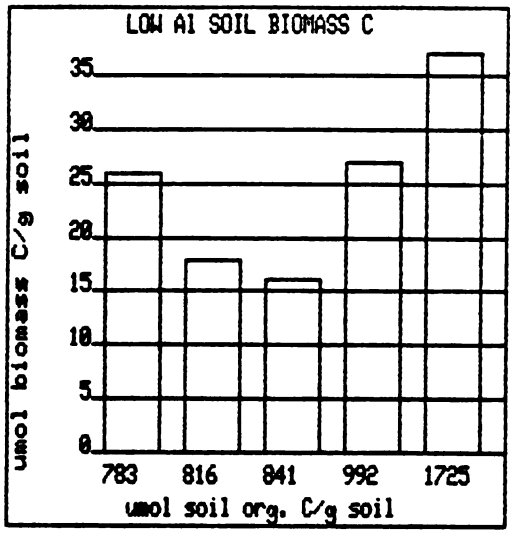

Fig 11a. The relationship between biomass C and soil organic C for the low aluminum soils.

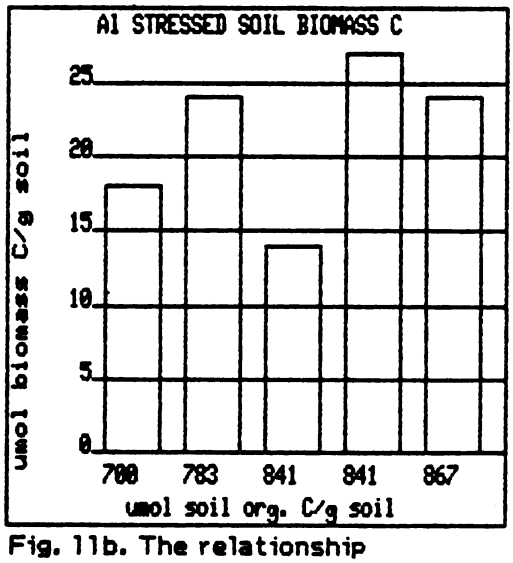

Fig. 11b. The relationship between biomass C and soil organic C for the aluminum stressed soils.

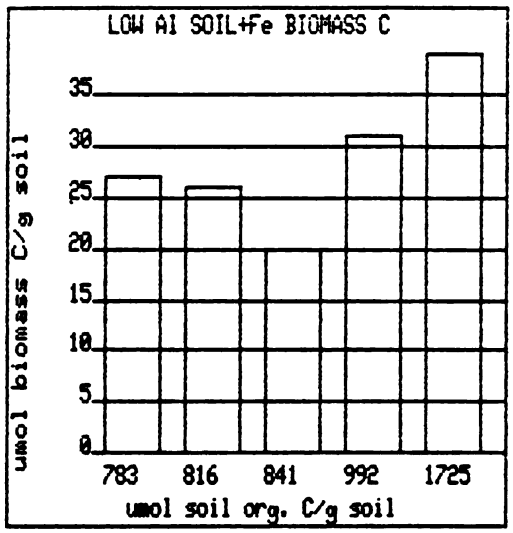

Fig. 12a. The relationship between biomass C and soil organic C for the Fe(III) amended low aluminum soils.

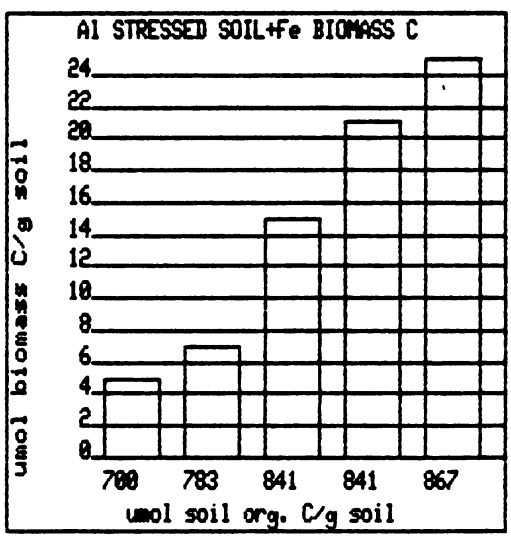

Fig 12 b. The relationship between biomass C and soil organic C for the Fe(III) amended aluminum stressed soils.

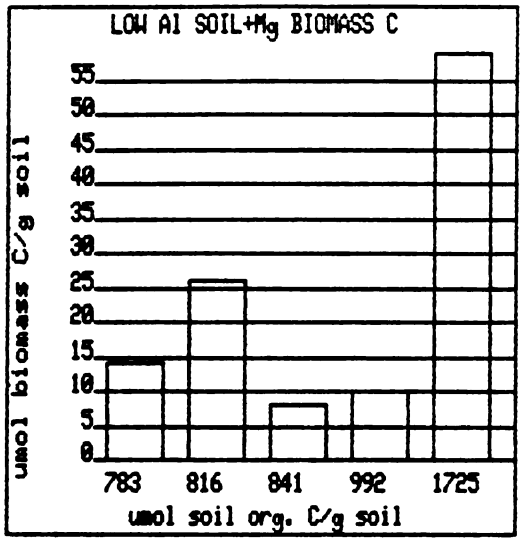

Fig 13a. The relationship between biomass C and soil organic C for the Mg amended low aluminum soils.

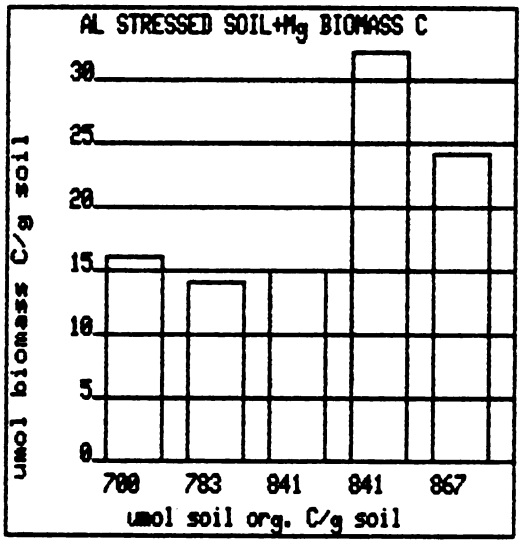

Fig 13b. The relationship between biomass C and soil organic C for the Mg amended aluminum stressed soils.

ENZYME ASSAYS:

Enzyme assays were conducted to help further describe the biological-biochemical parameters of biological processes under Al stress in nature. For reasons of aluminum soil chemistry, all enzyme assays were conducted without pH buffers. As most enzyme buffering regimes occur at pH > 5.5, effects caused by soluble aluminum species would be negated.

Pyrophosphatase:

Pyrophosphatase was the first enzyme activity assayed. Pyrophosphatase mediates the hydrolysis of pyrophosphate to two orthophosphates:

$$P_2O_7 + H_2O \longrightarrow 2HPO_4^-$$

The major interest in pyrophosphatase activity lays in the application of pyrophosphate as a fertilizer for agricultural soils. Pyrophosphatase activity has been correlated primarily with soil organic matter. This enzyme has generally exhibited an optimum activity at pH 8.0 (Tabatabai, 1982).

The substrate used for this assay was sodium pyrophosphate.

The pyrophosphatase assayed in low Al stressed soil showed no significant correlation with any of the factors measured in this study However, in the Al stressed soils, pyrophosphatase activity was significantly ($P < 0.05$) correlated with the glucose only biomass C estimate ($r = +0.887$). No relationship was found to exist between pyrophosphatase activity and exchangeable Al for either group of soils (Fig. 14a and 14b).

Phosphatase:

The phosphatase assayed was the monoesterase type, presumably an acid phosphatase. This enzyme catalyses the reaction:

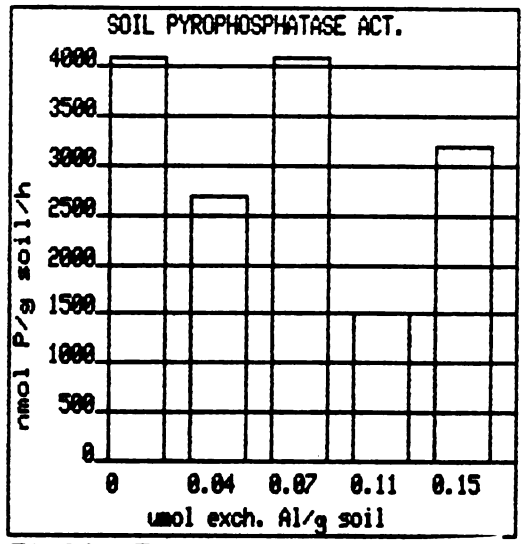

Fig 14a. The relationship between pyrophosphatase activity and exchangeable soil aluminum for the low aluminum soils.

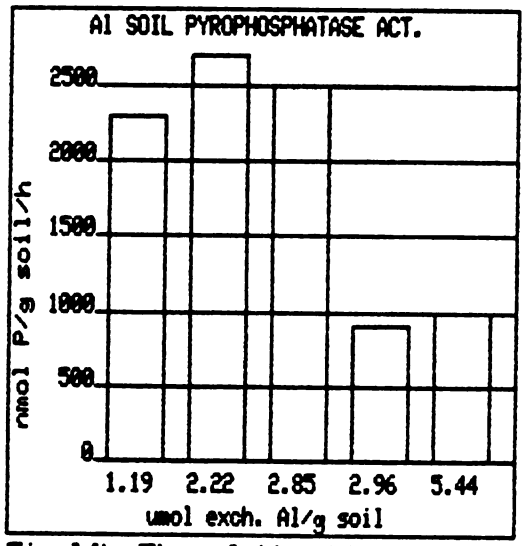

Fig. 14b. The relationship between pyrophosphatase activity and exchangeable soil aluminum for the aluminum stressed soils.

$$R\text{-}PO_4 + H_2O \longrightarrow R\text{-}OH + HPO_4^-$$

A variety of inhibitors including heavy metals will non-competitively inhibit phosphatase. Orthophosphate will competitively inhibit phosphatase activity also (Tabatabai, 1982). The substrate used in this assay was p-nitrophenol phosphate.

In the low Al stressed soils studied, a highly significant ($P < 0.05$) negative correlation ($r = -0.943$) existed between phosphatase activity and the orthophosphate concentrations for these soils. The soil which exhibited no phosphatase activity had recently been amended with a phosphate fertilizer prior to sampling. This soil had the highest orthophosphate concentration of all the soils examined. This lack of activity might suggest that the competitive inhibition cited above be invoked to explain the absense of activity. Also, a significant ($P < 0.01$) negative correlation ($r = -0.990$) between phosphatase activity and potassium, and a significant ($P < 0.05$) positive correlation ($r = +0.919$) between K and $PO_4^=$ were found in this study.

The Al stressed soil exhibited no significant correlations between phosphatase activity and the other soil factors measured, except dehydrogenase activity ($r=0.877$). Results of the phophatase assays for both groups of soils can be seen plotted against exchangable Al concentrations in Fig. 15a and 15b.

Sulfatase:

The sulfatase assay conducted was for the arylsulfatase type, breaking the O-S bond in the following reaction:

$$R\text{-}O\text{-}SO_3 + H_2O \rightarrow R\text{-}OH + H^+ + SO_4^=$$

Sulfatase activity has been shown to be correlated with soil organic matter content. Sulfatases are competitively inhibited by MoO , AsO ,

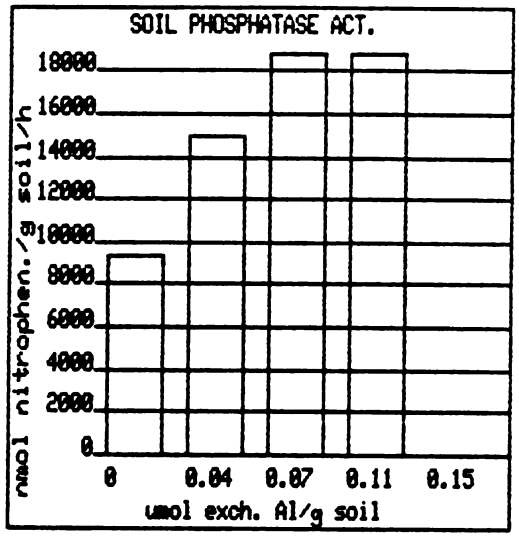

Fig. 15a. The relationship between phosphatase activity and exchangeable soil aluminum for the low aluminum soils.

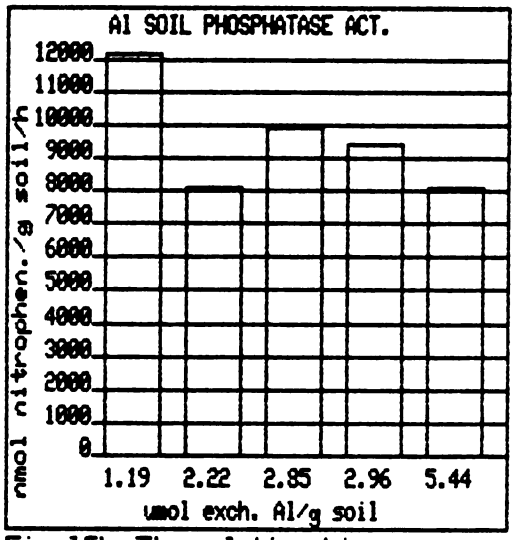

Fig. 15b. The relationship between phosphatase activity and exchangeable soil aluminum for the aluminum stressed soils.

and PO_4(Tabatabai, 1982). The substrate used in this assay was p-nitrophenol sulfate. Figures 16a and 16b show sulfatase activity plotted against exchangable Al concentrations for low Al and Al stressed soils, respectively.

With the low Al stressed soils, no significant correlation was found between sulfatase activity and any of the other variables measured. The soil exhibiting no sulfatase activity is the same soil which exhibited no phosphatase activity. Competitive inhibition is suggested for the same reasons given for phosphatases lack of activity.

However, in the Al stressed soils, sulfatase activity was significantly ($P < 0.05$) correlated with the exchangable Al and Mg, $r=(+)0.906$ and $r=(+)0.929$, respectively.

An explanation which might help to understand this association lay in the aluminum-phosphate soil chemistry. In this study, sulfatase activity shows a fairly high (though not significant) negative correlation with both phosphatase activity ($r= -0.847$) and soil orthophosphate concentration ($r= -0.858$). It is possible that the Al under acid soil conditions removes phosphate from the soil solution. This situation would relieve any competitive inhibition from PO_4 as cited above. The ability of Al under acid conditions to precipitate soluble orthophosphate is well documented (Lindsay, 1979). Also, the fixation of orthophosphates in soils by $Al(OH)_3^{\circ}$ has been demonstrated by Sims and Ellis (1983).

These types of mechanisms are suggested as an explanation for the significant positive correlation between sulfatase activity and Al. No beneficial effects have been cited in the literature for ei-

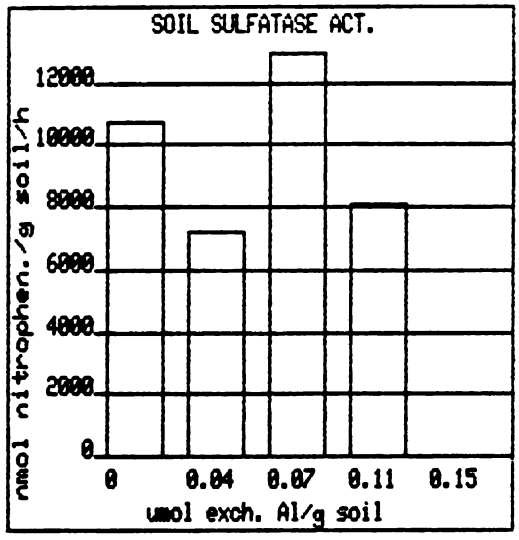

Fig. 16a. The relationship between sulfatase activity and exchangeable soil aluminum for the low aluminum soils.

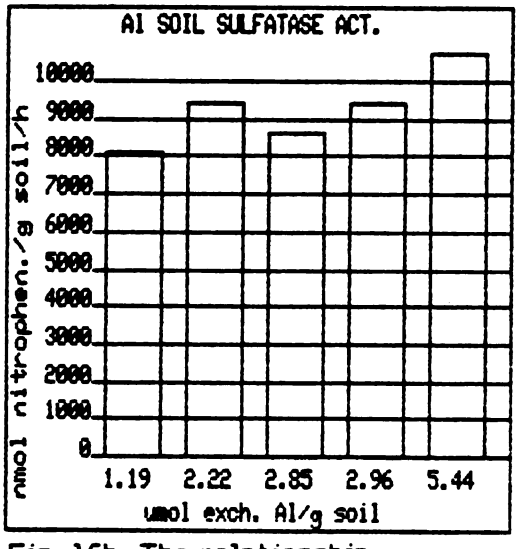

Fig. 16b. The relationship between sulfatase activity and exchangeable soil aluminum for the aluminum stressed soils.

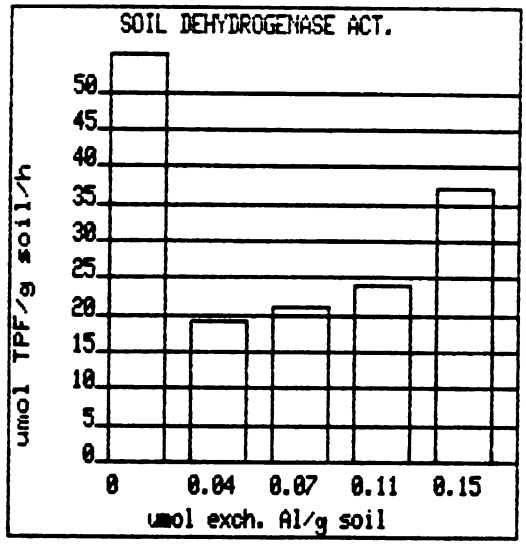

Fig. 17a. The relationship between dehydrogenase activity and exchangeable soil aluminum for the low aluminum soils.

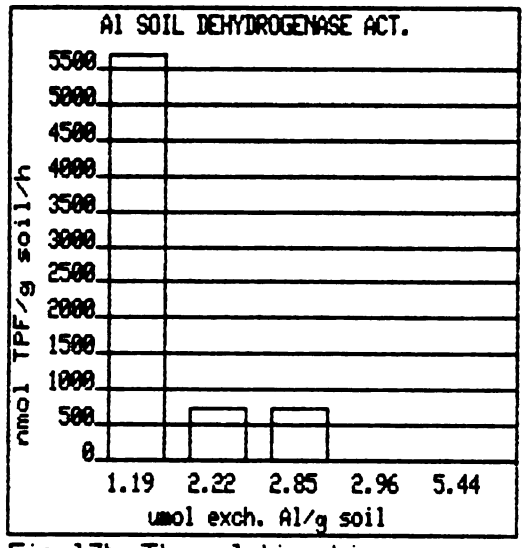

Fig. 17b. The relationship between dehydrogenase activity and exchangeable soil aluminum for the aluminum stressed soils.

ther Al or Mg directly related to sulfatase activity.

Dehydrogenase:

Dehydrogenase activity is described by the general reaction:

$$XH_2 + A \longrightarrow X + AH_2$$

Here, XH is an organic substrate, and A is a hydrogen-electron acceptor (Tabatabai, 1982). Dehydrogenase has been correlated with CO_2 evolution rates, proteolytic activity, and nitrification activity in soils examined by Skujins (1973). Triphenyl tetrazolium chloride was used as the hydrogen-electron acceptor.

The low Al stressed soils exhibited significant ($P < 0.05$) positive correlations for dehydrogenase activity with Mn concentrations ($r= 0.955$), soil organic C ($r= 0.950$), and biomass C ($r= 0.926$).

The Al stressed soils exhibited a significant positive correlation for both dehydrogenase activity with soil pH ($r= 0.983$), and phosphatase activity ($r= 0.877$). It would be interesting to speculate on the association between phophatase and dehydrogenase activity. The available phosphorous in Al stressed soils is likely limited by the aluminum species. The only phosphorous to become available might be organic-P cleaved by phosphatase. No such association was found between dehydrogenase and phosphatase activity in low Al stressed soils. Figure 17a and 17b show dehydrogenase activity plotted against exchangable Al for low Al and Al stressed soils, respectively.

SUMMARY AND CONCLUSIONS:

Data obtained from the artificial soil studies suggest a general reduction in the maximum velocities ($\overline{U}$) due to Al stress. This effect is much more pronounced with B. megaterium (B-12) than with Rhizobium

spp. (I-110).

The data from the Fe^{+3} and Fe^{+3} chelate studies demonstrate a definite interaction involving Fe^{+3} and Al under Fe induced Al stress conditions. The effect of Fe^{+3} was to intensify the Al stress effect on microbial activity.

The Al-biomass association data presented suggests a definite increase in the amount of Al associated with microbial biomass in the presences of Fe^{+3}. Surface interactions (precipitation or cation exch.) could be invoked to explain the Fe induced Al stress effects. However, exchange phenomena (Fe^{+3} displacing Al) is not supported by the Al-biomass association data which show increasing biomass Al in the presence of Fe^{+3}. Precipitation due to Fe^{+3}-Al interactions as the stress mechanism is not supported by data from the growth study. The last two treatments for both organisms in the growth study differ only in the reversal of the order of addition of Al and Fe^{+3}. Though possible, it does not seem probable that the order of addition would effect precipitation if it were the stress mechanism in this system. Yet marked differences in the stress response do occur relative to this reversal.

The Al stressed soils exhibited a generally hyperbolic response to increasing carbon substrate concentration as with the low Al stressed soils. The Fe^{+3} amended Al stressed soils, however, exhibited a dynamic character, similar to the Fe induced Al stress seen with the artificial soil system. Under Fe induced Al stress, the initial drop in CO_2 evolution activity occurred after the lowest Fe^{+3} concentration and leveled off for higher concentrations for the artificial soil system. The initial drop in activity was less pronounced with the

non-chelated Fe^{+3} amendements.

The Al stressed soil used in this study was a fine-loamy, mixed, mesic, Typic Hapludalf. Soils exhibiting classical Al stress problems are tropical and subtropical Oxisols. Oxisols characteristically are acid soils (pH 3.5 to 5.5), and are high in aluminum and iron oxides. Also, being acid in nature, one would be led to suspect these soils to have relative high concentrations of soluble iron and aluminum. Lindsay (1979) indicated that soils at pH 4.6 should yield soluble Fe^{+3} activity of approximately 10^{-11} M when in equilibrium with soil-Fe. Aluminum activity for Al^{+3} in equilibrium with gibbsite should be on the order of 10^{-6} M (Lindsay, 1979). These activities are not unlike the expected activities from concentrations used in this study.

The conclusion drawn from the data presented is that in the presence of Fe^{+3}, an increase in biomass associated Al occurs for *B. megaterium* (B-12) and *Rhizobium spp.* (I-110). Also an intensification of Al stress occurs in the presence of Fe^{+3} for these two test organisms. Therefore, it is suggested that the most likely mechanism for Fe induced Al stress is the import of Al intracellularly in association with Fe^{+3}. This effect might either be direct as in direct competition with Fe^{+3} assimilation or indirect being assimilated in association with other nutrients with their assimilation being accelerated by Fe^{+3}'s effect on metabolism. The final assessment of the effects of Fe on Al stress in microorganisms will have to include a more detailed examination of the specific chemical and growth kinetics of biological Fe-Al interactions. The separation of surface interactions and specific physiological effects is extremely difficult given the properties of Al's precipitation and exchange chemistry, and

the obvious biochemical complications arising from the association of Al with cellular components.

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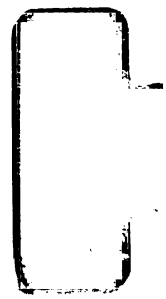

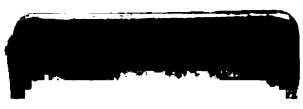


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