TRANSFORMATIONS OF NITROGEN AS OBSERVED IN EXTRACTABLE FRACTIONS OF SOIL AND SLUDGE SYSTEMS

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ABSTRACT

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"Active" N fractions were measured at intervals up to 16 weeks in incubating sludge and mixtures with soil or sand. Quick uptake of N by N-deficient oat seedlings was used to reveal short term changes in nutritional or toxic effects.

"Active phases" were recovered in two fractions: (1) direct extracts (suspensions) in 2N KCl and (2) the supernatant after autoclaving. Diffusable NH_3 and distillable NH_3 were determined in both fractions. Nitrate (NO_3^-) and nitrite (NO_2^-) were determined in direct extracts. In one series of experiments, volatile NH_3 was trapped in acid, Kjeldahl N was determined in the residue after autoclaving ("inactive phase"), and protein N in the "active phase" was estimated by difference as N not otherwise accounted for.

At the lowest rate of sludge addition (15 T/ha), mineralization and nitrification proceeded normally and N was utilized normally by the assay seedlings at all stages

of incubation. At higher rates (30 and 60 T/ha), utilization of N was adversely affected by excess NH_4^+ during periods when NO_3^- was depleted by reductive processes in the biosphere. During the first 8 weeks, while adequate organic energy sources were present, removal of NO_3^- and NO_2^- appeared to be due mainly to assimilatory reduction by adaptive heterotrophs. After initially available energy substrates were exhausted, it appeared that nitrification was sidetracked at the NO_2^- stage by reactions of HNO_2 with polyphenols, resulting in chemo-immobilization.

Exhaustion of organic substrates initially present was evidenced by rapid disappearance (lysis) of proteins, by rapid conversion to exchangeable (diffusable) and alkali labile (distillable) NH_3, and by rapid evolution of volatile NH_3. In initially acid sludge systems, further decreases in pH were evidence that oxidation of NH_3 by Nitrosomonas continued without interruption, even though neither NO_2^- nor NO_3^- accumulated.

Diffusable NH_3 and non-diffusable but distillable NH_3 fluctuated about a 1:1 ratio, indicating a common origin in reversible reactions characteristic of Maillard browning reactions between amino acids and sugars, or of aldol condensations of NH_3 with polyphenols.

Recoveries of N accounted for indicate that significant losses by bio-denitrification or chemo-denitrification did not occur.

Dedication

I dedicate this dissertation to my wife, Samira, and to my children, Marwa and Maisa, for earnestly and patiently waiting.

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INTRODUCTION

Large quantities of sludge are produced during treatment of municipal sewage. Disposal of the sludge in landfills, or by dumping at sea, or by incineration may present environmental problems.

Sludge materials contain most of the major, secondary and micronutrients necessary for plant growth. Because of the energy shortage and the high cost of fertilizers, sewage sludges are attractive as alternative fertilizers and soil conditioners. The feasibility of such use hinges primarily on the capacity of the sludges to supply nutrients readily without contributing to the accumulation of toxic constituents, especially heavy metals, in soils or in crops used for animal or human food.

The level of nutrients and potential toxicants in sludge, and their availability to plants, depends upon the source of the waste water, the nature and extent of waste water treatment, and the age and stability of the sludge. Heat-dried sludges produced by the "activated sludge" process are frequently sold by sewage works for use as manure on agricultural land, home gardens, and landscaped areas. These materials are commonly high in nitrogen (N) and often contain substantial quantities of phosphorus (P).

A great deal of current research is directed to developing criteria for estimating acceptable rates of sludge application from different sources under different conditions of soil, climate, vegetative cover, and management. In the case of nitrogen, these estimates are complicated by the number of biological, physical and chemical transformations that the different forms of N can undergo (mineralization, immobilization, volatilization, nitrification, denitrification), by differences in availability to plants of the different forms, and by their susceptibility to movement in runoff or leaching.

A major problem in controlling adverse or beneficial effects of N, where sludges are used as soil amendments, is estimating the effects of energy substrates that were not removed by sewage treatment. When these residual energy materials are added to soils, microbial numbers are greatly increased. The resulting systems are very dynamic. The relative concentrations of different forms of N can change quickly over time. Correspondingly rapid changes can be expected in the availability of N to crops and in possible toxicities due to rapid release of NH_3.

The behavior of nitrogen in soils amended with sludge has been studied by numerous researchers in the field, greenhouse and laboratory. Most often only changes in mineral forms have been followed. Nitrogen not accounted for has been attributed to immobilization, volatilization, or denitrification, or to some combination of these that

might have been expected under the conditions of the experiment. A very few ^{15}N studies with sewage effluent in soil systems have provided more specific evidence for immobilization, mineralization and denitrification. A few studies have shown production of N_2O from soil/sludge systems. Formation of N_2O provides specific evidence for denitrification.

Thus, our understanding of the behavior of N in soil/sludge systems is fragmentary. The available data are ambiguous and often contradictory. Greater detail is needed regarding changes in N status that occur during the first few weeks or months after application of sludges. Important transformations of N occur as it cycles through solid phase systems in the soil. These are not reflected by gross changes in organic N. A better understanding of changes that occur in the soil biosphere and how they progress over time can help in refining criteria for determining rates of application and management practices so as to minimize environmental hazards and maximize beneficial effects on crops.

The present research makes use of two approaches that do not appear to have been employed in studies of soil/sludge systems. The first is a greenhouse bioassay (Dement et al., 1959) in which an established root-mat of N-deficient oat seedlings is placed in contact with previously incubated mixtures of sludge with soil or sand. Subsequent growth and N-uptake over a two-week period

provide a sensitive measure of changes in availability of N during successive time intervals over a 14 to 16-week incubation. Short-term changes in toxicity may be reflected also.

The second approach is an attempt to focus on N fractions that recycle actively through the soil biosphere. After incubating for successive time intervals up to 16 weeks, sludge mixtures are subjected to a two-stage extraction. First, materials soluble or suspended in 2N KCl are removed. Then the residual solids are subjected to mild hydrolysis by autoclaving for 16 hours (Stanford and DeMar, 1970; Stanford, 1982). Diffusable NH_3 (MgO) and distillable NH_3 (NaOH) are determined in both the extract and the autoclaved hydrolysate. Nitrate and nitrite are determined in the extract.

LITERATURE REVIEW

Mineralization of Organic Nitrogen in Sludge

The fate of sludge-applied N in soil is very complex. The N can follow any of several alternative or sequential pathways: mineralization, nitrification, immobilization, fixation, plant uptake, denitrification, volatilization, convection, dispersion (Ryan et al., 1973). Of these, volatilization and denitrification can be considered as direct removals of N from the soil/sludge system. Plant uptake can be considered as a N-removal mechanism only if the plants are removed from the field. Nitrogen can be removed also in surface runoff or by leaching into subsurface drainage systems or to depths beyond the reach of plant roots.

All of these processes are greatly affected by the physical and chemical properties of the soil as well as by characteristics of the sludge being used. Thus, the type or sequence of pathways taken by N at one site may not result at another. Understanding the rates of N transformation and the fate of N in sludge-amended soils is very important to insure that the amounts of sludge applied provide sufficient available N for plant growth but do not liberate NO_3^- greatly in excess of plant needs.

Available N is normally defined as the amount of inorganic-N (NH_4^+ plus NO_3^-) in sludge, plus the amount of organic N in sludge that is mineralized during a cropping season. On the average, NH_4^+-N, NO_3^--N and organic-N components comprise 47, 3, and 50% of the "available" N in sludges, respectively (Sommers, 1977).

Wide differences in the rate of mineralization of organic-N occur when sewage sludges are added to soils. The rate of mineralization depends to a great extent on pre-treatment of the sludge, and on the rate and method of application, soil type, environmental conditions, and incubation time. Ryan et al., (1973) found that 4 to 48% of organic N in anaerobically digested sludge was mineralized to NO_3^--N + NO_2^--N in a 16-week incubation with sandy loam soil (Typic Argiudall) at rates up to 1880 ppm N on an oven-dry basis. The percent of N-mineralized decreased as the amount of N added to the soil was increased from 47 to 1880 mgN/kg soil. Ryan et al. concluded that mineralization of sewage sludge organic-N was more rapid under anaerobic than under aerobic conditions in soil. It appeared that nitrification and denitrification may have proceeded concurrently at high rates of sludge application, leading to net losses of N from these systems. However, the recovery of added N was almost quantitative at the low level of sewage sludge addition.

Sabey et al. (1975) added anaerobically digested sludge to a Nunn clay loam soil at rates ranging from 22.4 to 224

T/ha. They found that the amount of N-mineralized was 36 to 41% of organic N in the sludge at all application rates. About 300 ppm of NO_3^- -N accumulated in a one-month incubation period, while the NH_4^+-N level was low (<30 ppm) for all treatments and rates after the first month. They suggested that nitrification was more rapid than ammonification throughout most of the incubation period.

Numerous studies have shown that N uptake by plants increases with increasing application of sewage sludge (Hinesly et al., 1972; King and Morris, 1972a, 1972b; Sabey et al., 1975; and Sabey and Hart, 1975). This occurs because of increased amounts of available NO_3^--N when organic N is mineralized and when NH_4^+ is oxidized to NO_3^-.

Hence, based on plant uptake of N, Sabey et al., (1977), estimated that 4 to 29% of the sludge organic nitrogen can be mineralized and thus made available for crops. Magdoff and Amadon (1980) found that about 54% of organic-N added to sludge-amended corn soils was mineralized under laboratory conditions. The amount of N mineralized was well correlated with a chemical test of N-availability (autoclaving release of NH_4^+, similar to the assay used in the present study). Magdoff and Amadon concluded that the recovery of N in the corn at harvest plus soil NO_3^--N to a depth of 1.2 m was positively correlated with the estimated mineralization of organic N plus the inorganic N added.

The above result agrees with a similar finding obtained from field experiments by Kelling et al. (1977b) who

reported that up to 50% of the N applied was mineralized within three weeks after sludge application. Their study also showed that 25 to 15% of the added N was mineralized during the second and third year, respectively, after application. Residual rates reported by others are lower than this. Pratt et al. (1973) suggested a mineralization rate for organic N of 35, 10, 6 and 5% for the first, second, third, and fourth year, respectively, following application of sludge to agricultural land in California. Wisconsin guidelines (Keeney et al., 1975) estimate net organic mineralization to be 20 to 15, 6, 4, and 2% for the same periods of time.

Several studies have evaluated N mineralization for different types of sludges. Permi and Cornfield (1971) found that N mineralization increased almost linearly with time of incubation at a low level of sludge addition (0.5%), but at a high rate of 2% sludge addition there was an inhibitory initial effect followed by rapid mineralization. Total N mineralization was low (2.3 to 4.2% of organic-N) at 2% and 0.5% of sludge addition, respectively, in both anaerobically digested and activated sludges on sandy loam soils (pH 7.1) at 30^{o}C and six weeks of aerobic incubation. Lunt (1953) also found that N released during incubation from heat-dried sludges increased linearly with rate of application.

Stephenson (1955) reported that the amount of N mineralized in 41 days from raw, digested and activated sludges

was 7, 21, and 60%, respectively. Stewart et al. (1975) reported that sewage sludge solids mineralized considerably more slowly than did liquid sludges added to soils. Magdoff and Chromec (1977) showed that from 14 to 25% of the organic-N in anaerobically and 36 to 61% in aerobically digested sludges was mineralized during a 13-week laboratory incubation. In more recent work, Hsieh, et al., (1981) found that the mineralization potential was 30% of organic-N in activated sludges and 38% of digested sludges in 12% mixtures with soil.

From all of the above, it is obvious that sludge pretreatment, rate of application, time of incubation, soil type as well as soil chemical properties affect to a great extent the N-mineralization potential. Thus wide differences in mineralization of sewage sludge can occur.

Immobilization Of Mineral N In Presence Of Sludges

Mineralization and immobilization go on simultaneously in soils (Jansson and Persson, 1982). Organisms that decompose dead organic matter assimilate part of the N into their own cell proteins. If more N is present in available energy substrates than is needed for growth of the decay population, net mineralization will occur. If the available energy substrates include a large proportion of N-free substances (e.g. polysacchasides, fats, oils, waxes), the N in proteinaceous substrates will not be enough; if mineral N (NH_3, NH_4^+, NO_3^- or NO_2^-) is present, it will be drawn upon for assimilation, and net immobilization will occur.

The ratio of available energy to available N in the organic substrates can be approximated in terms of C/N ratio. A favorable balance between energy and N in plant materials is approached at a C/N ratio of about 25 (N content about 1.5 to 2.0%). Wider ratios (lower N contents) favor net immobilization, whereas narrower ratios favor net mineralization (Jansson and Persson, 1982).

Removal of mineral N into cells and tissues of soil organisms is referred to as biological immobilization. Mineral N can also be withdrawn from solution into the solid phase by non-biological mechanisms. These two types of immobilization as they relate to sludges will be discussed separately.

Biological Immobilization

Tester et al. (1979) reported that net mineralization of N in sludge compost decreased from 11 to 3% as the C/N ratio increased from 10 to 19. However, the C/N ratio of sewage sludges is usually less than 10 (King, 1977). As seen in the previous section, net mineralization usually occurs and large accumulations of NH_4^+ are observed during the first few days or weeks after sludges are applied.

Nevertheless, sewage sludges do contain substantial quantities of N-free energy substrates. Corresponding quantities of mineral N can be immobilized even though net increases occur in the mineral N pool (Sommers, 1977; Varanka et al., 1976). Terry et al. (1981) found that approximately 20% of the inorganic N initially present in a

soil amended with sewage sludge was converted into relatively stable forms of organic N.

The above studies confirm earlier conclusions by the same authors and others that extensive immobilization may occur during the first few weeks after sludge application and that this newly immobilized N appears in organic fractions that are relatively resistent to decomposition (Permi and Cornfield, 1969; Ryan et al., 1973; Terry et al., 1979). Although this N is only slowly mineralized, it must be taken into account in estimating residual release in subsequent years (Keeney et al., 1975; Pratt et al., 1973).

As an average for numerous studies, roughly one-third of the N in sewage sludges is present in mineral forms, mainly NH_4^+, although NO_3^- may occasionally be high (Kelling et al., 1977a,b; Magdoff and Amadon, 1980; Ryan et al., 1973; Sabey et al., 1975; Sommers, 1977; Sommers et al., 1976). Another third is in readily mineralized organic combinations, mainly proteins, and is referred to as "available organic N." The remainder is in resistant forms that are not readily mineralized.

At high rates of sludge application, the combination of mineral N already present plus that released quickly from "available" organic forms can result in accumulations of NH_4^+ or NH_3 that may be directly unfavorable for crops or that may inhibit nitrification at the NO_2^- step, giving rise to excessive concentrations of NO_2^- that can also have toxic effects on crops (Aleem and Alexander, 1960). Also, high

concentrations of NO_3^- can accumulate in excess of crop needs and increase the likelihood of NO_3^- contamination of surface and groundwaters (Magdoff and Amadon, 1980; Pratt et al., 1973; Stark and Clapp, 1980).

To reduce potential problems due to excessive net mineralization of N at high rates of sludge application, numerous studies have been made with simultaneous applications of organic matter low in nitrogen, such as straw, bark, wood chips and other carbonaceous refuse to increase C/N ratio and reduce the accumulation of inorganic N to levels greater than can be utilized by crops (King et al., 1977; Yoneyama and Yoshida, 1978).

Sabey et al. (1977) observed N deficiency and significantly slower growth of crops where a 1:1 mixture of bark and sludge was used directly as a soil amendment. Prior composting can help to minimize deficiencies due to tie-up of N or P by the added carbon sources (Terman et al., 1973; Tester et al., 1977). Epstein et al. (1978) found that composting with carbonaceous refuse decreased mineralization of organic N in a digested sludge from 42 to 7%, and from 43 to 4% in a raw sludge. This study also showed that additional immobilization occurred when the composts were applied to soils. Tester et al. (1979) observed immobilization when fescue was planted immediately after amending soils with sludge compost plus fertilizer N and P; however, the immobilized N and P were remineralized after four months and thus became available to the grass.

Non-biological Immobilization

Immobilization of N in soils is due primarily to assimilation of inorganic N compounds (NH_3, NH_4^+, NO_2^-, NO_3^-) into organic constituents and products of heterotrophic organisms (Jansson and Persson, 1982). However, inorganic N species can be removed from the available mineral N pool by non-biological mechanisms.

Ammonium (NH_4^+) can be fixed in the interlayer spaces of expanding three-layer silicate clays (Nommik and Vahtras, 1982). The reaction is promoted by increasing pH above 5.0 and by drying or freezing. Potassium (K^+) competes for the same interlayer sites, but exchange of one cation for the other is very much slower than in surface exchange. Fixed NH_4^+ is only slowly available to microorganisms or plants.

Ammonia (NH_3) and nitrous acid (HNO_2) can both enter into non-enzymatic reactions with organic matter (Nommik and Vahtras, 1982; Nelson, 1982). A part of the N fixed in these reactions is resistant to hydrolysis by acids or alkali, and it is released only slowly by microbial decomposition.

Mechanisms for these reactions are poorly understood (Broadbent and Stevenson, 1966). Studies with model compounds indicate that organic structures that react readily with NH_3 or HNO_2 are mainly aromatic, such as polyphenols and polyhydric carboxylic acids. These compounds are found in lignin or its degradation products, and in the "lignin-derived" or "aromatic-based" fractions of humus (fulvic acids, humic acids, and humin).

The reactions between NH_3 and phenols probably involve free radical intermediates (semiquinones) formed by univalent oxidation of phenols (Nommik and Vahtras, 1982). Initial products of these reactions are very reactive and condense readily to form polymers with N incorporated in heterocyclic rings. Condensation of NH_3 in organic combinations is promoted at alkaline pH where phenols oxidize autocatalytically. However, free radicals are formed also by microbial phenoloxidases and dehydrogenases. Thus, fixation of NH_3 by organic matter can proceed in the acid pH range at rates permitted by the equilibrium: $NH_3 + H^+ \rightleftharpoons NH_4^+$ (Nelson, 1982).

Fixation of N from nitrite by humic substances, lignin and other phenolic compounds apparently involves reactive nitrogen oxides formed by the spontaneous decomposition of the undissociated acid in the acid pH range: $2HNO_2 \rightleftharpoons NO + NO_2 + H_2O$. Nitric oxide (NO) and nitrogen dioxide (NO_2) are electron-deficient free radicals. These and the nitrosonium cation (NO^+), another product of nitrous acid decomposition at low pH, enter readily into addition reactions with phenols (Mortland and Wolcott, 1965).

In model systems, reactions of nitrous acid with phenols give rise to various nitrated or nitrosated products. These are readily reduced to amines that can rearrange or condense to form heterocyclic structures in which N is resistant to chemical or enzymatic hydrolysis. These reactions take place most readily at pH values less

than 5.0. However, fixation of N from added NO_2^- has been observed at soil pH levels up to 7.8 (Bremner and Fuhr, 1966). An important consideration here is that the H ion concentration of water films on colloidal surfaces can be lower by 100-fold than the bulk of a soil/water slurry (Nelson, 1982).

Losses Of N From Soil/Sludge Systems

Immobilization removes N from the mineral N pool, but the N remains in the system and may be released again at some future time. Net losses of N from soils occur in runoff, by leaching, by volatilization of NH_3 and by denitrification. Only the last two mechanisms are of interest to the present study.

Volatilization of NH_3

Several studies have evaluated the volatilization of NH_3 from sludges and other nitrogenous wastes when applied to soils. Koelliker and Miner (1973) estimated that 10 to 50% of total N in anaerobically digested sludge produced in Ontario is ammoniacal. Approximately 1% of the ammoniacal N (NH_4^+ plus NH_3) in aqueous solution at pH 7.2 and 20°C occurs as NH_3. However, physical and chemical properties of waste solids may significantly reduce the proportion of the ammoniacal N in the NH_3 form from that predicted for very dilute aqueous systems (Hashimoto and Ludington, 1971).

Peterson et al., (1973) studied NH_3 volatilization and NH_4^+ fixation by sludge fertilized calcareous strip-mined

material with pH 7.8 and 28% clay content. After two weeks of incubation, up to 14.7% of added NH_4^+ was water-soluble, 8.7% was exchangeable, and 35% was fixed by the clay fraction. By difference, the NH_3 volatilization was calculated to be about 50% regardless of sludge application rate, with most of this loss occurring during the first week.

King (1973) found that, in 18 weeks, 16 to 22% of the N incorporated into the soil as liquid sludge and 21 to 36% of the N in surface-applied sludge was lost (unaccounted for). Only a small part of this loss was NH_3 volatilization. King concluded that denitrification was the major pathway of N loss.

Schwing and Puntenney (1974) suggested that 25% of the ammoniacal N in the sludge was lost by volatilization from sewage sludge applied in the field. In a laboratory study, however, Ryan and Keeney (1975) reported that the amount of NH_3 volatilized from surface-applied liquid sludge ranged between 11 to 66% of the added NH_4^+ -N. These volatilization losses decreased as the clay content of the soil increased. Volatilization rate increased with increasing rate of sludge application and with repeated application of sludge.

Curnoe[1], in other laboratory studies, reported that increasing temperature or soil pH caused an increase in NH_3 volatilization from anaerobically digested sewage sludge

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[1]W.E. Curnoe. 1975. Ammonium volatilization from sewage sludge applied to the soil surface. M.S. Thesis. Univ. of Guelph, Guelph, Ontario, NIG2W1.

while increasing clay plus silt content in the soil resulted in decreasing NH_3 volatilization. Other factors of much lesser importance were the initial moisture content and relative humidity. He concluded that from 5 to 25% of the NH_4^+-N in applied sludge was lost as NH_3 in 40 days, depending on the levels of the five factors mentioned above.

Lauer et al. (1976) estimated that 61 to 99% of the ammoniacal N present in manure, surface applied in the field, was lost as NH_3 over 5 to 25 days. Most of the losses occurred in the first few days following application. They suggested that the rate of drying was related most closely to the rate of volatilization. Terry et al. (1978) reported that NH_3 volatilization increased from 9 to 35% of added NH_4^+ -N as the air flow rate increased from 20 to 511 ml/min. However, incorporating the wastes into the surface 7.5 cm of the soil reduced NH_3 losses (King, 1973; Sommers et al., 1979).

Beauchamp et al. (1978) reported that, during a five-day experimental period in May, 60% of the 150 kg ammoniacal N/ha applied in sludge was volatilized, while 56% of 89 kg ammoniacal N/ha applied was volatilized in a seven-day experimental period in October. These losses from sludge compare favorably with losses of NH_3 from solid manure of 61 and 99% over time periods of 5 to 25 days in field experiments described by Lauer et al. (1976). On the other hand, McGarity and Rajaratnam (1973) estimated that only 9.4% of the N applied as urine (118 kgN/ha) was

volatilized during a six-day period in a closed system in the field.

Sommers et al., (1979) found that <1% of the applied NH_4^+-N was lost through NH_3 volatilization in Kokomo sludge. They concluded that the loss of added N as NH_3 was low, because no air was flowing over the soil surface. However, higher NH_3 losses were observed where 90 T/ha of sludge was applied all at once or split into two 45 T/ha of sludge applications in soils incubated at 30°C and in soils inoculated with fungal mycelium. They suggested that denitrification and/or immobilization are the major mechanisms for removing N from the mineral pool in soil treated with sewage sludge.

In more recent studies, Feigin et al. (1981) reported that about 17% of the tagged, mineral ^{15}N in solution and 24% of the effluent-tagged ammonium-^{15}N were lost, apparently through both denitrification and volatilization. They concluded that simultaneous application of C and N in the sewage effluent was probably responsible for the increased losses of N through denitrification in the effluent-tagged ammonium-N treatment. The losses of tagged fertilizer-N (ammonium-sulfate-^{15}N) by denitrification were relatively low and the applications of the sewage effluent did not increase these losses despite additional available C. They suggested that the greater losses of effluent-tagged ^{15}N were probably due to both denitrification and volatilization, where these volatilization losses could stem

from the existence of some NH_3 in the slightly alkaline effluent or mineral solutions. However, NH_3 would have represented <10% of the total mineral-N in the solution (Lance, 1972). The volatilization from the fertilizer was largely prevented by carefully covering it with soil prior to the seeding of plants.

Losses Due to Denitrification

Denitrification refers to the reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to gaseous nitrogen, either as molecular N_2 or as an oxide of nitrogen (mainly N_2O). The principal mechanisms in nature for reducing NO_3^- are biological. However, numerous workers have observed the formation of gaseous molecular N_2 and/or oxides of nitrogen where nitrite (NO_2^-) has been added to well-aerated acid soils where biological denitrification would not be expected. These reactions are non-enzymatic and have been referred to as chemo-denitrification (Nelson and Bremner, 1969; Reuss and Smith, 1965; Smith and Clark, 1960; Wullstein and Gilmour, 1964). In the following review, bio-denitrification and chemo-denitrification will be discussed separately.

Bio-denitrification: By definition, biological denitrification is "dissimilatory" nitrate reduction, where NO_3^- serves as the terminal electron acceptor in the oxidation of organic substrates. The role of NO_2^- which is formed as an intermediate is distinctly different than in "assimilatory" nitrate reduction where NO_2^- is reduced to

NH_3, which is then incorporated into amino acids and proteins.

The capability for reducing NO_3^- or NO_2^- to gaseous forms has been identified in a large number of species of anaerobic facultative bacteria--both autotrophic and heterotrophic (Firestone, 1982). Other organisms may play a minor role. Bollag and Tung (1972) found that nitrite, but not nitrate, can be converted to N_2O (nitrous oxide) by two species of soil fungi. However, several types of microbial N-metabolism, including nitrification and NO_3^- reduction to NH_4^+ (assimilatory nitrate reduction) can result in gaseous N-oxide production (N_2O, NO, or both). Side reactions incidental to NO_2^- reduction may be responsible (Ritchie and Nicholas, 1972, 1974; Yoshida and Alexander, 1970).

Enzyme systems involved in NO_3^- reduction are adaptive. However, the adaptation is rapid and takes place in a few hours, particularly where NO_3^- is the principle available form present. Not all NO_3^- reducers are denitrifiers. To avoid confusion, most microbiologists identify denitrification as a facultative respiratory process present in a limited number of bacterial genera (Firestone, 1982). Since NO_2^- reduced in the respiratory process is not assimilated, NO_3^- is utilized less efficiently for growth by denitrifiers than by other NO_3^- reducers (Volz and Starr, 1977).

The rate of N loss by denitrification is influenced by a number of soil environmental factors, mainly pH, temperature, dissolved O_2, O_2 diffusion rate and readily decomposable organic matter (Cady and Bartholomew, 1960; Fewson

and Nicholas, 1961; Nommik, 1956; Woldendorp, 1963, 1968). The essential conditions are a shortage of oxygen and a supply of readily available energy substrates. The usual substrates in nature are organic, although a few autotrophic species, such as sulfur oxidizers, can use NO_3^- for respiration in the absence of O_2.

Biological denitrification increases rapidly from 2^{o} to $25^{o}C$ and then more gradually to an optimum near $60^{o}C$. The optimum pH appears to be near neutral to slightly alkaline (Bremner and Shaw, 1958; Nommik, 1956; Stanford et al., 1975). Wijler and Delwiche (1954) found that, below pH 7, N_2O was the major gaseous product. Above pH 7, N_2O was produced but was reduced quickly to N_2.

Although biodenitrification is an anaerobic process, it appears that a very low threshold level of dissolved O_2 may be necessary (Nommik, 1956). Also, denitrification may occur in apparently well-drained, well-aerated soils where saturated pores or rapidly decomposing organic matter can provide anaerobic micro-habitats for denitrifying bacteria (Broadbent and Clark, 1965; Hsieh et al., 1981; King, 1973; Ryan and Keeney, 1975; Woldendorp, 1963, 1968).

Until very recently, there have been no specific procedures for quantifying denitrification. Losses of N have been estimated as N not accounted for (Allison, 1965). The stable isotope, ^{15}N, has been used as a tracer in laboratory studies and in the field, but results are subject

to misinterpretation (Broadbent and Clark, 1965). Measurement of N_2O in the soil atmosphere provides specific evidence for denitrification but does not account for N_2 (Ryden and Lund, 1980). A recent development is the use of acetylene to inhibit the enzyme that reduces N_2O to N_2, so that N_2O can now be used as a quantitative measure of denitrification in both laboratory and field studies (Firestone, 1982). Another development by the same group of researchers is the use of the short-lived radiactive isotope, ^{13}N, in laboratory studies.

The newer methods have not been used in studies with sludge or other wastes. The stable ^{15}N has been used to tag mineral N in sewage effluents (Feigin, 1981). Although 24% of the tagged N was lost, the proportion lost by volatilization or denitrification was not determined. Measurement of N_2O has been used as evidence for denitrification in soils amended with cattle manure (Guenzi et al., 1978) and with sludge (Mosier et al., 1982). However, measured losses of N_2O were low relative to N not accounted for.

In a number of studies, estimates of denitrification losses from sludge applications, based on N not accounted for, have ranged up to 38% of the applied N (Epstein et al., 1978; Kelling et al., 1977b; King, 1973; Ryan and Keeney, 1975). Where volatilized NH_3 was not measured, these estimates involved a subjective evaluation by the authors of the relative importance of experimental and environmental

factors that might have favored denitrification over volatilization of NH_3.

The above estimates for denitrification losses from sludge compare with estimates of denitrification losses from fertilizer N that range from 0 to 70% (Craswell, 1978; Kissel and Smith, 1978; Kowalenko, 1978; Rolston et al., 1976, 1979). Losses of fertilizer N from some irrigated California soils, based on evolution of N_2O, ranged from 95 to 233 kg N/ha/yr (Ryden and Lund, 1980). Average losses of fertilizer N, based on numerous lysimeter studies, range from 15 to 20% (Nelson, 1982).

Chemo-denitrification: In addition to biological denitrification in soils, some workers have observed formation of gaseous molecular nitrogen and/or gaseous oxides of nitrogen in soils under conditions not conducive to bio-denitrification (Nelson and Bremner, 1969; Reuss and Smith, 1965).

These losses of N have often been associated with accumulation of NO_2^- and several studies have provided strong presumptive evidence that significant gaseous loss of applied N can occur through chemical reactions of NO_2^- formed by nitrification of NH_4^+ and NH_4^+-forming fertilizers in acidic to mildly alkaline soils (Broadbent and Clark, 1965). These reactions involve the undissociated nitrous acid (HNO_2) and, therefore, require an acid environment. The reactions become significant at pH below 5.0. Even in less acid soils, the necessary low pH levels may exist at

microsites associated with actively nitrifying bacterial populations, since the Nitrosomas reaction produces both NO_2^- and H^+.

It has been postulated that certain metallic cations in their reduced state (Cu^{1+}, Fe^{2+}, Mn^{2+}) may reduce NO_2^- to NO or N_2O in soils (Wullstein and Gilmour, 1964). These authors speculated that chemo-denitrification is an important process for loss of gaseous nitrogen from soil.

Bremner and Blackmer (1978) have shown that small amounts of N_2O are emitted during nitrification of urea and NH_4^+ fertilizers in aerobic soils. The emission of N_2O is not the result of biological denitrification but appears to be a "side-tracking" reaction during the oxidation of NH_4^+ to NO_2^-. No N_2O is emitted when urea is added to sterile soils or to soils treated with a nitrification inhibitor, and the amount of N_2O emitted is related to the NH_4^+ -N nitrified.

Nelson (1982) has reviewed the evidence concerning chemical reactions of NO_2^- as pathways for N loss from soils. He summarized major mechanisms that may play a role in chemo-denitrification as follows:

1) Self-decomposition of nitrous acid in acidic and neutral soils under aerobic and anaerobic conditions to produce nitric oxide (NO) and nitrogen dioxide (NO_2) (Nelson and Bremner, 1970).

2) Reaction with organic matter groups such as phenolic groups in lignin, lignin degradation products and humic substances to produce nitrous oxide (N_2O) and N_2. These reactions are most rapid below pH 5.0 but have been observed in soils at pH 7.8. An important consideration is that water films surrounding colloidal surfaces in soils are often 100 times more acidic than in a bulk soil/water slurry.

3) Reaction of nitrous acid with transition metal cations such as Mn^{2+} or Fe^{2+} (Wullstein and Gilmour, 1964).

4) Reaction of nitrite with ammonium or hydroxylamine (Allison, 1963).

5) Reactions of nitrous acid with compounds containing free amino groups (Smith and Chalk, 1980).

6) Reaction of nitrous acid with clay minerals (Wullstein and Gilmour, 1964; Mortland, 1965).

The reactions cited are really reactions of undissociated nitrous acid (HNO_2) and are favored by acid pH. How important they are in nature is not known. The ones that are most likely are those between HNO_2 and phenolic compounds (lignin, or its degradation products, and soil humic substances).

Effects Of Sludge On Crop Yields

Beneficial Effects

The value of sewage effluents, sludges and composts as sources of nutrients for production of crops has been assessed in numerous studies (Barrow, 1955; Bear and Prince, 1947; Day and Tucker, 1958; Duggan and Wiles, 1976; Fraps, 1932; Garner, 1962, 1966; Mitchell et al. 1978a; Muller, 1929; Noer, 1926; Stucky and Newman, 1977; Terman et al., 1973).

Beneficial effects of sludge applications are most often related to N and/or P content (King, 1981; Mays et al., 1973). The ratio of N:P:K in a typical municipal sludge is about 11:8:1 (Sommers, 1977). Thus, K is often

deficient and may limit crop response, unless supplemental fertilizer is used to give a favorable balance of nutrients (Bunting, 1963; Sikora et al., 1980; Vlamis and Williams, 1961).

Much of the N in sewage effluents may be available to crops in the first season (Day and Kirkpatrick, 1973; Day et al., 1962a,b). In sludges, however, only a third to one-half of the N is directly available. The remainder is organic, of which 10 to 30% decomposes the first year after being incorporated into soil. The rate of decomposition and the release of available N decreases in subsequent years (Hinesly et al., 1979; Miller, 1974; Milne and Graveland, 1972; Peterson et al., 1973). When sludges are composted with added carbonaceous wastes, the availability of N may be further reduced by immobilization (Bunting, 1963; Mays et al., 1973; Sikora et al., 1980).

Except where the availability of N is reduced by immobilization or where nutrient imbalances are not corrected by supplemental fertilization, the availability of N and P in sewage effluents and sludges compares favorably with commercial fertilizers (Boswell, 1975; Day et al., 1962a,b; Coker, 1966a,b). Crop responses may be particularly favorable in soils where soil pH and fertility levels are low (Sheaffer et al., 1979), and during seasons of relatively unfavorable weather conditions (Hinesly et al., 1979). Due to continuing release of nutrients as waste organics decompose, residual benefits from sludges or sludge composts

continue over longer periods of time than where equivalent amounts of N and P are applied as mineral fertilizers (Hinesly et al., 1979; Sikora et al., 1980).

Sludges may contain substantial quantities of heavy metals (Berrow and Webber, 1972; Lunt, 1953; Page, 1974). A number of these are essential micronutrients (Cu, Fe, Mn, Zn). Boron, another essential nutrient is also present in varying concentrations, depending upon the source of the sludge. In certain situations, where one or more of these is deficient in the soil, it is possible that specific micronutrient responses may contribute to increased yields of crops where sludges are applied (Dowdy and Larson, 1975). However, as is evident from literature cited earlier, most direct comparisons of sludge applications with commercial fertilizers indicate that the primary yield response is to the N supplied in sludge or, less frequently, to P.

Adverse Effects

The response of crops to sludge applications varies with source of sludge, rate of addition, plant species, soil type, weather conditions, and management practices. An important factor is the time allowed for sludge to equilibrate with the soil before a crop is planted.

In literature encountered in the present review, optimum yield responses have been reported for rates ranging from 7.5 to 180 T/ha (Hinesly et al., 1972, 1979; Kelling et al., 1977a,b; Milne and Graveland, 1972; Sheaffer et al., 1979; Vlamis and Williams, 1961). However, the efficiency

of N removal from sludge by crops decreases with increasing rate of application (Coker, 1966a; Kelling et al., 1977a; King and Morris, 1972a; Stewart et al., 1975). Also, as sludge inputs are increased, there is an increased possibility that crops may be injured during germination or in seedling stages by excess soluble salts, or that excessive uptake of heavy metals may result in toxic effects on the crops themselves or on livestock or humans that consume them.

Soluble salts: Adverse effects of very high levels of sludge addition (150 T/ha or more) on yields of crops such as corn and rye have been ascribed to soluble salt effects (Cunningham et al., 1975; Hinesly et al., 1972). No data on soluble salts were presented, but yield reductions were associated with dry growing conditions.

Excessive concentrations of soluble salts are particularly damaging to germinating seedlings. Reductions in forage yields of rye, sorghum and sudan grass were observed in the first season after application at rates of 30 and 60 T/ha (Kelling et al., 1977a). Soil type was a factor, since optimum yields were obtained with 7.5 T/ha on a silt loam soil and at 15 T/ha on a well-drained sandy loam.

Soluble salt effects can be reduced by allowing time for leaching and equilibration with soil after addition of sludge before planting a crop. Reductions in yields of sorghum and sudan grass seeded shortly after incorporation of sludge were ascribed to poor germination (Sabey and Hart,

1975). Where wheat was seeded three months after similar rates of sludge were incorporated, yields were increased.

Crops such as lettuce appear to be particularly susceptible to adverse effects of sludge (John and VanLaerhoven, 1976), whereas coastal bermuda grass appears to be tolerant to high levels of both soluble salts and heavy metals (King and Morris, 1972a; Touchton et al., 1976).

Heavy metal toxicity: Much recent work has focused on effects that heavy metals in sludge can have on plant yields (Berrow and Webber, 1972; Page, 1974; Webber, 1972). Under certain conditions, these substances can be taken up in abnormal concentrations by plants and cycled into food chains leading to animals and man. The fate of heavy metals in sludges after incorporation in soil is an area of much recent discussion and research (Dowdy and Larson, 1975; King, 1981; Page, 1974).

Metals appear in sludge in a wide variety of forms, organic as well as inorganic. Different forms of a given metal can vary greatly in their availability to plants or microorganisms. Metals complexed with small organic molecules are often taken up more readily than mineral forms of the same element. On the other hand, complexes formed with organic ligands of high molecular weight will not be available, except as the macromolecules are broken down by enzyme action or by changes in pH, redox potential or other environmental factors.

There is a high degree of variability between sludges, not only in total metal content but in form (Sommers, 1977). Silveira and Sommers (1977) found that the distribution of Cu, Zn, Cd, and Pb in exchangeable, DTPA-extractable, and HNO_3-extractable fractions is not similar for different sludges. This variability also occurs over time at a given treatment plant, and seems to be a function of digester efficiency and the composition of the incoming sewage. Further changes will occur after sludges are applied on the land, and these changes will be influenced strongly by soil type, management and weather (John and VanLaerhoven, 1976).

There is general agreement that Cu, Ni and Zn pose the greatest threat to crop yield and quality (Cunningham et al., 1975; Lunt, 1953; Merz, 1959; Mortvedt and Giordano, 1975). Of these, Cu appears to be about twice as toxic as Zn, while Ni may be as much as eight times as toxic (Webber, 1972). High concentrations of Cd and Pb may be detrimental to plants but, along with Cu and Zn, they are also a potential hazard to animals, including man, in the food chain (Chaney, 1973; Haghiri, 1973; Sommers, 1977; Silveira and Sommers, 1977).

Several soil variables may influence the toxic effects of trace metals. These include organic matter content, kind and amount of clay, and pH (Bunzl et al., 1976; Gadd and Griffiths, 1978; Sinha et al., 1978).

Interactions of Sludges With Soil Systems

Physico-chemical Effects

It is generally agreed that sludge applications improve chemical and physical conditions in soils (Duggan and Wiles, 1976; Evans, 1968; Hinesly et al., 1979; Hortenstine and Rothwell, 1968; Mays et al., 1973; Muller, 1928; Terman et al., 1973). Increases in moisture holding capacity and cation exchange capacity have been shown, as well as decreases in bulk density and compression strength. These effects may be temporary, depending upon the rate of sludge addition and the extent to which soil organic matter levels are increased residually.

An important role of the residual humified organic matter is to retain and stabilize mineral nutrients and heavy metals and moderate their availability to plants (Bloomfield et al., 1976; Nishita et al., 1956). The stabilizing effect of sludge applications depends upon the nature of complexing organic matter in the sludge and its stage of decomposition (Dowdy and Larson, 1975).

Soil pH Relationships

Additions of sludge to acid soils may have the effect of raising pH. In very acid soils this may be a primary factor increasing crop yields (Sheaffer et al., 1979; Terman et al., 1973). In less acid to neutral soils, particularly soils that are poorly buffered, additions of sludge may lower pH temporarily because of the acidity produced during

nitrification of N introduced with the sludge (Hinesly et al., 1972, 1979; John and VanLaerhoven, 1976).

Soil pH is an important factor affecting the availability and potential toxicity of heavy metals. In general, solubility and uptake by plants increases with decreasing pH (Cunningham et al., 1975; Dowdy and Larson, 1975; Mitchell et al., 1978b; Page, 1974). Increasing pH reduces availability by favoring reactions that lead to insoluble precipitates or complexes.

Inverse relationships between soil pH and extractability and/or plant uptake of Cd, Mn, Ni and Zn from sludge have been shown (Bloomfield and Prudeau, 1975; John et al., 1972; John and VanLaerhoven, 1976; Mahler et al., 1978). The last authors suggest that the solubility of Cd and Zn at higher pH are controlled by their carbonates or phosphates.

Many investigators have reported success in reducing the solubility and/or toxicity of sludge-applied heavy metals by liming the soil (John and VanLaerhoven, 1976; King and Morris, 1972a,b; Terman et al., 1973; Webber, 1972). Bloomfield and Prudeau (1975) found that liming decreased extractable Ni and Zn, but had no effect on the solubility of Cu. This they ascribed to the higher affinity of Cu for organic matter (cf. Mitchell et al., 1978).

Soil Microbial Populations

Addition of sludge to soil normally stimulates the rapid development of a large and heterogenous population of organisms. A number of major groups of microflora are

represented (bacteria, actinomycetes, fungi, algae), as well as various fauna, ranging from micro to macro forms (e.g. protozoa, nematodes, insects).

The majority of soil organisms are heterotrophic, i.e., they derive energy and structural carbon from the decomposition of organic substances. The heterotrophic biomass is primarily responsible for mineralization and immobilization of N in soils (Jansson, 1971). Nitrogen mineralization is defined as the transformation of N from the organic state to inorganic forms (NH_3 or NH_4^+). Nitrogen immobilization is defined as the transformation of inorganic N compounds (NH_4^+, NH_3, NO_3^- , NO_2^-) into the organic state through assimilation into cellular proteins and other nitrogenous products of microbial metabolism. The two processes work in opposite directions, breaking down and building up organic matter.

Whenever moisture and temperature are favorable, mineralization and immobilization processes go on simultaneously. A portion of the nitrogen in available organic substrates is assimilated into the cellular materials of the decomposing population. This recycling of substrate N into microbial cells and products has been referred to as the "internal N cycle" (Jansson, 1958). In later publications by the same author, a more descriptive term, "mineralization-immobilization turnover" (MIT), has been used (Jansson, 1971; Jansson and Persson, 1982).

If available substrates are high in carbohydrates relative to proteins, NH_3 released by deamination of proteins will be recycled closely. In addition, net immobilization of N already present in the external mineral N pool may occur. On the other hand, if available substrates are relatively high in proteins, NH_3 released by deamination will exceed the requirement of the heterotrophic population, and net mineralization (ammonification) will occur.

The intensity of recycling through the heterotrophic population will change over time as carbon is respired as CO_2 from more readily decomposed substrates (e.g. carbohydrates, proteins), and as less readily broken down materials are exposed (e.g. cellulose, lignin). Such changes in the nature of available substrates lead to changes in the kinds of organisms that can grow or remain active. As a result, the "soil microbial population" is really a succession of populations. One group of organisms grows, exhausts substrates that it can use, then dies off and is replaced by another.

Such population succession will result in fluctuating demand for mineral forms of N in the system. Ammonia or ammonium ($NH_3 \overset{H^+}{\rightleftharpoons} NH_4^+$) appear to be taken up preferentially by heterotrophic organisms, although NO_3^- or NO_2^- can be used by many.

An important group of organisms that can utilize NO_3^- or NO_2^- are the denitrifying bacteria. These are heterotrophs that are basically aerobic in their metabolism, but which

can adapt to anaerobic conditions by using NO_3^- or NO_2^-, instead of O_2, as terminal electron acceptors. The essential adaptation permits them to reduce NO_2^- to gaseous N_2O and/or N_2. This transformation is referred to as "dissimilatory nitrate reduction" to distinguish it from "assimilatory nitrate reduction" where NO_2^- is reduced to NH_3 which is then incorporated into amino acids and cellular proteins (Alexander, 1961).

Two specialized groups of nitrifying bacteria are mainly responsible for the presence of NO_2^- and NO_3^- in the external mineral N pool. These are autotrophic organisms that use CO_2 as their source of structural carbon. The nitrosomonas group derives its energy for reducing CO_2 and for growth from the oxidation of NH_3 to NO_2^-. The nitrobacter group oxidizes NO_2^- to NO_3^-. Both groups are aerobic (i.e., they require O_2). However, they can remain active over a wide range of moisture conditions, up to nearly complete saturation.

In well-drained soils, the active heterotrophic populations are also mainly aerobic. Nevertheless, anaerobic conditions can develop in micro environments where O_2 diffusion is restricted, or where the demand for O_2 is high in the vicinity of rapidly decomposing organic matter. In particular, the interior of aggregated clumps of sludge may remain anaerobic for considerable periods of time, even though the bulk of the soil volume remains well-aerated

(Bremner and Douglas, 1971; King, 1973; Ryan and Keeney, 1975).

Thus, aerobic and anaerobic processes can proceed simultaneously. The proportion of anaerobic to aerobic environments increases as moisture content increases. However, a major shift from aerobic to anaerobic metabolism occurs only as soils approach complete saturation.

The energy content of raw sewage is reduced substantially during treatment. Nevertheless, the sludge recovered after treatment still contains a wide range of organic substrates (Broadbent, 1973). At heavy rates of application, competition between important heterotrophic and autotrophic populations for mineral forms of N can be intense. A differential effect on a given N transformation, due to toxicants in the sludge or to soil or management variables, can affect the final result in terms of environmental protection or the output of useful plant products. The nature and intensity of probable interactions will be influenced by a number of soil variables, including organic matter content, kind and quantity of clay, moisture level and structural characteristics that affect aeration.

Talburt and Johnson (1967) concluded that the sensitivity of microorganisms to toxic metal ions may vary. Some species are known to develop resistance by genetic or phenotypic changes, resulting in exclusion or metabolism of the toxic ions (Ashida, 1965). Metal-tolerant organisms have been isolated from soils where high concentrations

occur naturally and from soils that have been polluted by heavy metals (Hartman, 1974). A few studies have investigated the influence of metal in soils or sludge on N transformation and have reported somewhat mixed results. Wilson (1977) found that activity of nitrifiers was inhibited by sludge containing high levels of Cd, Pb, and Zn. Permi and Cornfield (1969) found that ammonification was not affected by 1000 ppm Cu or 100 ppm Cr, but 10,000 ppm Cu greatly reduced ammonification. Quraishi and Cornfield (1973) reported that addition of up to 10,000 ppm Cu stimulated N mineralization and nitrification during incubation of sandy loam soil treated with 200 ug/gm N as dried blood. The maximum stimulating effect was at 1000 ppm Cu. In contrast to this, Liang and Tabatabai (1977, 1978) found inhibitory effects on mineralization and nitrification from 19 trace elements added at a level of 5 umol/gm soil.

Soil temperature, moisture status and pH markedly affect soil biological processes, hence affect sludge decomposition and N transformation. Miller (1974) concluded that soil temperature was the major factor affecting the rate of sewage sludge decomposition. He stated that the rate of decomposition of sewage sludge was largely independent of differences in soil texture or chemical properties. Soil moisture content did not affect the rate of sludge decomposition in a sandy soil, but saturated conditions reduced it in silt loam soil and almost completely stopped it in a clay soil.

Terry et al. (1979) found that initial soil pH in the range of 6.3 to 7.5 had little effect on the rate of sludge decomposition. Soil moisture tension in the range of -0.25 to -1 bar also had little effect. However, increasing soil temperature speeded up the decomposition rate of sewage sludge.

In a follow-up study, Terry et al. (1981) found that the nitrification process was faster in sludge amended soil with initial pH 7.5 than at pH 6.0 or 6.3. The nitrification rate of sludge amended soil was increased more at soil moisture tensions of -0.25 and -0.5 bar than at -1.0 bar. Temperature had a strong effect on the mineralization of sludge-organic N and on immobilization of added inorganic N. Both mineralization and immobilization rates increased as temperature increased from 15 to 30^{o}C. About 40% of added sludge organic N was mineralized in silt loam soil at 21^{o}C after 168 days of incubation, but only up to 26% of added NH_4^+-N was immobilized.

MATERIALS AND METHODS

The objectives of the research were to evaluate a quick uptake plant assay as a means for following changes in availability of N during the first four months after incorporation of sludges in soil, and to relate these changes in availability to changes in forms of mineral and organic N that might be considered to represent actively cycling components of the biosphere.

Sewage Sludge

A commercially available, heat-dried municipal sludge (Milorganite®) was used. The lot used was relatively high in total N (6.2 to 6.6%). The N was mainly organic. In 2N KCl extracts, 170 ugN/g was found as NH_4^+, and 10 ugN/g as NO_2^- plus NO_3^-. The organic carbon content was about 21%, giving a C/N ratio of about 3.4. About 40% of the sludge solids were organic, and about one-third of the organic matter was humate (fulvic plus humic fractions extractable with alkali).[2]

® = Trade name registered by the Milwaukee Sewerage Commission, Milwaukee, Wisconsin.

[2] = Organic fractionation and analyses by Dr. Guang-Ji Zeng, Academy of Agricultural Science, Heilongjiang Province, China.

Heavy metals were not of direct concern in this study, and no analyses were performed. The possibility that interactions involving heavy metals may have influenced results must be borne in mind. John and VanLaerhoven (1976) found about 500 ug/g each of lead, copper and iron in Milorganite, together with about 150 ug/g each of zinc and manganese, and lesser quantities of cadmium (72 ug/g) and nickel (18 ug/g).

Soil

The soil used is classified as Granby sandy loam (mixed, mesic, typic Haplaqualls). The experimental lot used contained 1.89% organic matter and 0.113% Kjeldahl N. The pH in water (1 to 2 suspension) was 7.2. The soil contained 11 ug/g of available P (Bray P_1) and 78, 1226 and 90 ug/g respectively K, Ca and Mg exchangeable to 1 N NH_4OAC.

Silica Sand

Mixtures of sludge with soil or with sand were compared in parallel experiments. A commercial source of silica sand was used. It was fine textured (<0.25 mm), and had a pH of 6.8.

Experimental Mixtures

Before mixing, bulk lots of air-dry soil or sludge were passed through a 10 mesh (2 mm) sieve and thoroughly homogenized. Mixtures representing sludge rates of 15, 30, and 60 T/ha (6.69, 13.38, and 26.67 g/kg) were then prepared by adding the appropriate quantities of Milorganite to 250 g of

soil or sand. For comparison with the mixtures, soil alone, sand alone and sludge alone were also dispensed in 250 g unit lots. After mixing, these experimental unit lots were stored air-dry until the beginning of progressively shorter incubation periods as described below.

Greenhouse Experiment

The greenhouse experiment was set up in accordance with a completely random design to consider the following factors:

9	mixtures: sludge alone, soil alone, sand alone, and mixtures of soil or sand with sludge at rates of 15, 30, and 60 T/ha.
4	incubation times: 0, 4, 8, and 16 weeks.
2	assays: biological (quick uptake by oats) and chemical (analyses for forms of N in fractions obtained by direct extraction or mild hydrolysis by autoclaving).
4	replications.
288	total experimental units

For this experiment, the mixtures described in the previous section were dispensed in polyethylene bags to prevent loss of water during incubation. The bagged mixtures were then placed in plastic 12 oz. ice cream cartons. At the beginning of an incubation period, randomly selected unit lots were activated by adding deionized water to approximately 0.3 bar water potential (90% of field capacity). Moisture samples taken at 0, 8, and 16 weeks showed very little variation ($\pm$ 4%) from the initial values.

In order to have all samples ready for assay on the same day, unit lots were activated in the reverse order of incubation times, beginning with those to be assayed after 16 weeks. The samples were then incubated in the dark at 30°C.

Plant Bioassay

A quick uptake procedure described by Dement et al., (1957) was used to detect changes in availability of N during the course of incubation. The assay crop was oats (Avena sativa L., var. Garry).

Nitrogen deficient seedlings were grown in silica sand cultures. Fifteen days before the assay date, 40 oat seeds were planted in 450 g of sand in nested pairs of 12 oz. plastic cartons, the bottom of the inner carton having been removed. The cultures were kept at about 90% water holding capacity by addition (3 x 50 ml) of Hoagland's minus N nutrient solution (Hoagland and Arnon, 1950) plus water as needed to maintain constant weight.

Fifteen days after planting, the oat seedlings were visibly N deficient, but a vigorous root mat had developed at the bottom of the inner container. At this time, the cultures were transferred to place the root mat in contact with a quadruplicate series of all experimental mixtures previously activated and incubated for periods of 0, 4, 8, and 16 weeks. A second quadruplicate series was processed for chemical assays to be described later.

The transplanted cultures were allowed to grow in contact with the experimental mixtures for another two weeks, at which time the shoots were harvested and dried at 60°C for 24 hours. The dried tissues were ground to pass a 40 mesh screen and stored in sealed paper envelopes until they could be weighed and analyzed for N content, using a micro-Kjeldahl procedure, excluding nitrate, described by Jackson (1960, pp. 185-190).

Incubation Experiment

In the greenhouse experiment described above, much of the input N was not accounted for. Losses may have occurred by volatilization of NH_3 or by denitrification, or N may have been immobilized by condensation into "inactive" forms resistant to mild hydrolysis by autoclaving. Also, the data indicated that the systems were very dynamic and that more frequent sampling would be needed to detect important short term changes.

Accordingly, a second experiment was set up to consider the following factors:

5	mixtures: sludge alone, soil alone, sand alone, and mixtures of soil or sand with sludge at the 60 T/ha rate.
9	incubation times: increasing in two-week increments from 0 to 16 weeks.
2	NH_3 trapping treatments: samples were incubated in sealed one qt. mason jars; half of the jars were equipped with acid traps to collect volatilized NH_3.
4	replications.
360	total experimental units.

In this second experiment, the plant bioassay was not employed. All experimental units were activated on the same day by adding deionized water to about 90% of water holding capacity (0.3 bar). Incubation was at 25°C.

Quadruplicate units were sacrificed for chemical analyses, beginning at zero time and at two-week intervals thereafter to 16 weeks.

Chemical analyses were the same as in the greenhouse experiment, except that trapped NH_3 was measured and residual N in the residue after autoclaving was also determined.

Chemical Assays

The objective of chemical assays in this study was to focus on fractions and forms of N that may reflect, rather directly, important changes due to metabolic activities in the biosphere. A two-stage extraction was employed:

1) Direct extraction with a neutral salt (2N KCl or saturated $CaSO_4$) to recover soluble, exchangeable, and readily suspendable materials.

2) Mild hydrolysis by autoclaving to remove materials not displaced by direct extraction but retained in the solid phase by relatively labile mechanisms.

Direct Extractions

In both experiments, extractions were performed immediately at the end of each incubation period. The incubated samples were mixed thoroughly before subsampling

for analysis. In the greenhouse experiment, parallel subsamples were extracted in 2N KCl or in saturated $CaSO_4$. In the incubation experiment (second experiment), only 2N KCl was used.

Exactly 25 g of moist soil was extracted with 25 ml of the extractant by shaking for one hour. The samples were then centrifuged (8 min at 3,000 rpm, or 400 xg). The supernatant was recovered by decanting and made up to 75 ml with the extractant. In the first experiment (greenhouse experiment) these extracts were frozen and stored at $-4^{o}C$ until analyses could be performed. In the second experiment, analyses for the different forms of N were undertaken immediately after extraction.

Hydrolysis by Autoclaving

Residual materials after extraction with saturated $CaSO_4$ were not processed further. Residual solids after extraction with 2N KCl were resuspended and transferred completely with 25 ml 2N KCl to 120 ml pyrex glass bottles.

These were loosely capped and autoclaved for 16 hours at $121^{o}C$. The samples were shaken to suspend and transfer them completely to centrifuge tubes. After centrifuging (8 min at 400 xg), the supernatants were decanted and made up to 75 ml with 2N KCl. They were then stored in plastic bottles at $-4^{o}C$ until analyses could be conducted for distillable and diffusable N.

It should be noted that this procedure deviates from the procedure described by Stanford (1969) and Stanford and

Dement (1969, 1970). These authors carried out the autoclaving hydrolysis in 0.01M $CaCl_2$, rather than 2N KCl. In preliminary studies, negligible differences in hydrolyzable N were found between these two autoclaving media. The 2N KCl was selected since it is commonly used as an extractant for exchangeable NH_4^+, and its use eliminated the need for two different extracting solutions.

Forms of N in Extracts and Autoclaved Hydrolysates

Nitrite and Nitrate: These were determined only in direct extracts with 2N KCl. The extracts of sludge alone or its higher rate mixtures with soil or sand were frequently deeply colored. To remove the dark colored pigments, 20 ml of the extract was passed through 1 g of activated charcoal on Whatman No. 42 filter paper.

Nitrite (NO_2^-) and nitrate (NO_3^-) were determined in clear extracts, using procedures prescribed for the Technicon Autoanalyzer II for analysis of water and wastewater. In these procedures, a colored complex of NO_2^- is measured photometrically before and after catalytic reduction of NO_3^- to NO_2^-, using cadmium as catalyst. Values obtained are reported as ugN/g moist sample.

Diffusable N: A micro-diffusion method adapted by Stevenson (1965) from one described by Bremner and Shaw (1955) and Bremner (1965c) was used to determine pre-formed NH_4^+ in direct extracts and NH_4^+ released by hydrolysis in the supernatants after autoclaving.

The determinations were carried out in Conway microdiffusion dishes, 75 mm in diameter by 19 mm high (Microchemical Specialty Co., Berkeley, Calif.). A gum arabic fixative (gum arabic plus glycerol in K_2CO_3 solution) was used to provide a seal between the dish and the lid. At the time of opening the unit for titration, pressure differentials can cause popping of droplets of the alkaline fixative into the boric acid used to collect NH_3. To avoid this, lids with vent holes that could be closed with a rubber stopper were used.

The stopper was moistened with water before inserting into the lid. Just before use, a thin coating of the gum arabic was applied to the outer rim of the microdiffusion unit with a small brush. Then a 3 ml aliquot of extract or autoclaved hydrolysate was pipetted into the outer chamber of the unit and, into the center well, 1 ml of 4% boric acid containing methyl red plus bromcresol green as indicator. Approximately 3 ml of freshly prepared 11% MgO suspension was added to the outer chamber from a rapid delivery pipette. The unit was closed immediately by placing the lid on the coated rim of the dish with firm pressure and a slight sliding motion.

The contents of the outer chamber were then mixed thoroughly by sliding the unit on the bench with an easy circular motion. Complete diffusion of NH_3 into the boric acid was accomplished in 36 hours at $30^{\circ}C$.

Before opening the unit for titration, the rubber stopper was removed to relieve any pressure differential between the inside and outside of the dish. The lid was then removed, again with a gently sliding motion. One ml (1 ml) of deionized water was added to the boric acid in the center well before titrating with standardized 0.02 to 0.08 N H_2SO_4. A microburette was used and a thin glass rod to stir the solution and transfer droplets from the tip of the burette during the titration. The color change at the end point was from green to permanent faint pink. Results were expressed in ug N/g of moist sample.

Distillable N: As used in this present study, NH_3 recovered by distillation with NaOH will include the NH_3 determined separately by microdiffusion, plus NH_3 released from amino sugars and other alkali-labile compounds, the nature of which is largely unknown (Bremner, 1965b; Stevenson, 1965, 1982).

Three to 5 ml of extract or hydrolysate was diluted with 20 ml of distilled H_2O in a micro-Kjeldahl flask (100 ml). Ten ml (10 ml) of 10 N NaOH was added. Steam distillation was continued (about 4 min) until about 30 ml of distillate had been transferred to a flask containing 5 ml of 4% boric acid plus methyl red-bromcresol green indicator. Collected NH_3 was titrated with standardized H_2SO_4. Results were recorded as ug N/g of moist sample.

Amino sugar N: The difference between distillable and diffusable N is reported as amino sugar-N. This designation

is widely accepted in the soils literature and will be used here as a matter of convenience.

As noted earlier, extracts and hydrolysates of sludge and higher rate mixtures with soil or sand were frequently colored by humic pigments. Thus, the N reported as amino sugar N undoubtedly includes N released from peripheral non-α-NH_2 and amide groupings on fulvic and humic acids (Stevenson, 1982). Non-α-NH_2 on amino acids such as arginine, tryptophan, lysine and proline is also released as NH_3 in alkali.

Amino sugars in soils appear to be primarily products of microbial synthesis. They are important constituents of microbial cell walls and extracellular mucopolysaccharides (Alexander, 1961). In the present study, the N in the "amino sugar" fraction is considered to represent nitrogenous microbial products other than protein (Stanford, 1982).

Residual N

Total N in the residue after autoclaving was determined by a micro-Kjeldahl procedure (Bremner, 1965a). Residual solids after centrifuging were oven dried (105^{o}C, 24 hr) and thoroughly mixed. An aliquot of the dried residue was ground to pass a 100 mesh sieve. A 400 mg sample was transferred to a 100 ml Kjeldahl flask, moistened with 2 ml of deionized water, and allowed to stand for 30 minutes before adding 1.0 g of mixed K_2SO_4 plus $CuSO_4$ and selenium

catalyst and 3 ml of 36 N H_2SO_4. Digestion was continued at a boil for 3 to 4 hours after the digest had cleared.

After cooling, the sample was diluted with 20 ml deionized H_2O. Ammonia liberated by addition of 10 ml 10 N NaOH was steam distilled into 4% boric acid containing methyl red-bromcresol green indicator. The collected NH_3 was titrated with standardized H_2SO_4, and values are reported as ug N/g of the original moist sample.

Volatilized NH_3

In the incubation experiment, volatilized NH_3 was determined by acid trapping in one quadruplicate series of sealed Mason jars for each treatment. A second quadruplicate series of jars was sealed but not trapped.

In the trapped series, a beaker containing 40 ml of 0.5 N H_2SO_4 was placed on top of the soil or mixture in each jar at the beginning of the incubation. The jars remained sealed during incubation. At the end of each incubation period, the appropriate jars were opened and samples sacrificed for chemical assay.

At this time, a 5 ml aliquot of the trapping acid was placed in a 100 ml distillation flask, together with 10 ml 10 N NaOH. The trapped NH_3 was steam distilled into 4% boric acid and titrated with standard acid, as above.

N Not Accounted For

In trapped samples, substantial quantities of input N could not be accounted for by the sum of the various N

fractions plus volatilized NH_3. Important forms of N not measured in extracts or autoclaved hydrolysates would have been compounds having α-NH_2 groups in suspended microbial cells and products and skeletal N in fulvic and humic acids.

pH Determinations

The pH in water (1:2 suspensions) was determined by glass electrode in the materials and mixtures as prepared and at the end of each incubation period. A Sargent-Welch Model 5060 pH meter was used.

Statistical Treatment of Data

Analysis of variance was conducted in accordance with completely random sampling in split or split-split designs. Actual values or log transformations were used as appropriate for ranges of values encountered (Little and Hills, 1978). Significance of differences was tested by Duncan's multiple range test. This test is more conservative than the LSD where more than two treatments are compared. Software and microcomputer processing facilities of the Department of Crop and Soil Sciences, Michigan State University, were used.

RESULTS AND DISCUSSION

Greenhouse Experiment

In this experiment, the quick uptake plant assay with N-deficient oat seedlings was used to evaluate changes in availability of N or possible toxic effects during decomposition of sludge. Parallel series of incubated samples were subjected to chemical assay to characterize active forms of N that the roots of the transplanted seedlings would encounter.

N Uptake Studies with Oats

Data for dry weight, N content and N uptake in tops of oat seedlings are given in the Appendix (Tables 3 to 8). Significant effects were associated with rates of sludge addition and a marked interaction of rates with weeks of incubation.

Data for dry weight are presented graphically in Figure 1. Maximum yields of dry matter were obtained where oat roots were placed in contact with pure sludge that had not been incubated. However, changes occurred during incubation that depressed growth. Yields with sludge alone and with additions of 30 or 60 T/ha to soil or sand were reduced significantly at 4 and 8 weeks of incubation. Yields were trending upward again by 16 weeks.

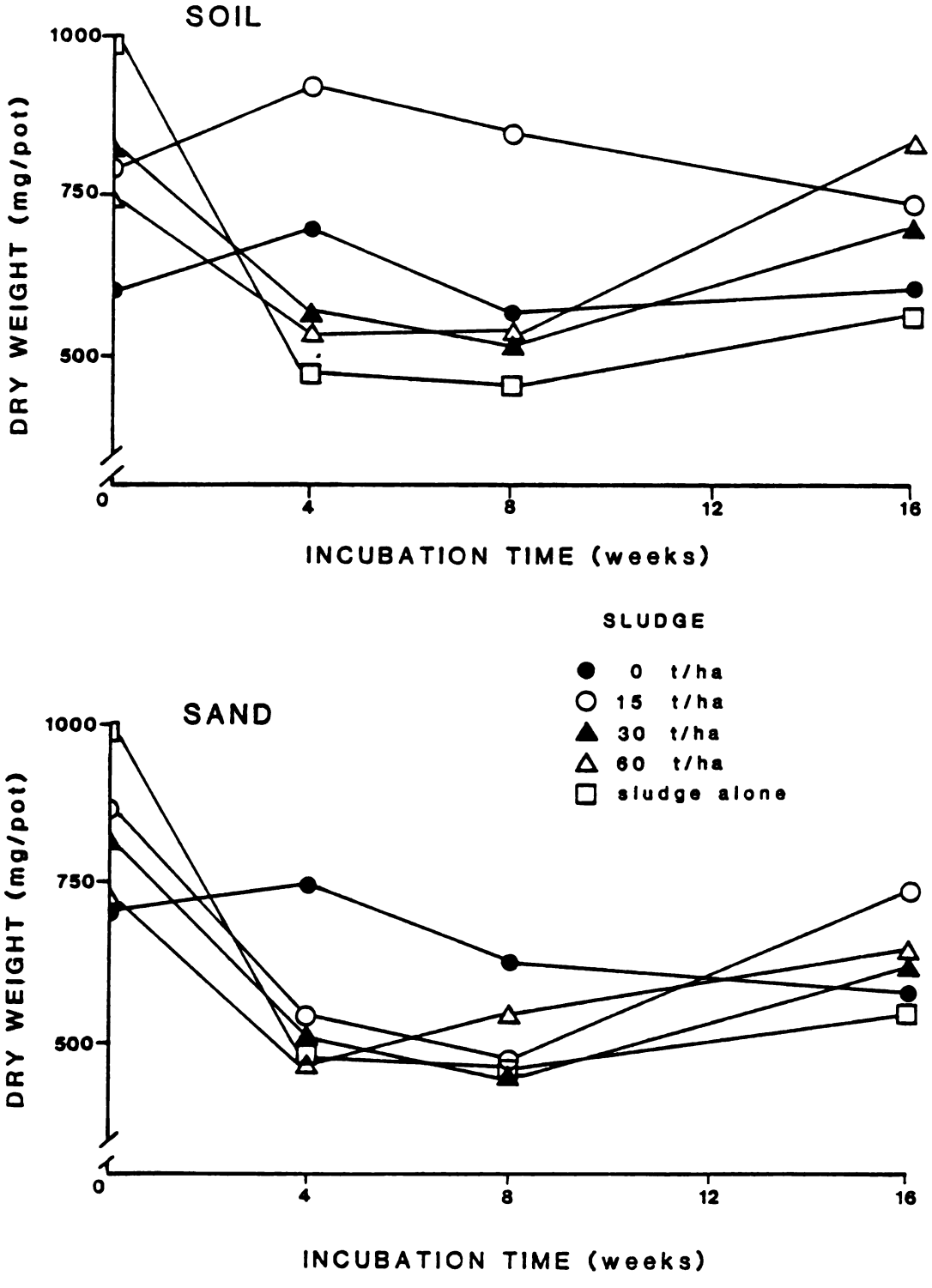

Figure #1. Dry weight of oat seedlings after 2 weeks' contact with previously incubated mixtures of sludge with soil or sand.

The yield depression associated with early stages of decomposition occurred also at the lowest rate of addition (15 T/ha) in sand systems, but not in soil. At this rate, the yield response in soil increased to a maximum at four weeks, then declined slowly as incubation time increased.

The percent N in tops (Figure 2) was greater in the presence of sludge at all rates than in soil alone or sand alone. At the beginning of incubation, N content was generally related to the level of sludge addition. However, it appeared that assimilation of N by the seedlings was affected adversely by changes that occurred during the first four weeks of incubation in sludge alone. A similar reduction in N content at four weeks occurred at the 60 T/ha rate in soil.

Effects on growth and N content are both reflected in the data for nitrogen uptake (Figure 3). It is apparent that the performance of the assay crop was influenced mainly by inhibitory effects associated with early stages of incubation. At the lowest rate of sewage addition (15 T/ha), these inhibitory effects were greatly reduced in soil.

Forms of N in Rooting Media

At the beginning of each two-week plant assay period, a parallel series of similarly incubated samples was extracted with 2N KCl and then autoclaved in 2N KCl for 16 hours. The objective was to identify "active" forms of N that might influence growth and N uptake by the oat seedlings. As a procedural variation, direct extracts in

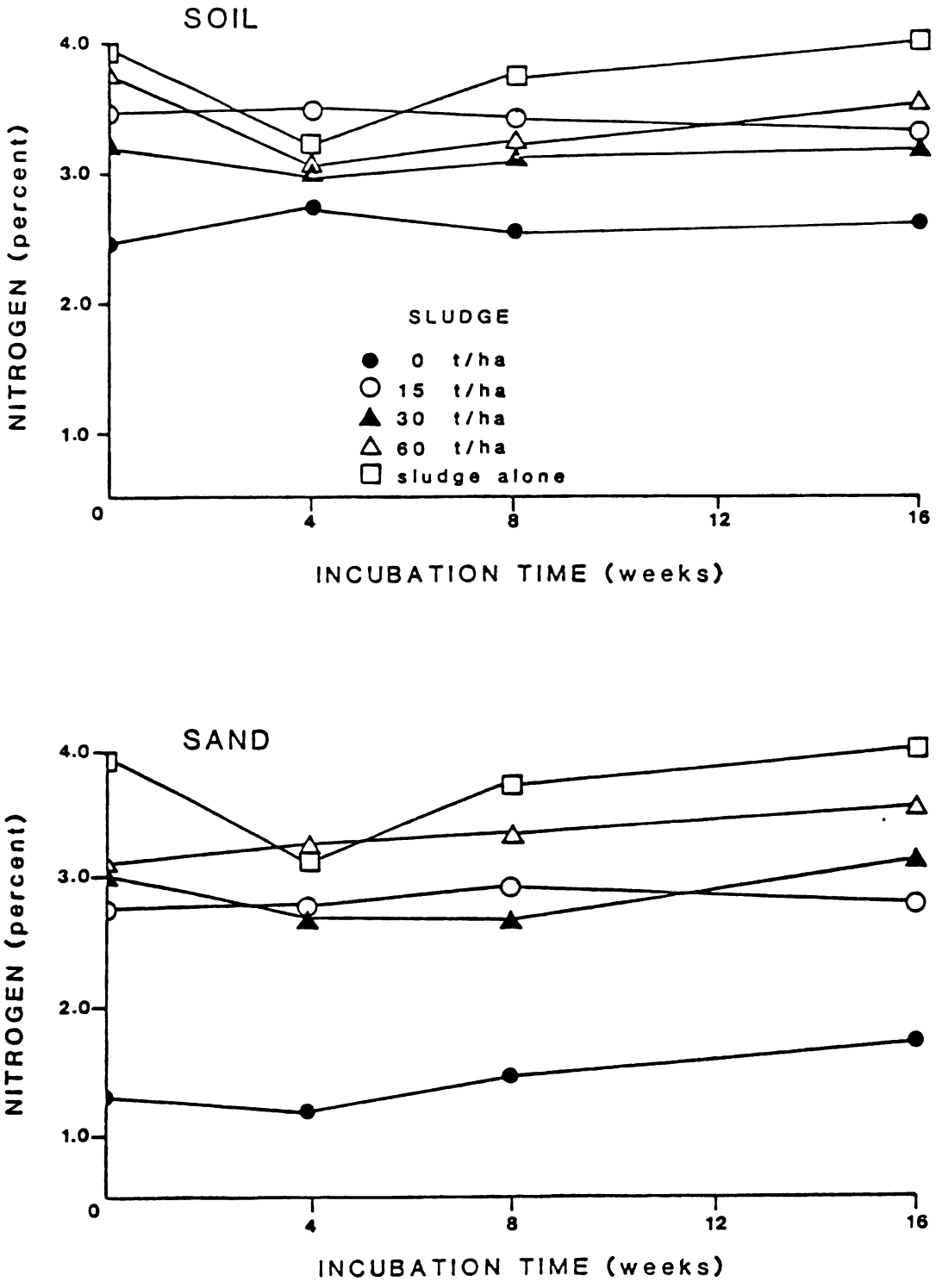

Figure #2. Nitrogen concentration of oat seedlings after 2 weeks' contact with previously incubated mixtures of sludge with soil or sand.

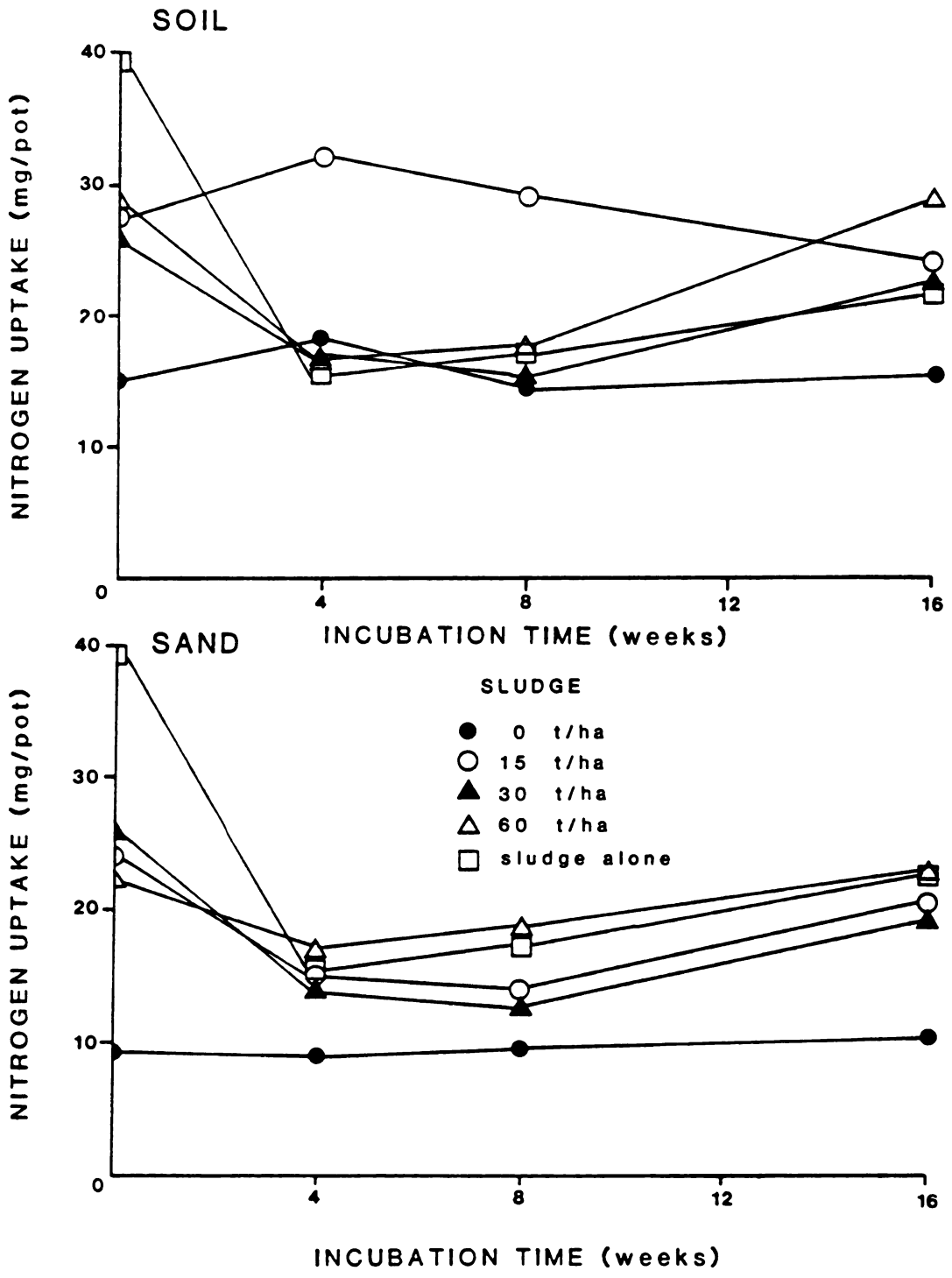

Figure #3. Nitrogen uptake by oat seedlings after 2 weeks' contact with previously incubated mixtures of sludge with soil or sand.

saturated $CaSO_4$ (approximately 0.01 M) were compared with those in 2N KCl.

Sludges alone: Data for NH_4^+ and "amino sugar-N" recovered from samples of sludge that had not been diluted with soil or sand are given in Tables 9 and 10 in the Appendix. Significant differences were associated with methods of extraction and interactions with incubation time. The data are presented graphically in Figure 4.

Large quantities of NH_4^+ and "amino sugar-N" were released during incubation in forms that were directly extractable in 2N KCl and in saturated $CaSO_4$. Approximately equal quantities of NH_4^+-N and amino sugar-N appeared in KCl extracts through the eighth week of incubation, after which N in the amino sugar fraction continued to increase while NH_4^+ declined.

In $CaSO_4$, quantities of NH_4^+ were significantly higher than in KCl in the second sampling and again after 16 weeks of incubation (Table 9), while recoveries of N in the amino sugar fraction were lower in the second and third samplings (Table 10). This indicates that some of the NH_4^+ that might otherwise have been diffusable in the presence of MgO was trapped at sites that were occluded by contraction of polymeric structures due to the high ionic strength of the 2N KCl. This trapped NH_4^+ was released by subsequent distillation in NaOH and thus appeared in the amino sugar fraction.

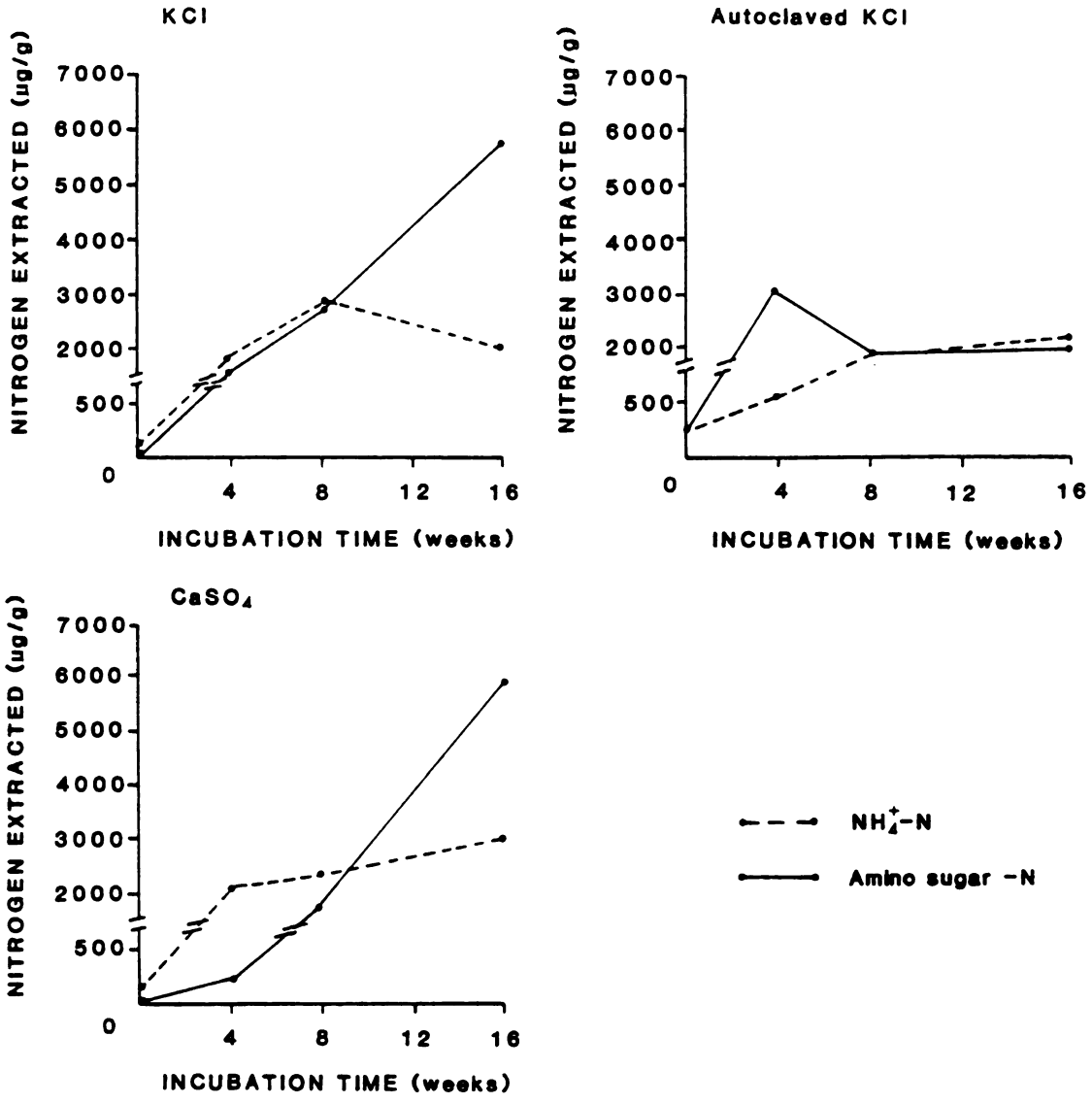

Figure #4. NH_4^+-N and amino sugar N fractions in sludge alone.

At zero time, a much larger proportion of total NH_4^+ and amino sugar-N was released by autoclaving than could be extracted directly with KCl. As incubation progressed, however, the proportion directly extractable increased. After 16 weeks, approximately three-fourths of the N associated with amino sugars was directly extractable (Table 10).

After four weeks of incubation, two-thirds of the N hydrolyzed by autoclaving (Figure 4) appeared in the amino sugar fraction. In later samplings, the hydrolysates contained similar quantities of N as NH_4^+ and in the amino sugar fraction (cf. Tables 9 and 10).

Analyses for NO_2^- and NO_3^- were performed only in direct KCl extracts. Small amounts were found. These data will be discussed in a later section.

Sludge mixtures: Data for diffusable N ($NH_4^+ \xrightarrow{MgO} NH_3$) in direct extracts of sludge mixtures with soil or sand are presented in Tables 11 and 12 in the Appendix. The results for soil systems are plotted in Figure 5 and for sand systems in Figure 6.

As was the case for sludge alone, NH_4^+ directly extractable in $CaSO_4$ was frequently higher than in KCl (Tables 11 and 12). Quantities released during incubation were directly related to rates of sludge addition and reached peak values at four to eight weeks (Figures 5 and 6). At the 60 T/ha rate, the maximum recovery in KCl at eight weeks was 550 ug/g in soil and 480 ug/g in sand. These values compare with a maximum at this time of 2700

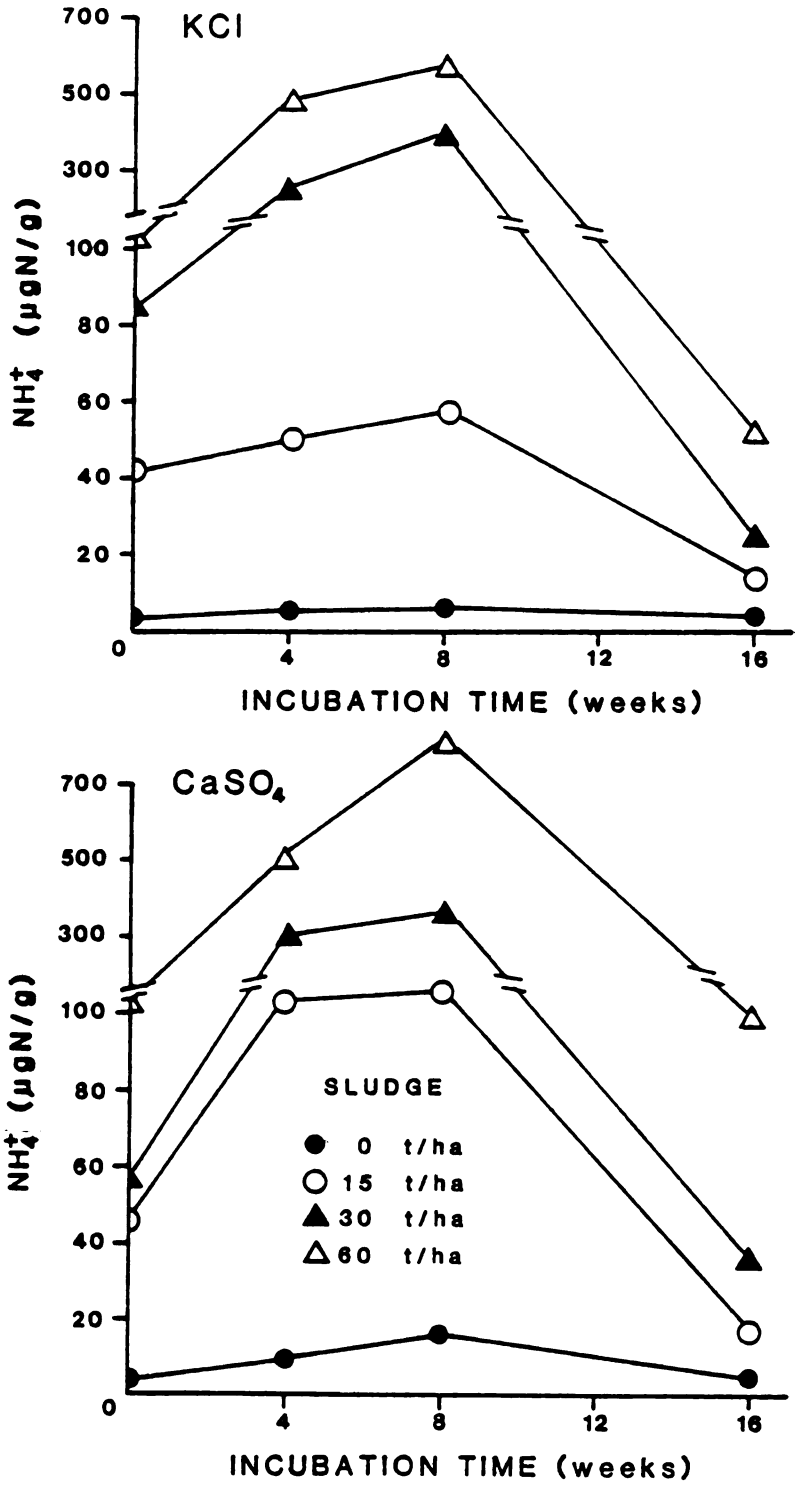

Figure #5. Directly extractable NH_4^+-N in soil/sludge mixtures.

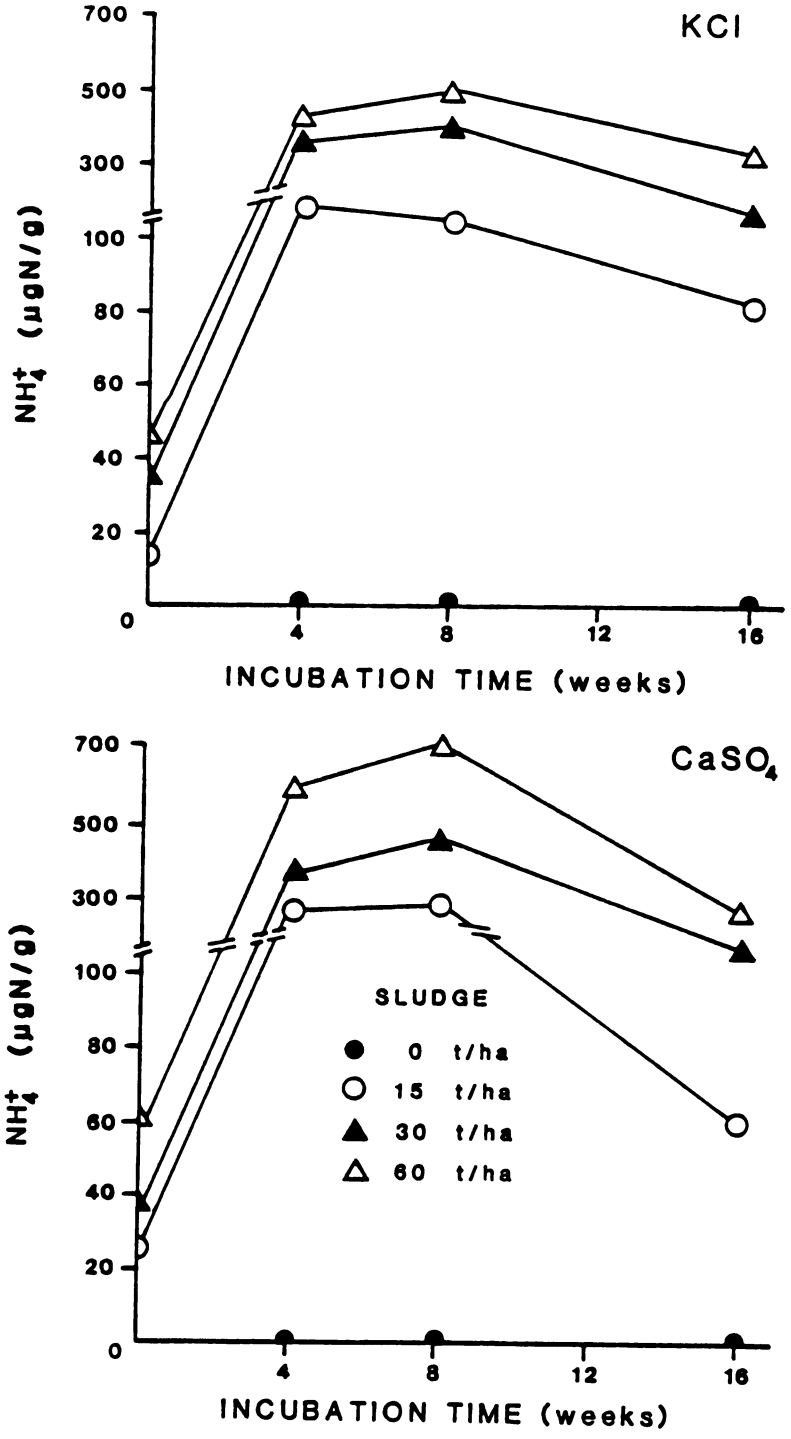

Figure #6. Directly extractable NH_4^+-N in sand/sludge mixtures.

ug/g in sludge alone (cf. Figure 4). After this peak, recoveries declined more rapidly and to lower values at 16 weeks in soil systems (Figure 5) than in sand (Figure 6), or in sludge alone (Figure 4).

Data for non-diffusable, alkali-distillable N (amino sugar fraction) in direct extracts are given in Tables 13 and 14 in the Appendix. The data are plotted in Figures 7 and 8. In general, patterns of change in this fraction were similar to those for directly extractable NH_4^+ (cf. Figures 5 and 6), but peak quantities at eight weeks were very much less in most systems (cf. Tables 13 and 14 with 11 and 12).

Recoveries of the amino sugar fraction in $CaSO_4$ were frequently very different than in KCl, sometimes more, sometimes less (Tables 13 and 14). These erratic differences between the two extractants may reflect dynamic changes in polymeric or particulate fractions formed during decomposition, and in their susceptibility to flocculation at the high ionic strength of the KCl. Flocculated materials removed by centrifugation might be expected to appear in the hydrolysate obtained later by autoclaving the residue.

Data for NH_4^+ and the amino sugar fraction in hydrolysates obtained by autoclaving are tabulated in the Appendix (Tables 15 and 18). They are presented graphically in Figures 9 and 10.

The materials subjected to autoclaving in 2N KCl had previously been extracted with 2N KCl. Thus, the NH_4^+ released by autoclaving from soil systems (Figure 9) may be

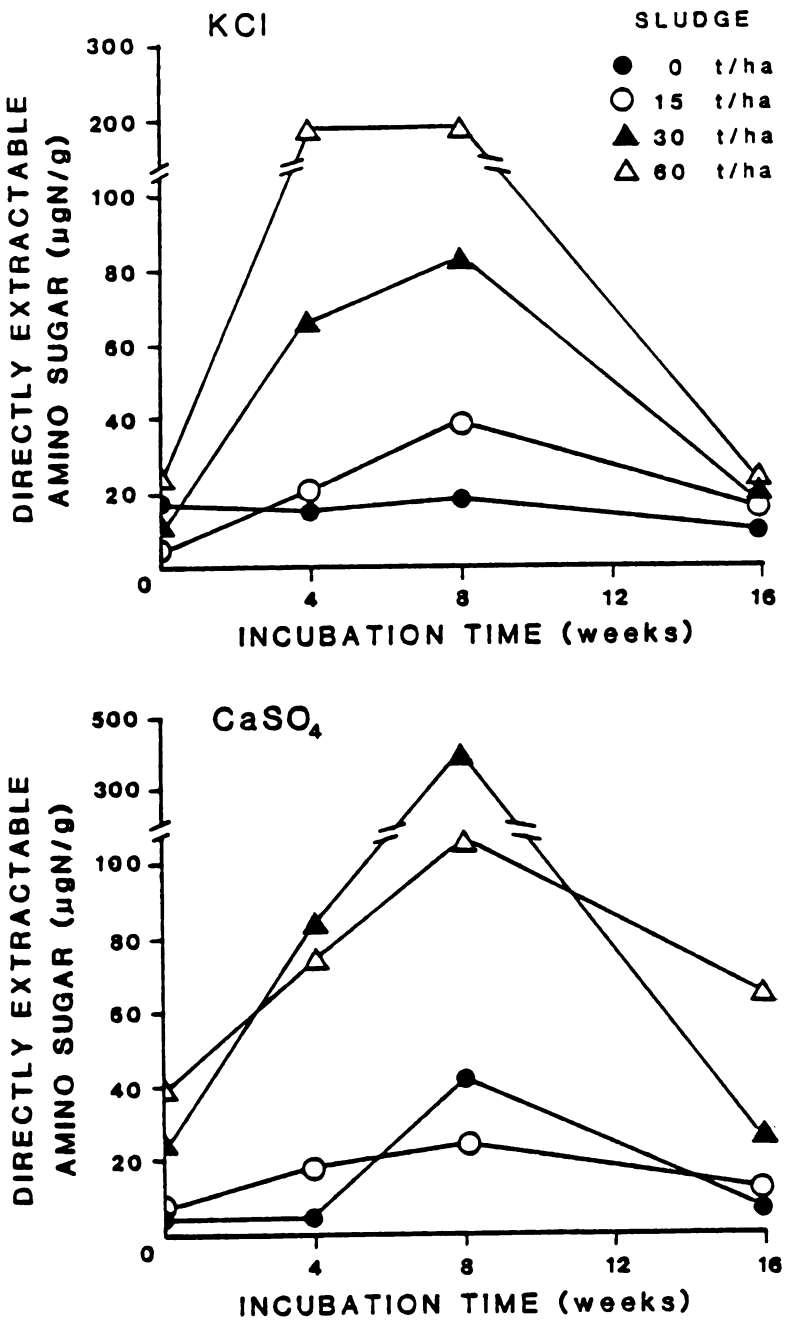

Figure #7. Directly extractable amino sugar N fractions in soil/sludge mixtures.

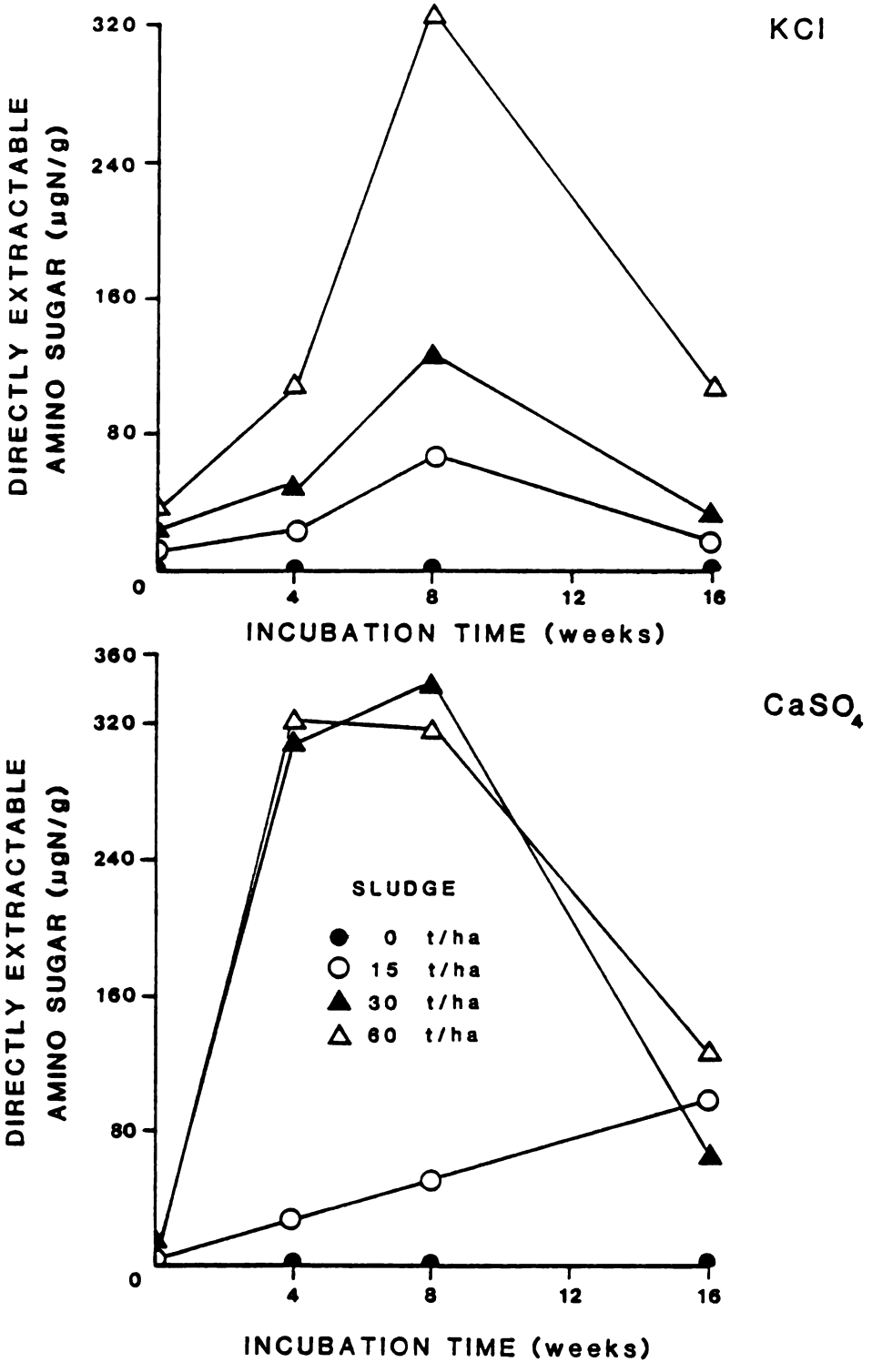

Figure #8. Directly extractable amino sugar N fractions in sand/sludge mixtures.

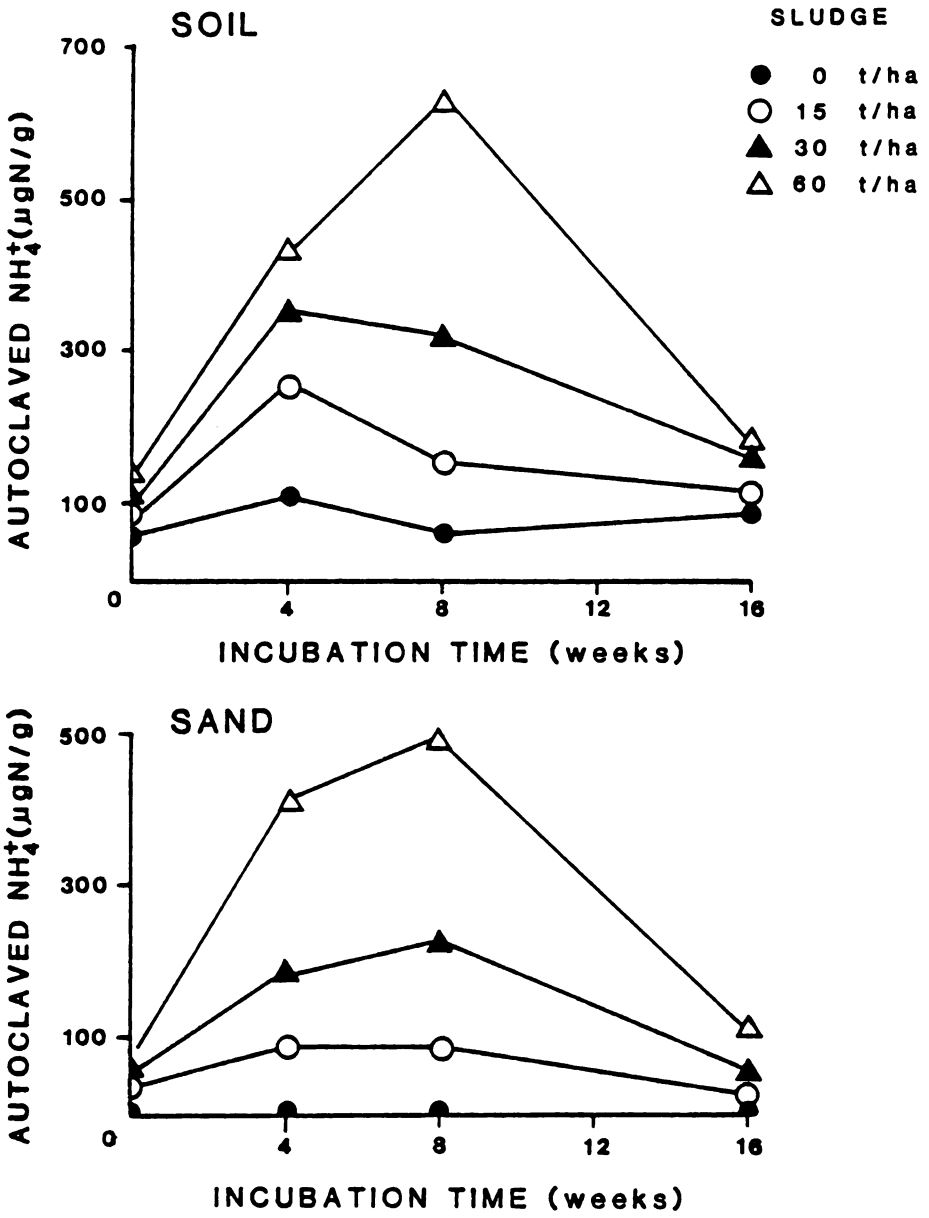

Figure #9. NH_4^+-N released by autoclaving from soil/sludge and sand/sludge mixtures.

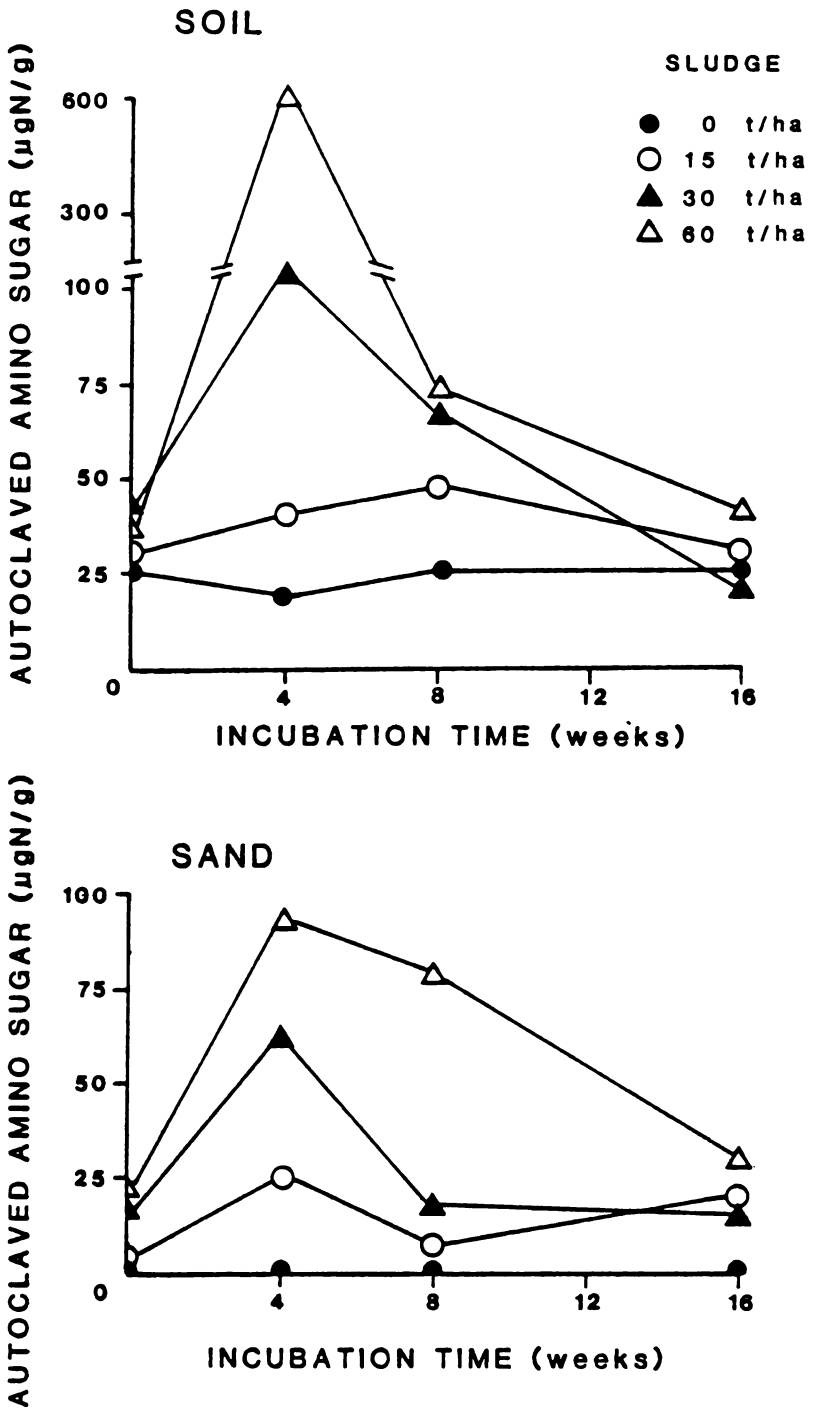

Figure #10. Amino sugar N fractions released by autoclaving from soil/sludge and sand/sludge mixtures.

compared directly with NH_4^+ extracted directly in KCl (Figure 5). Patterns of change were essentially similar in that recoveries before and after autoclaving reached maximum values at four to eight weeks of incubation. The same was true for sand systems (cf. Figures 6 and 9).

The autoclaved amino sugar fraction (Figure 10) peaked sharply at four weeks of incubation at the higher rate of sludge addition, as it had in sludge alone (cf. Figure 4). Autoclaved NH_4^+ (Figure 9) tended to peak 4 weeks later. This suggests that some of the "amino sugar N" in autoclaved fractions was converted sequentially to diffusable NH_4^+.

It should be noted that no N was recovered from sand alone in any of the fractions of Figures 6, 8, 9, or 10.

Nitrite plus nitrate: Both NO_3^- and NO_2^- were determined in direct extracts in 2N KCl. At zero time, NO_2^- was encountered at concentrations ranging from traces to 12 ug N/g. This maximum concentration was found in soil amended with 60 T/ha sludge. Initial concentrations declined quickly, and only trace quantities ranging up to 3 ug N/g were found in any system during subsequent incubation.

For this reason, NO_2^- is not reported separately. The sum of N recovered as NO_3^- plus NO_2^- is reported for sludge and its mixtures with soil or sand in Tables 19 and 20 in the Appendix. The data are presented graphically in Figures 11 and 12.

Nitrification was rapid in soil at the lowest rate of sludge addition (15 T/ha). In the first four weeks of

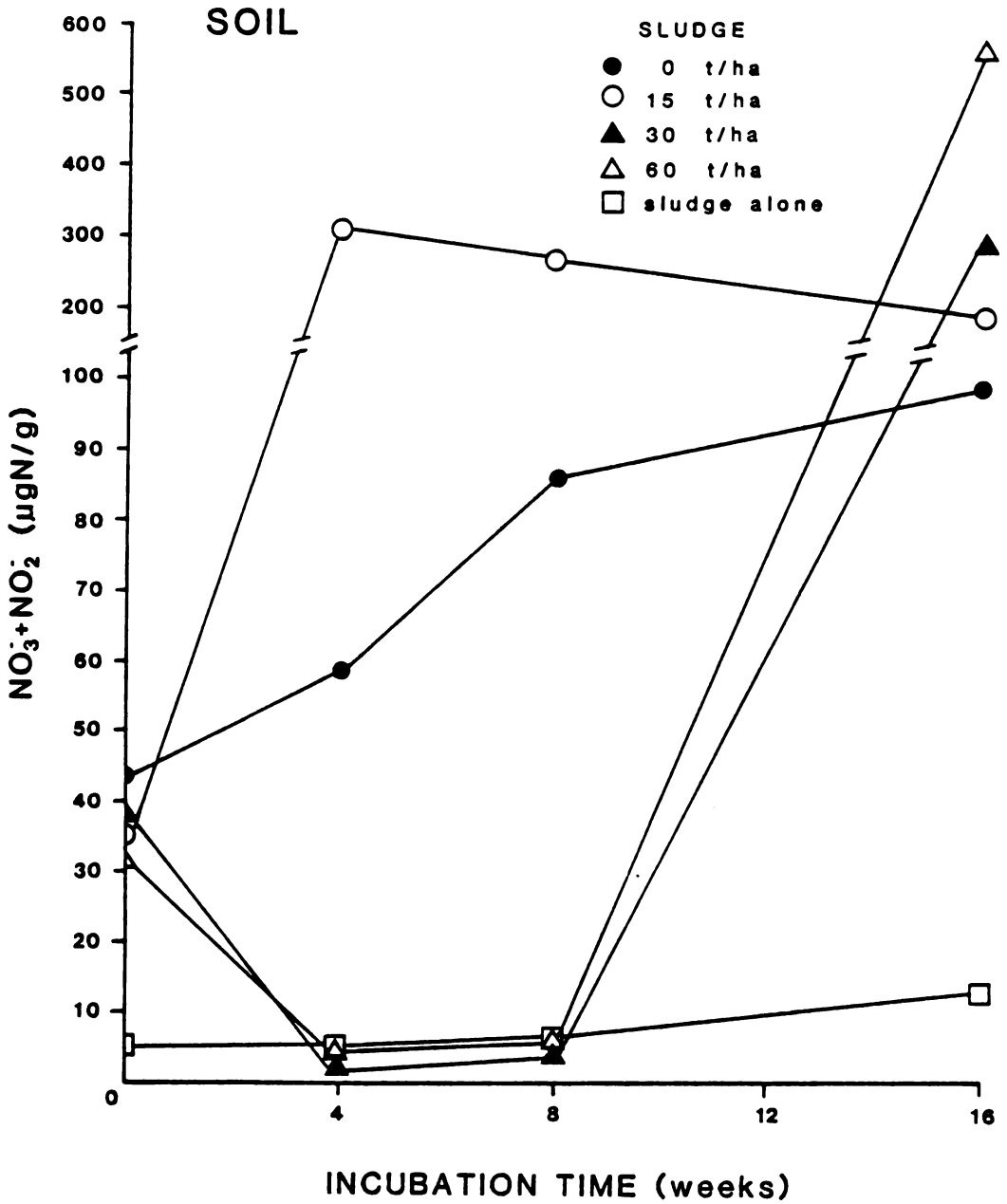

Figure #11. Nitrate plus nitrite in soil/sludge mixtures and sludge alone.

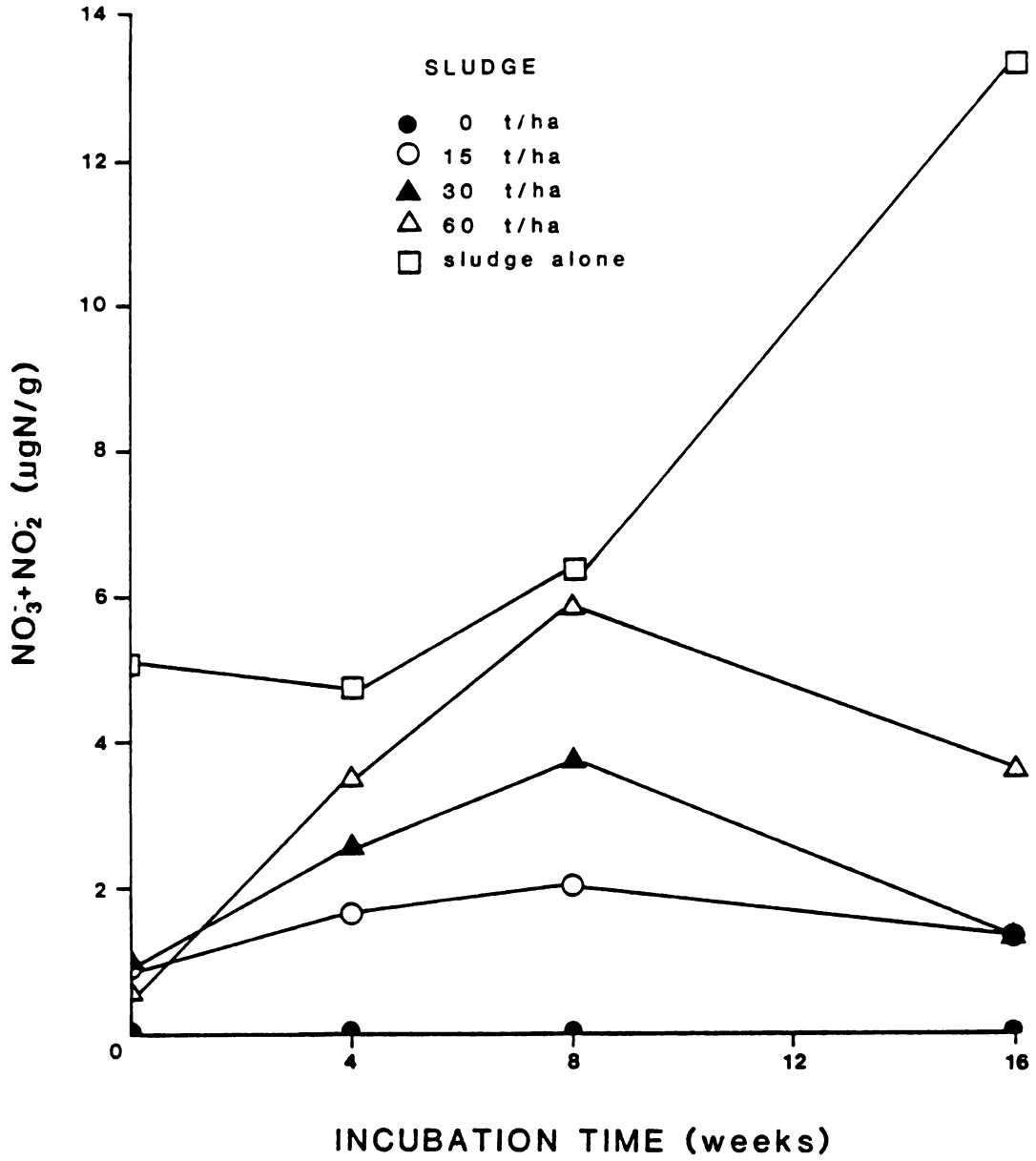

Figure #12. Nitrate plus nitrite in sand/sludge mixtures and sludge alone.

incubation, NO_3^- increased to a maximum 308 ugN/g, after which it declined slowly to 190 ugN/g at 16 weeks (Figure 11). Nitrification proceeded more slowly, but continuously in soil alone, reaching a maximum of 98 ugN/g as NO_3^- at 16 weeks.

By contrast, NO_3^- plus NO_2^- initially present in soil containing 30 or 60 T/ha of sludge largely disappeared in the first four weeks. Nitrate accumulation was seriously interfered with through the eighth week of incubation. During the last eight weeks, NO_3^- accumulated again at rates similar to those observed during the first four weeks at the 15 T/ha sludge addition.

Nitrification was strongly inhibited in sludge alone (Figure 11). However, significant increases in NO_3^- did occur between the fourth and 16th weeks of incubation, reaching a maximum value of 13 ugN/g (Figure 12 and Table 19). Levels of NO_3^- plus NO_2^- in mixtures of sludge with sand were lower in all samplings than in sludge alone. In these mixtures, significant increases occurred during the first eight weeks of incubation and significant decreases thereafter.

It should be noted that NO_3^- was the principle anionic form of N encountered at all times in all systems except sand alone, where none was found. Nevertheless, the presence of NO_2^- even in trace amounts, indicates that the levels of NO_3^- encountered represent equilibria controlled by nitrification and opposing reactions in which NO_2^- is an

intermediate. Thus, periods when net losses of NO_3^- + NO_2^- occurred in the systems of Figures 11 and 12 may be ascribed to one or more of several processes which consumed NO_3^- or NO_2^- more rapidly than they were being formed by nitrifying bacteria. Probable consuming reactions include biological immobilization, biodenitrification, chemodenitrification and chemical fixation of NO_2^- (Firestone, 1982; Nelson, 1982).

Considerations Regarding Toxic Effects

In Figure 11, the apparent inhibition of nitrification between the fourth and eighth weeks of incubation at 30 and 60 T/ha of sludge coincides with a period when diffusable NH_4^+ in KCl extracts of the same soil/sludge systems was extremely high (Figure 5). The pH of the soil was initially 7.2 and, as will be seen in data from the second experiment, may have been increased by as much as one pH unit by ammonification at these high rates of sludge addition.

Thus, the equilibrium $NH_3 \overset{H^+}{\rightleftharpoons} NH_4^+$, would have favored NH_3 concentrations that might have inhibited oxidation of NO_2^- to NO_3^- by Nitrobacter (Aleem and Alexander, 1960). However, NO_2^- did not accumulate -- only trace quantities ranging up to 3 ug N/g were found. It is possible that Nitrosomonas types ($NH_4^+ \longrightarrow NO_2^-$) were also inhibited. However, systems were already present that had removed NO_3^- and NO_2^- initially present by the fourth week of incubation (Figure 11). Also, net nitrification resumed abruptly after eight weeks and proceeded at the same high rate observed earlier in the 15 T/ha system. Conversion of NH_4^+

to NO_3^- between eight and 16 weeks was essentially stoichiometric (cf. Tables 11 and 19 in the Appendix).

These results suggest that oxidation of NH_4^+ by Nitrosomonas proceeded without interruption during the period when neither NO_2^- or NO_3^- accumulated, but NO_2^- was removed as rapidly as formed, by reactions leading to denitrification and/or to immobilization in organic forms. The data collected in this experiment do not permit further speculation on this point.

With regard to inhibitory effects on growth and assimilation of N by oat seedlings (Figures 1 to 3), it does appear that adverse effects of dominantly NH_4^+ nutrition were involved. In both soil and sand systems, toxic effects on oats at four and eight weeks occurred where diffusable NH_4^+ in the rooting media was very high (Figures 5 and 6) and NO_3^- was very low (Figures 11 and 12).

Incubation Experiment

No attempt was made in this second experiment to repeat the plant assays. Incubations were repeated, with the following variations: soil, sand, and sludge alone were compared with mixtures at only the 60 T/ha rate of sludge; incubations were carried out in sealed glass containers rather than in polyethylene bags; 5N H_2SO_4 was used to trap volatilized NH_3 in one set of containers but not in the other; the incubation temperature was a little lower (25°C rather than 30°C). Incubated samples were extracted with 2N KCl, then autoclaved in 2N KCl, as in the first experiment,

and the same forms of N were determined. In addition, changes in pH were followed, and Kjeldahl N was determined in the residue after autoclaving.

Changes in pH

The addition of sludge (initial pH 4.62) caused the initial pH to decrease from 7.38 and 6.80 in soil and sand, respectively, to 6.85 and 5.23 (Table 1). After four weeks of incubation, the pH for all treatments had increased. These increases were associated with large increases in diffusable NH_4^+ in direct KCl extracts of sludge alone and the two mixtures, but not in soil or sand alone. In fact, in sand alone, no N was found at any time during the incubation.

These initial pH increases in soil and sand cannot be ascribed to ammonification or to additions of alkalinity in the deionized water (pH 6.2) used to activate the samples prior to incubation. It appears that the deionized water acted as a mild extractant and favored equilibria that led to dissociation of cations in excess of anions.

Trapping of ambient free NH_3 had no consistent effect on pH changes during incubation. As will be pointed out in a later section, periods of net nitrification in individual systems were accompanied by decreased pH. Decreases in NO_3^- plus NO_2^- were accompanied by increases in pH or no change in pH values.

Table 1. Changes in pH during incubation of trapped and not trapped systems involving soil, sand, sludge and mixtures. †

Trapped ‡ vs. not trapped	Medium	Incubation time in weeks								
		0	2	4	6	8	10	12	14	16
T	Soil alone	7.38	--	7.81	7.82	7.22	7.78	7.82	7.69	8.04
NT	" "	7.38	--	7.48	7.46	7.39	7.31	7.29	7.78	7.65
T	Soil/sludge	6.85	--	8.11	8.40	8.30	8.51	8.56	8.39	8.59
NT	" "	6.85	--	8.20	8.20	8.37	8.71	8.73	8.46	8.47
T	Sand alone	6.80	--	7.60	7.40	7.18	7.91	7.55	7.43	7.58
NT	" "	6.80	--	7.68	7.60	7.28	7.49	7.56	7.55	7.45
T	Sand/sludge	5.23	--	6.63	6.60	6.65	6.16	5.69	5.81	5.94
NT	" "	5.23	--	7.06	6.98	6.63	6.39	6.22	6.26	6.42
T	Sludge alone	4.62	--	5.58	5.08	4.82	4.93	4.83	4.86	5.12
NT	" "	4.62	--	5.22	5.10	4.84	4.83	4.85	4.98	5.17

† = Trapped samples were incubated with an acid (H_2SO_4) trap to remove ambient NH_3-N.

‡ = Means for quadruplicate samples.

Immobilization vs Denitrification

Nitrogen recoveries for trapped samples are presented in Tables 21 to 24 of the Appendix. At zero time, the sum for all measured forms of N, including volatilized NH_3, represented 92 to 95% of calculated inputs, based on total N in soil, sand, or sludge and the quantities used in each system. Percentage recoveries decreased to values as low as 62% (soil/sludge, Table 22) at four to eight weeks and then increased to values that were at times equal to or greater than at the beginning of incubation.

Quantities of N (ug/g) not accounted for are given in Table 2. Negative values for soil alone at 12 to 16 weeks might suggest that N fixation occurred to replace N lost earlier by denitrification. However, these negative values could be accounted for by no more than a 6% error in determining N in the original soil (cf. Table 21).

Considering the data for all systems, there is no evidence that important net losses of N by denitrification occurred. Rather, it appears that much of the N not accounted for at various times during incubation was retained (immobilized) in forms that were not measured.

Forms of N Not Measured

Forms of N that were not measured were those that were resistant to alkaline hydrolysis when direct extracts and autoclaved hydrolysates were distilled in NaOH. These would have included α-amino N in microbial cells and cellular debris, and structural N in fulvic and humic acids

Table 2. Nitrogen not accounted for in the total for fractional forms found in soil, sludge, and sludge mixtures.

Medium	Time of incubation in weeks								
	0	2	4	6	8	10	12	14	16
	μ gN/g								
Soil alone	55.8	61.7	200	112	97.9	101	-16.8	-36.0	-63.0
Soil/sludge	221	505	1031	823	505	261	144	281	265
Sand/sludge	123	199	262	295	46.4	17.4	160	29.8	353
Sludge alone	2928	2553	1914	2806	5007	2712	846	1177	2575

(Stevenson, 1982). Both the direct extracts and the hydrolysates were colored by humic substances and also undoubtedly contained microbial cells and other particulates of colloidal size that were not removed by centrifuging for eight minutes at 400 xg.

Diffusable NH_4^+ and Amino Sugar Fractions

Totals for extracts plus hydrolysates: In Figures 13 and 14, changes in N not accounted for are compared with diffusable NH_4^+ or amino sugar fractions as the sums recovered in direct extracts plus the hydrolysate after autoclaving. In soil alone and in the soil/sludge system, N not accounted for increased quickly to maximum values at four weeks (Figure 13). In the sand/sludge mixture and in sludge alone (Figure 14), peaks occurred at six or eight weeks, and sharp increases occurred again in the last sampling at 16 weeks.

The patterns of change in N not accounted for are consistent with an expected initial increase in microbial numbers and α-amino N in cellular proteins (Miller, 1974; Broadbent, 1973). The much earlier increase in numbers indicated for soil alone and the soil/sludge mixture reflect their higher pH (Table 1). Also, it is likely that the soil provided an inoculum of zymogenous organisms with a wider range of enzymatic capabilities than were present in the sludge. Increases near the end of incubation might reflect:

a. growth of a successional population;

b. conversion of labile N to alkali-stable structural N in fulvic and humic acids;

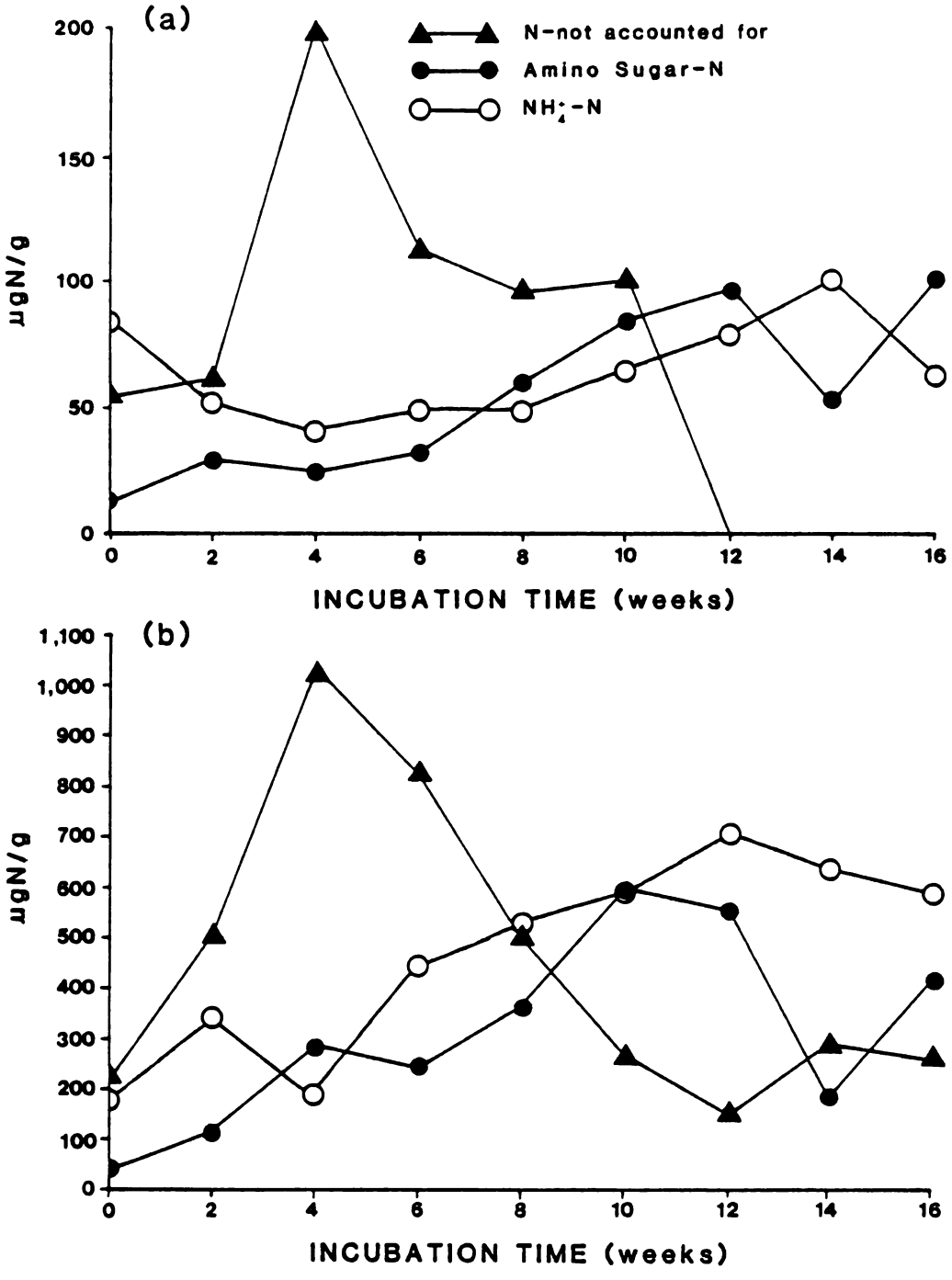

Figure #13. Summed recoveries (extracts plus hydrolysates) of diffusable NH_4^+ and amino sugar fractions compared with N not accounted for in soil alone (a) and soil/sludge mixtures (b). Data for trapped samples only.

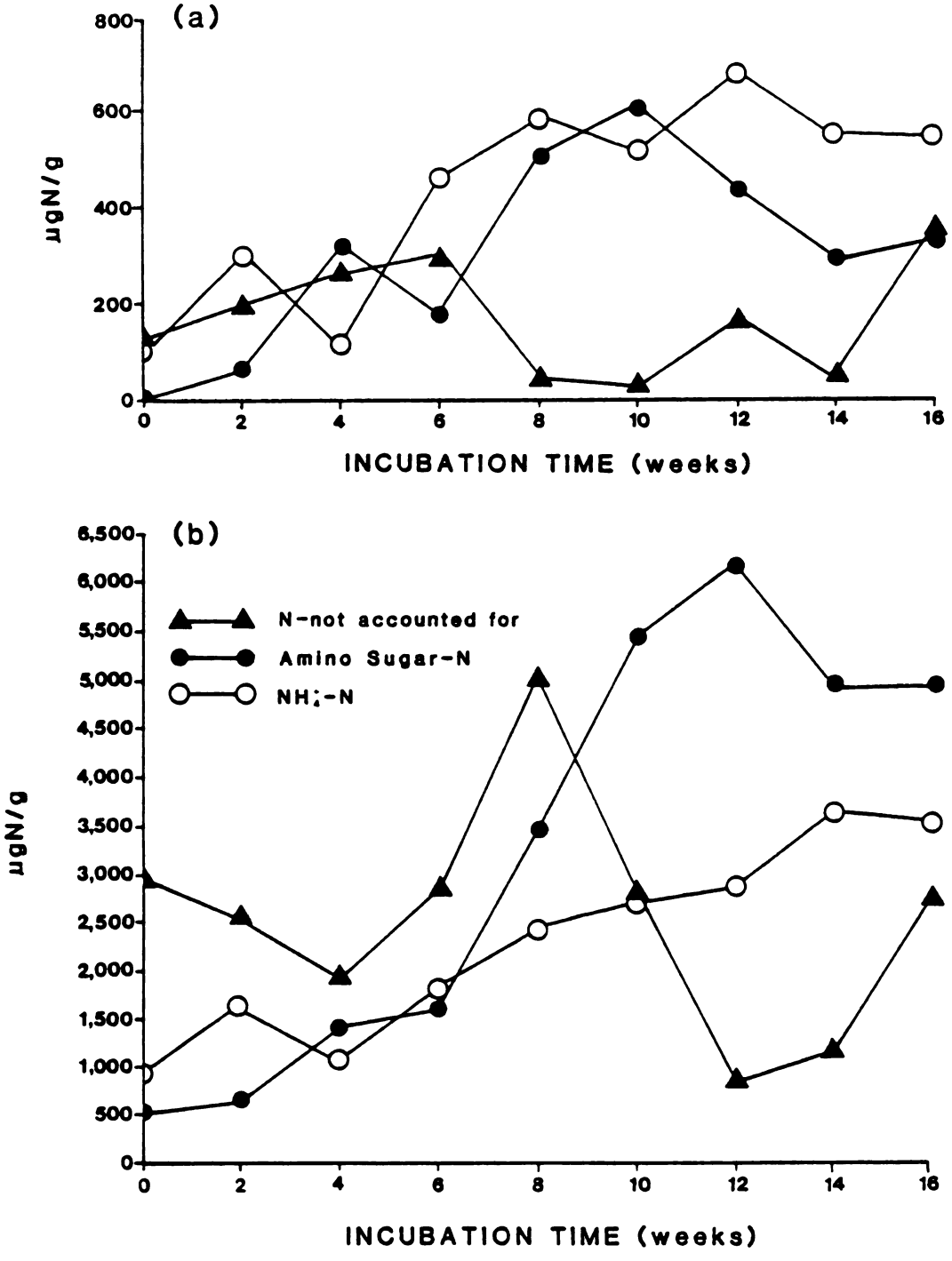

Figure #14. Summed recoveries (extracts plus hydrolysates) of diffusable NH_4^+ and amino sugar fractions compared with N not accounted for in sand/sludge mixtures (a) and sludge alone (b). Data for trapped samples only.

c. losses of N by denitrification.

Amino sugars are important constituents of microbial cell walls and extracellular mucopolysaccharides (microbial "gums" or "mucilages"). The N in this fraction (Figures 13 and 14) increased to peak values considerably later than N not accounted for. Mature or senescent microbial populations might be expected to continue producing extracellular polysaccharides for a period after active growth in numbers had ceased.

However, the indicated maximum quantities of amino sugar N appear unreasonably high in relation to the indicated earlier levels of microbial protein. Also, major increases in this fraction appear to have coincided with rapid and extensive lysis of microbial proteins.

The intimate mixture of enzymes, amino acids, sugars and phenols in cellular autolysates is highly reactive. It is likely that much of the alkali labile N that appeared during and after lysis was in the form of amides and amines in humic acid precursors formed by Maillard reactions between amino acids and sugars, and by analogous condensation and degradation sequences involving amino acids or NH_3 and polyphenols (Nommik and Vahtras, 1982; Stevenson, 1982).

Diffusable NH_4^+ in direct extracts is simply NH_4^+ exchangeable to 2N KCl. It represents net release of NH_4^+ by ammonification, which is the first step in mineralization. Diffusable NH_4^+ in the autoclaved supernatants probably represents ammonified N that was retained initially by

electrovalent exchange also, but at sites that were later covered up by hot water soluble products of metabolism. Increases in diffusable NH_4^+ reflect increases in surface area and number of oxidized acidic sites capable of retaining NH_4^+ by electrovalent exchange.

Increases in the total for categories of N depicted in Figures 13 and 14 were derived largely from forms of N that were initially resistant to hydrolysis by autoclaving ("residual N" in Tables 21 to 24 of the Appendix). Thus, decomposition resulted in the transfer (mobilization) of N from "inactive" to "active" organic fractions. Some of this N was further mineralized to NH_4^+, NO_2^- and NO_3^- recovered in the direct KCl extract.

Some N may have been lost by denitrification. However, such losses would appear to have been small compared with immobilization in microbial cells and products in "active" fractions extractable with KCl or released by autoclaving.

Extracts and hydrolysates compared: Data for the amino sugar fractions in direct extracts and autoclaved hydrolysates are presented in Tables 25 to 30 of the Appendix. Data for diffusable NH_4^+ appear in Tables 31 to 36. Data for samples where ambient NH_3 was removed by acid trapping are presented in Figures 15, 16, and 17.

Fractional recoveries for soil alone (Figure 15a, b) may be compared with their sums in Figure 13a. In both the extract and the autoclaved hydrolysate, NH_4^+-N and N in the

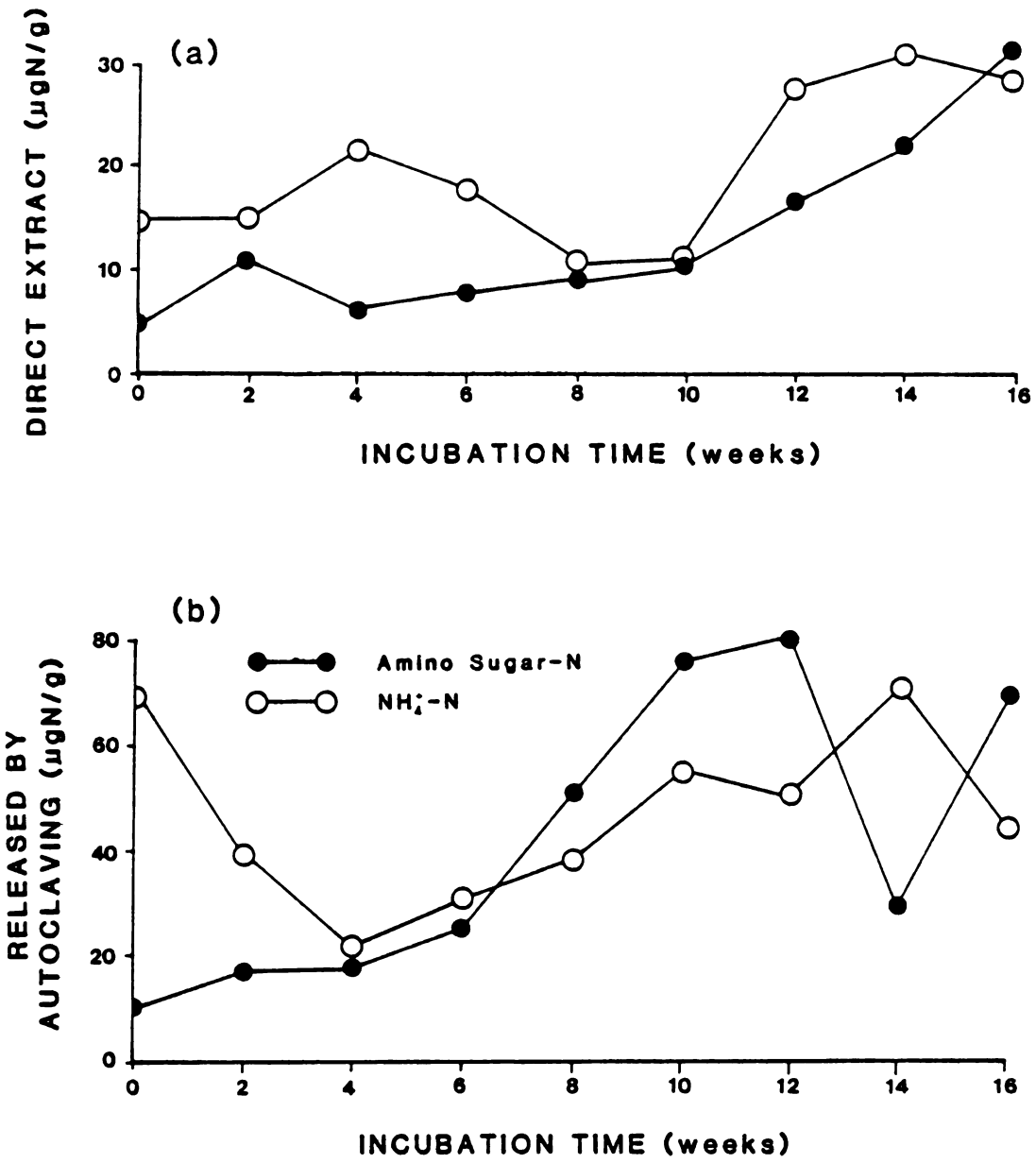

Figure #15. Amino sugar N fractions and NH_4^+-N in direct extracts (a) or released by autoclaving (b) from soil alone (data for trapped samples only).

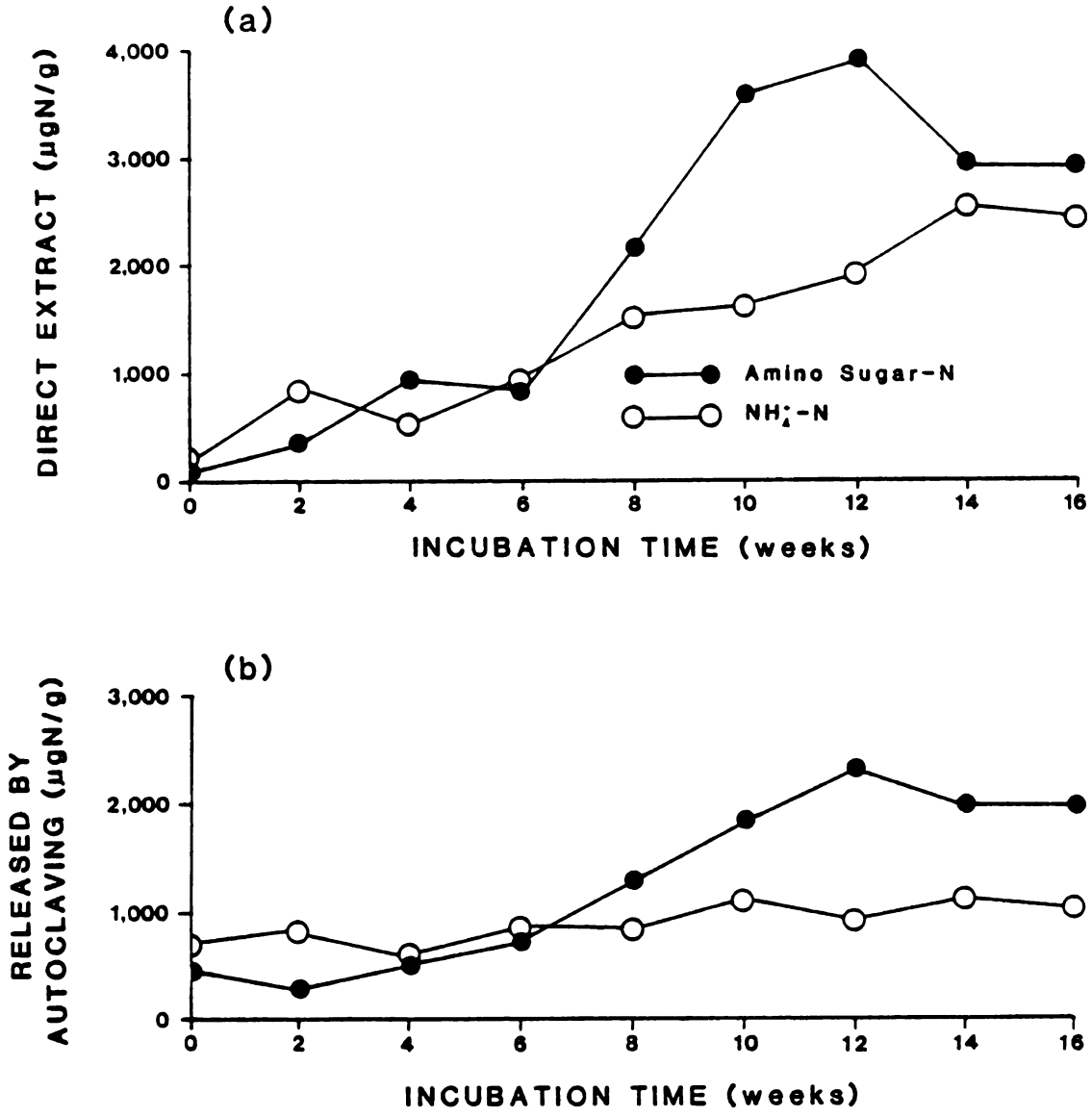

Figure #16. Amino sugar N fractions and NH_4^+-N in direct extracts (a) or released by autoclaving (b) from sludge alone (data for trapped samples only).

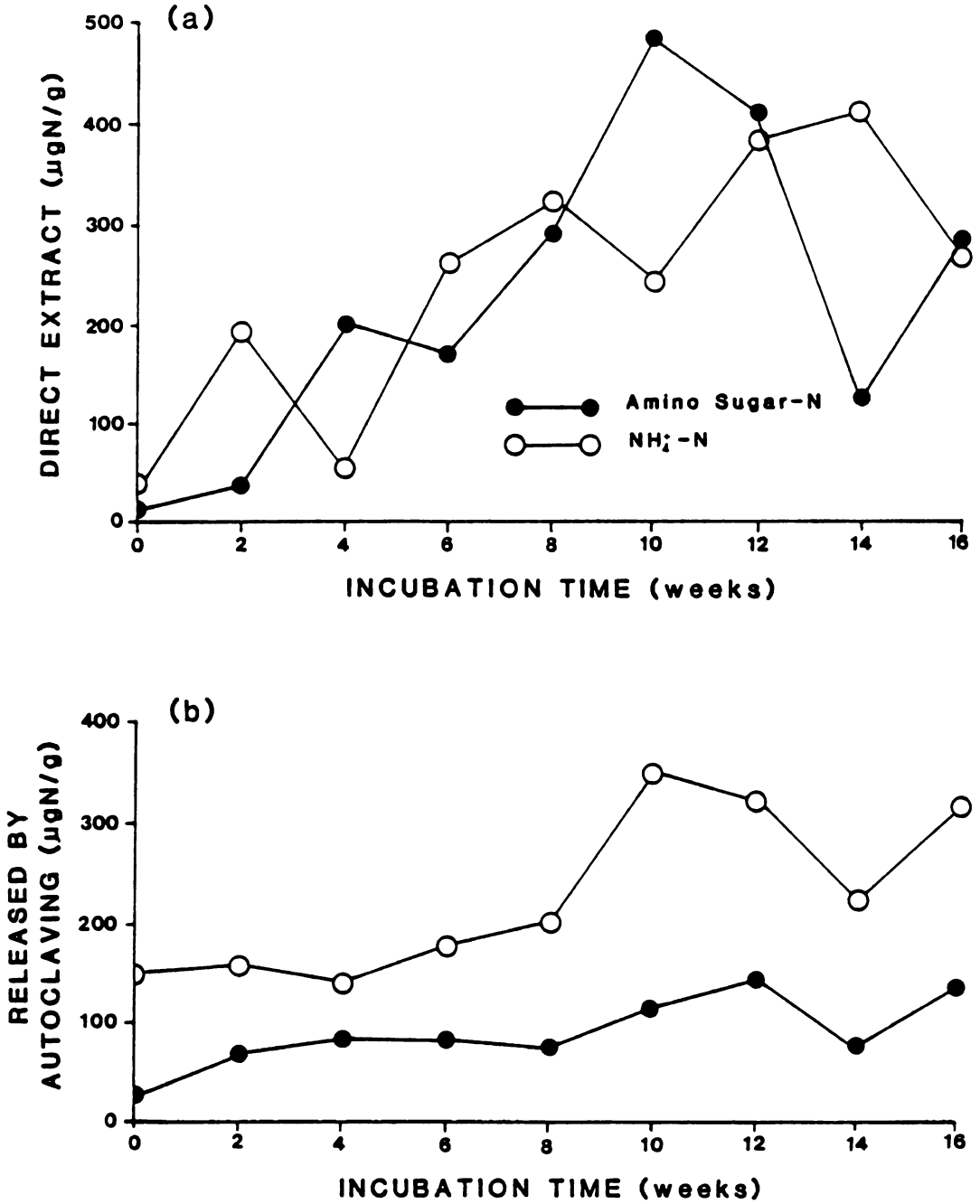

Figure #17. Amino sugar N fractions and NH_4^+-N in direct extract (a) or released by autoclaving (b) from soil/sludge mixtures (data for trapped samples only).

amino sugar fraction were recovered periodically in about equal quantities. A similar tendency for these two categories of N to return periodically to equal quantities is apparent in the direct extract of sludge alone (Figure 16a) and the soil/sludge mixture (Figure 17a). Reciprocal increases and decreases in these two forms of N in direct extracts of the sand/sludge system were very similar to those for soil/sludge in Figure 17a. (cf. Figure 19a,b).

This tendency for diffusable NH_4^+ and N in the amino sugar fractions to fluctuate about a 1:1 ratio suggests a basic stoichiometry between sites active in electrovalent exchange of NH_4^+ and sites capable of condensing reversibly with NH_3 (Lindbeck and Young, 1965; Mortland and Wolcott, 1965; Nommik and Vahtras, 1982).

Effects of Acid Trapping

The above results for trapped samples probably represent rather well what might be expected under well aerated field conditions where ambient NH_3 could diffuse out of the soil. On the other hand, results with samples where ambient NH_3 was not removed by acid trapping indicate that surface interactions can be influenced significantly by local concentrations of NH_3 in microenvironments.

Probable sources of volatile NH_3: Very small amounts of volatile NH_3 were recovered from sludge alone (Figure 18). This reflects the effect of low pH on the $NH_3 \overset{H^+}{\rightleftharpoons} NH_4^+$ equilibrium (cf. Table 1). Significantly larger quantities of NH_3 were recovered from soil alone (Figure 18 and Table

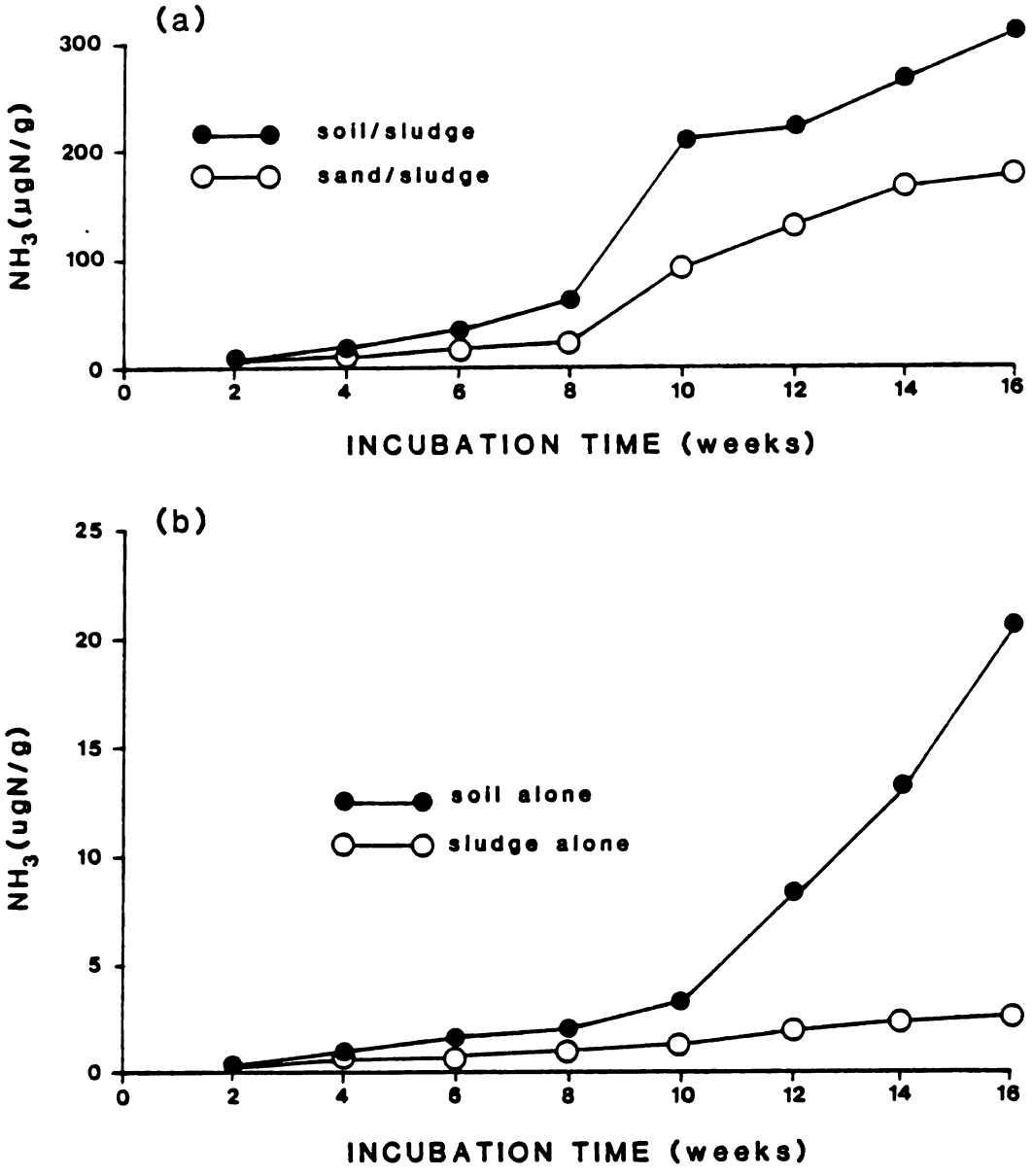

Figure #18. Volatilized ammonia collected from trapped systems.

37). The rate of release increased sharply after eight weeks.

Much larger quantities of volatile NH_3 were produced in the two mixtures, and variance was analyzed separately (Table 38). Again, rates increased sharply after eight weeks (Figure 18).

If one compares the data in Figure 18 with those in Figures 13a, b and 14a, it will be seen that major releases of volatile NH_3 from soil alone and the two mixtures occurred after major decreases in size of microbial populations (as inferred from N not accounted for). Rapid release of NH_3 began as diffusable NH_4^+ and alkali-labile N (amino sugar fraction) approached maximum values. Later releases were accompanied by large reciprocal fluctuations of alkali-labile N and diffusable NH_4^+ in the hydrolysate from soil alone (Figure 15b) and in the direct extract from soil/sludge (Figure 17a) and sand/sludge (Figure 19a, b).

In autoclaved hydrolysates of the soil/sludge mixtures (Figure 17b), the two forms of N tended to accumulate in parallel fashion, but much larger quantities were retained as diffusable NH_4^+. The same was true for trapped samples of the sand/sludge mixture (cf. Figure 20a and 20b).

In sludge alone, N that might have come off as NH_3 at higher pH was apparently retained mainly in alkali-labile forms in both the KCl extract and the hydrolysate (Figure 16a, b).

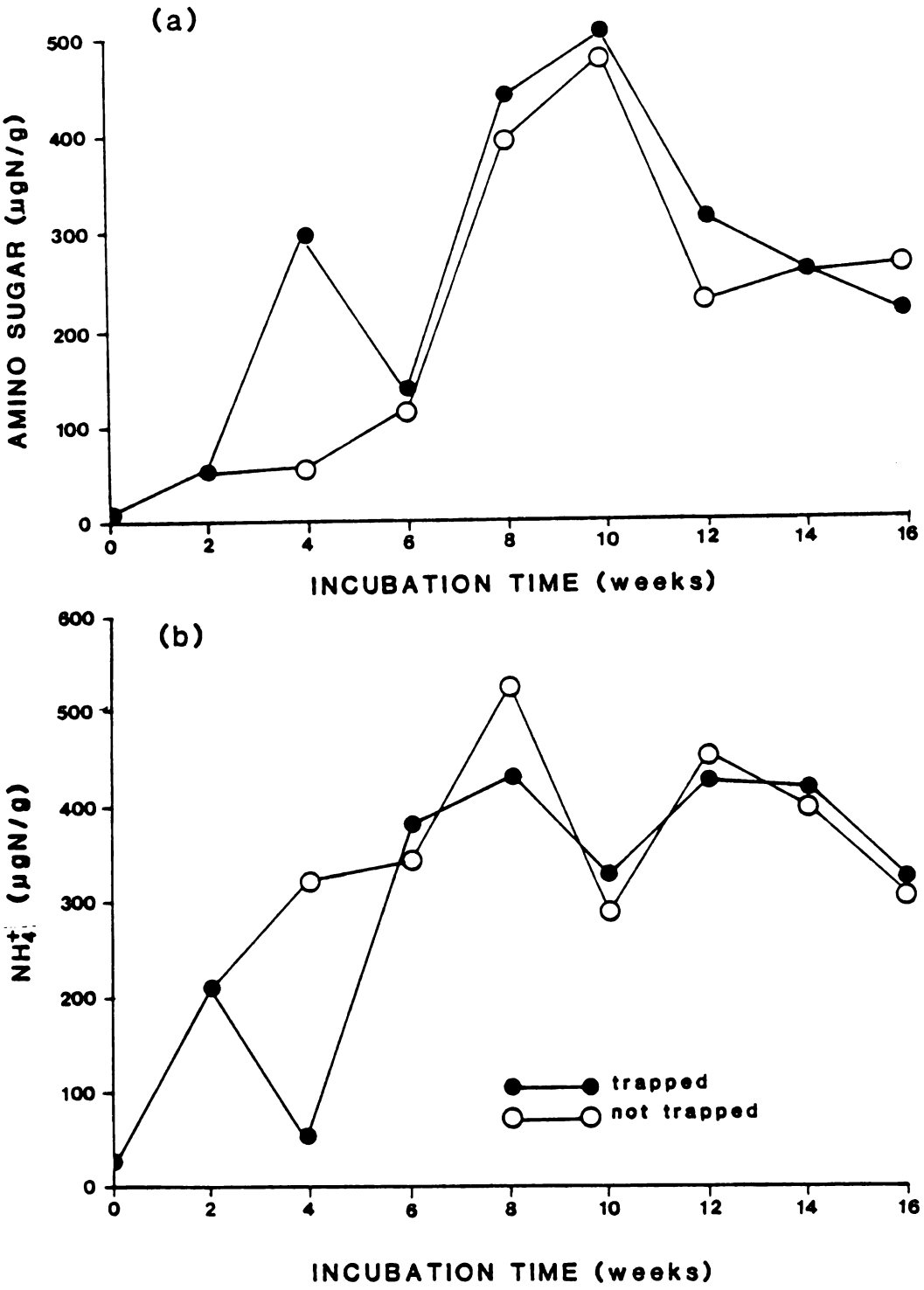

Figure #19. Amino sugar N fractions (a) and NH_4^+-N (b) in direct extract (2NKCl) of sand/sludge mixture.

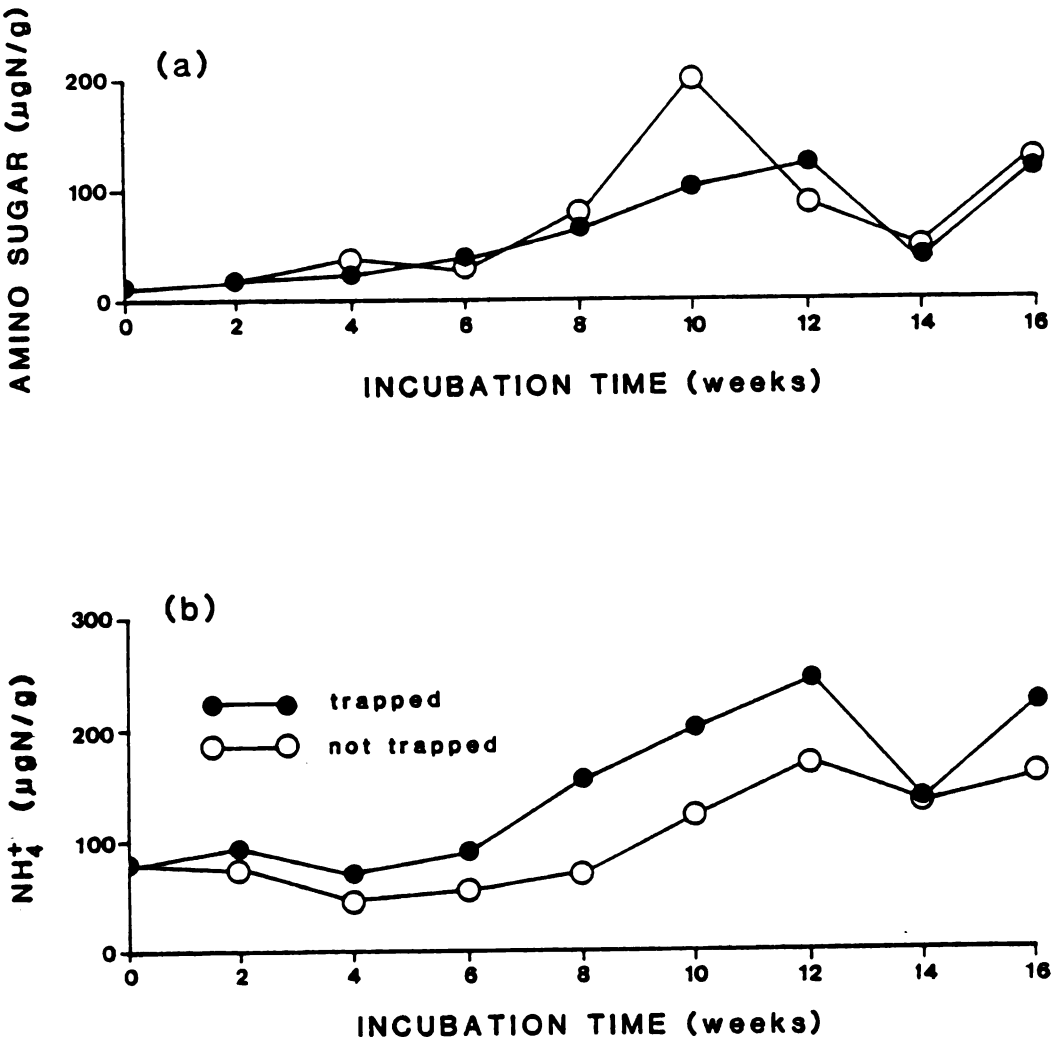

Figure #20. Amino sugar N fractions (a) and NH_4^+-N (b) released by autoclaving from sand/sludge mixture.

The above relationships indicate that rapid release of volatile NH_3 occurred only after saturation of sites capable of retaining NH_4^+ by electrovalent exchange or NH_3 by reversible condensation reactions. The rate of volatilization would appear to have been controlled primarily by equilibria involving reversibly condensed NH_3 (the "amino sugar" fraction). Reciprocal variations in this fraction and NH_4^+ diffusable in the presence of MgO probably reflect cyclic changes in oxidation state of organic functional groups and/or changes in degree of protonation associated with periods of net nitrification or net reduction of nitrite (Alexander, 1977; Nommik and Vahtras, 1982).

Effects of trapping on NH_4^+ and "amino sugar" fractions: In sludge alone, average recoveries in fractions obtained by direct extraction (Tables 29 and 35) or by autoclaving (Tables 30 and 36) were very similar for trapped and not trapped samples, although significant differences in patterns of accumulation did occur. In particular, the sharp reciprocal fluctuations of NH_4^+ and the amino sugar fraction at four weeks in the direct extract of trapped samples (Figure 16a) was less marked where ambient NH_3 was not removed (Tables 29 and 35). Later increases in extractable NH_4^+ were greater in trapped samples (Table 35), whereas alkali-labile N reached higher maximum values where ambient NH_3 was not removed (Table 29).

In the two mixtures, the large reciprocal fluctuations observed at four weeks in direct extracts of trapped samples

did not appear in not trapped samples (Figures 17a and 19a, b; cf. Tables 27 and 33). Later reciprocal changes occurred more or less simultaneously in trapped and not trapped samples.

Materials released by autoclaving were apparently less directly influenced by changes in the active biosphere (Figures 17b and 20a, b). Except for sharp dips at 14 weeks, violent fluctuations did not occur. In trapped samples of soil/sludge, N accumulated less rapidly in hydrolyzable fractions during the first half of the incubation than in not trapped samples (Tables 28 and 34). Early accumulations were similarly retarded in both trapped and not trapped samples of sand/sludge. In later samplings, hydrolyzable NH_4^+ (Table 34) fluctuated over a higher range of values in trapped than in not trapped samples. Ranges for hydrolyzable "amino sugar" N (Table 28) were similar in trapped and not trapped samples during the latter half of the incubation.

In soil alone, hydrolyzable fractions were apparently associated with surfaces directly accessible to active microbial populations. Hydrolyzable NH_4^+ initially present was utilized similarly in trapped and not trapped samples during the first four weeks (Figure 15b and Table 32). Later reciprocal fluctuations with hydrolyzable "amino sugar" N (cf. Table 26) were similar to those observed in direct extracts of soil/sludge (Figure 17a) and sand/sludge (Figure 19a, b), and were observed in both trapped and not

trapped soil. The principal effect of trapping was in the direct extracts, where alkali-labile N (Table 25) and NH_4^+ (Table 31) tended to decrease during the latter half of incubation in not trapped samples but continued to increase in trapped samples.

In summarizing the above observations, it would appear that an early effect of the NH_3 retained in not trapped systems was to delay the development of alkali-labile sites at surfaces accessible to direct extraction. This suggests that NH_3 initially adsorbed by the reaction $NH_3 + HX \rightleftharpoons NH_4X$ may have served to buffer surface sites against oxidative changes leading to condensation reactions with NH_3 (Mortland and Wolcott, 1965; Nommick and Vahtras, 1982). As will appear in the next section, fluctuations of NH_4^+ and alkali-labile N in extracts and hydrolysates may have involved interactions between heterotrophic and autotrophic organisms.

Effects of acid trapping on NO_3^- plus NO_2^- and on residual N: As noted earlier, NO_2^- was encountered in all samples, except sand alone, but usually in trace amounts (Tables 21 to 24 in the Appendix).

In soil alone, the sum of NO_3^- plus NO_2^- was very much higher throughout incubation in samples where ambient NH_3 was not removed by trapping (Figure 21a and Table 39). This differential was established during the first two weeks and can be accounted for by reciprocal changes in residual N (Figure 23a and Table 41). At the same time, the pH of

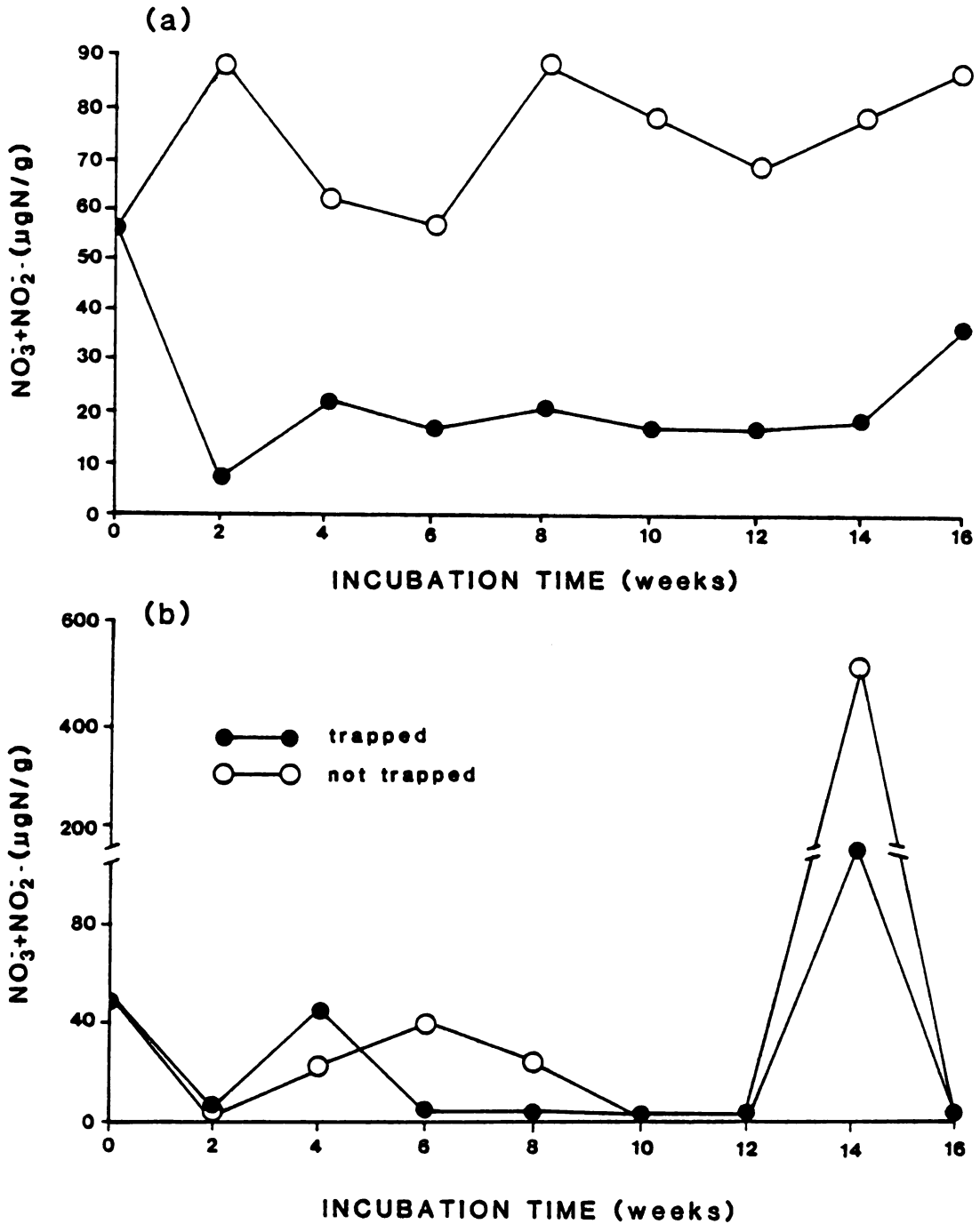

Figure #21. Nitrate plus nitrite in soil alone (a) and soil/sludge mixture (b), incubated with and without an acid trap to remove ambient NH_3.

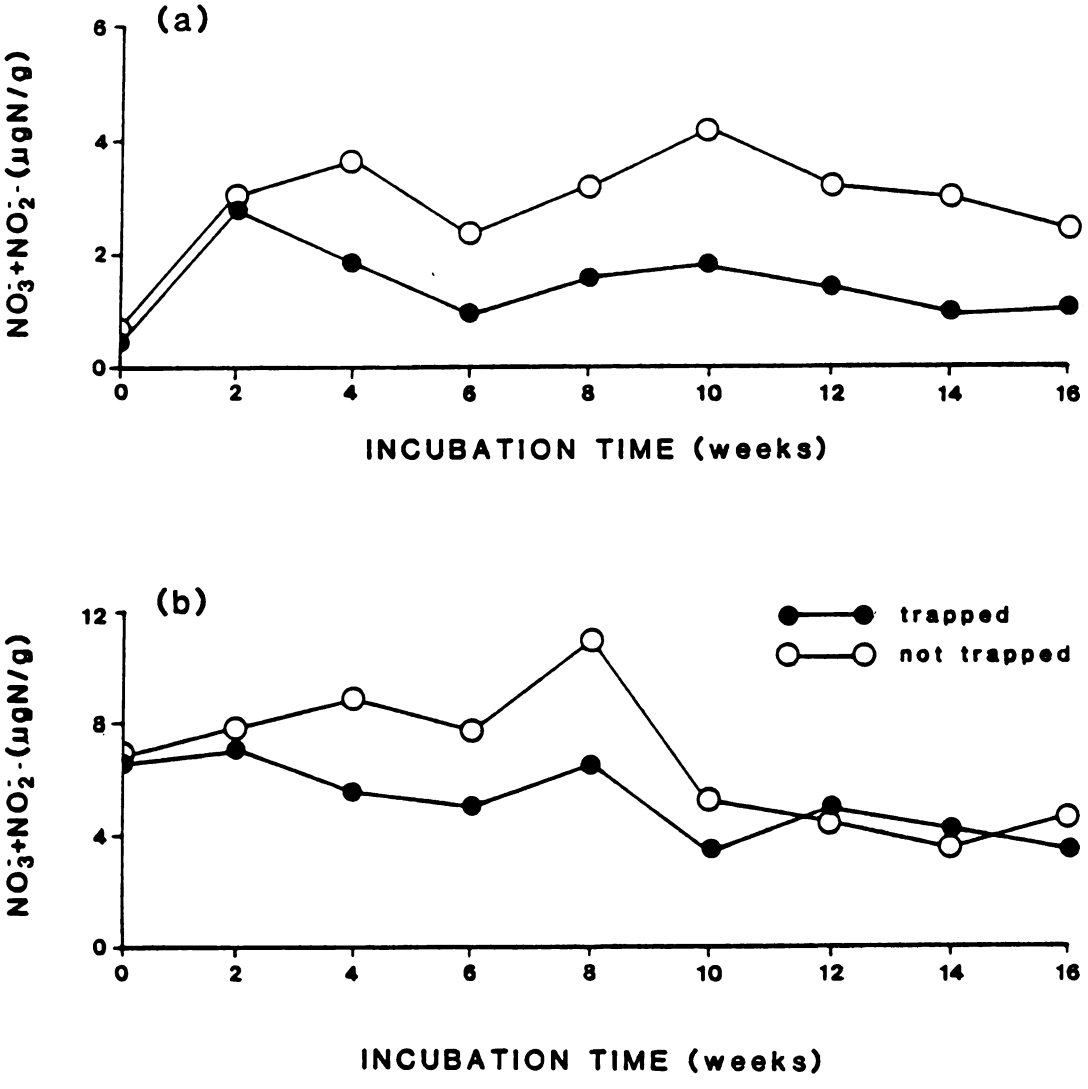

Figure #22. Nitrate plus nitrite in sand/sludge (a) and sludge alone (b), incubated with and without an acid trap to remove ambient NH_3.

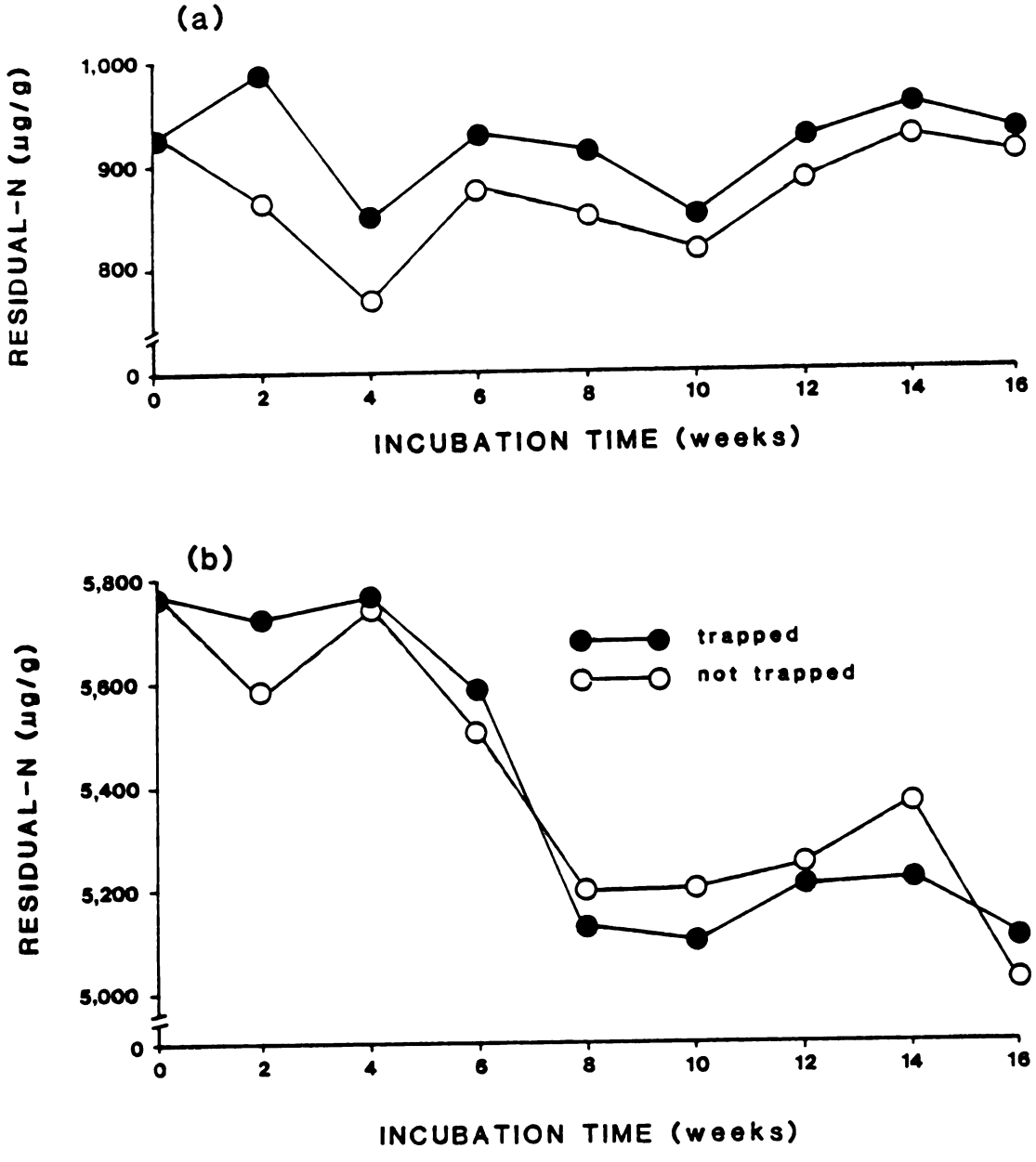

Figure #23. Residual nitrogen after autoclaving in soil alone (a) and sludge alone (b), incubated with and without an acid trap to remove ambient NH_3.

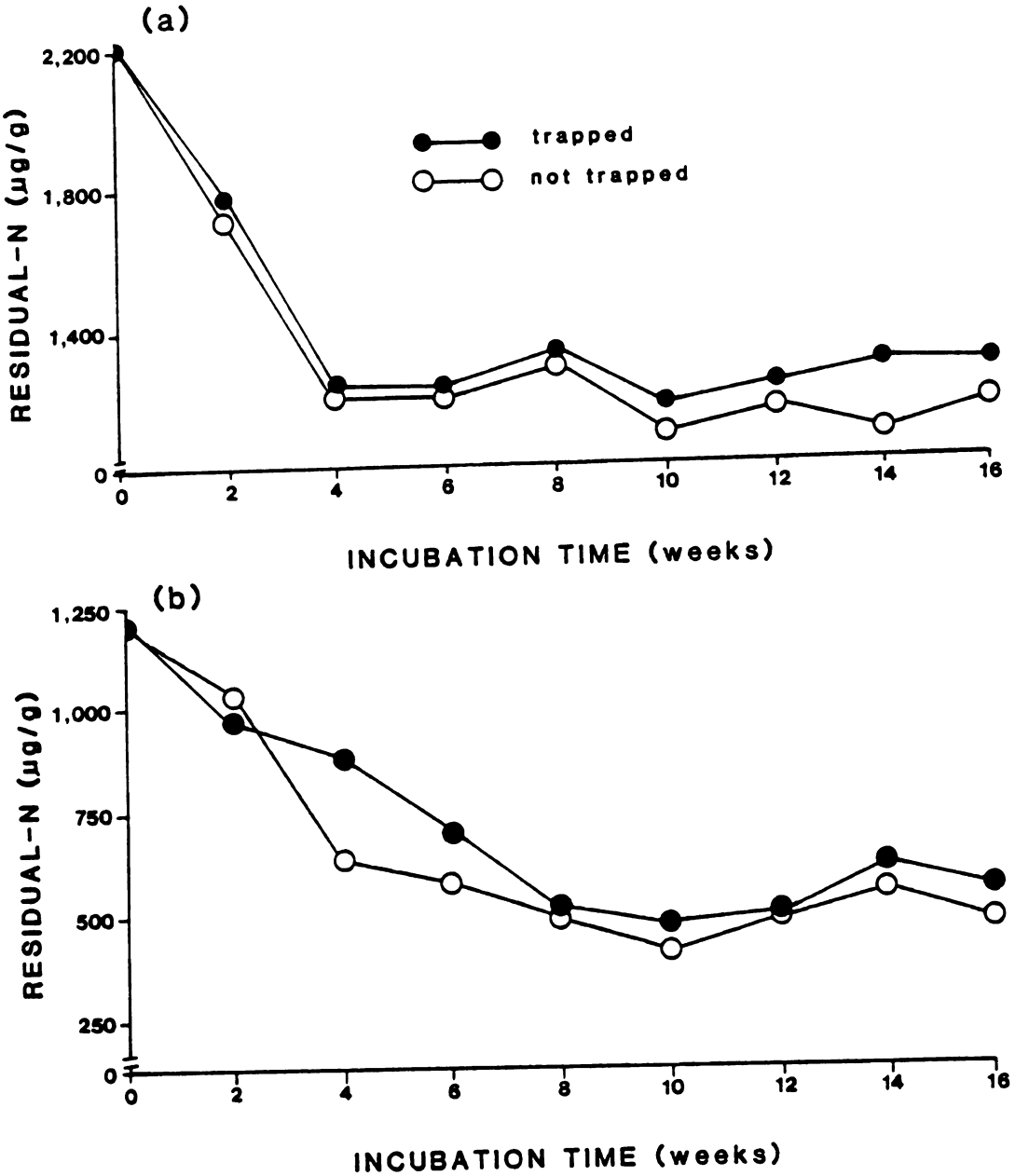

Figure #24. Residual nitrogen after autoclaving in soil/sludge (a) and sand/sludge (b), incubated with and without an acid trap to remove ambient NH_3.

trapped samples increased and remained higher than in not trapped samples in most later samplings (Table 1). This is consistent with the release of alkalinity that occurs when NO_3^- is reduced biologically (Alexander, 1961; Broadbent 1973).

Thus, it would appear that removal of ambient NH_3 may have reduced the availability of N to organisms requiring NH_3 or NH_4^+, thereby favoring the early enrichment of the heterotrophic population with types capable of reducing NO_3^- and NO_2^-.

It is apparent from Figures 21 and 22 that active nitrifying populations were present in all systems. Increasing CO_2 concentration in the sealed incubation containers would have been increasingly favorable for these autotrophic organisms (Bremner and Douglas, 1971; Eno, 1960). It is possible that the removal of ambient NH_3 reduced the availability of NH_3 or NH_4^+ to Nitrosomonas types responsible for the first step of nitrification, thereby lowering the rate of the overall transformation in trapped samples. In any case, it is apparent that the quantities of nitrified N found reflect a cyclically changing equilibrium between rates of nitrification and rates of reduction of NO_3^- and/or NO_2^-.

Dissimilatory reduction (denitrification) may have occurred in systems involving sludge (Tables 22 to 24). However, such losses would appear to have been minor relative to processes leading to incorporation of reduced N

into solid phase components. It is likely that NO_3^- and NO_2^- were first reduced assimilatively and incorporated into microbial cells. The very low pH values that developed in sludge alone after six weeks (Table 1) would have been favorable for chemical fixation of NO_2^- (Nelson, 1982). However, the patterns of change in N not accounted for in Figures 13 and 14 indicate that major transfers of N among solid phase components involved first an early rapid uptake by microbial cells that could be suspended by shaking in the direct extract and/or by autoclaving in the hydrolysate.

It is clear from Figures 23 and 24 that much of the nitrogen that appeared as microbial cells and extractable or hydrolyzable fractions came from materials that were initially resistant to hydrolysis by autoclaving. In the case of soil alone, a substantial additional source was NH_4^+ susceptible to release by autoclaving (Figure 15b). Sludge apparently contained substantial quantities of extractable or hydrolyzable N in alkali-stable substrates (N not accounted for in Figure 14b). These "active phase" substrates were metabolized before any major mobilization of more resistant forms in the "inactive phase" occurred (Figure 23b).

The electrically neutral NH_3 molecule is transferred across cell membranes more readily than charged species such as NH_4^+, NO_3^- or NO_2^-. Removal of NH_3 would account for differentially greater utilization of NO_3^- or NO_2^- in trapped samples (Figures 21 and 22). It would have also influenced

surface interactions at the interface between the solution phase and accessible surfaces in the solid phase. Preferential utilization of NH_3 over NH_4^+ undoubtedly contributed to differential effects of trapping on the distribution of N among the various NH_4^+ and "amino sugar" fractions in Tables 25 to 36.

SUMMARY

Systems responsible for the extensive changes in forms of N observed in this study are obviously complex. Transformations and equilibria that may have been involved are outlined in Fig. 25.

The dynamic nature of the observed transformations derives from readily available energy materials present initially in soil and/or sludge. Estimating levels and probable effects of available energy in soils or in organic materials added to soils is a major unsolved problem in predicting the fate of N and other nutrients or toxicants in field situations (Broadbent, 1973; Jansson and Persson, 1982).

The soil in this study had been air dried, and the sludge had been dried by heating. Before use, both were ground to pass a 2 mm sieve. Drying and grinding would have exposed energy substrates to support the flush of microbial growth evidenced by increases in N not accounted for during the first several weeks of incubation (Figs. 13 and 14).

In the soil (C/N $\simeq$ 9/1) and in the sludge (C/N $\simeq$ 3.4/1), removal of structural C as CO_2 by heterotrophic respiration would have led to net mineralization of N (Jansson and Persson, 1982). Mineralized N appears first as

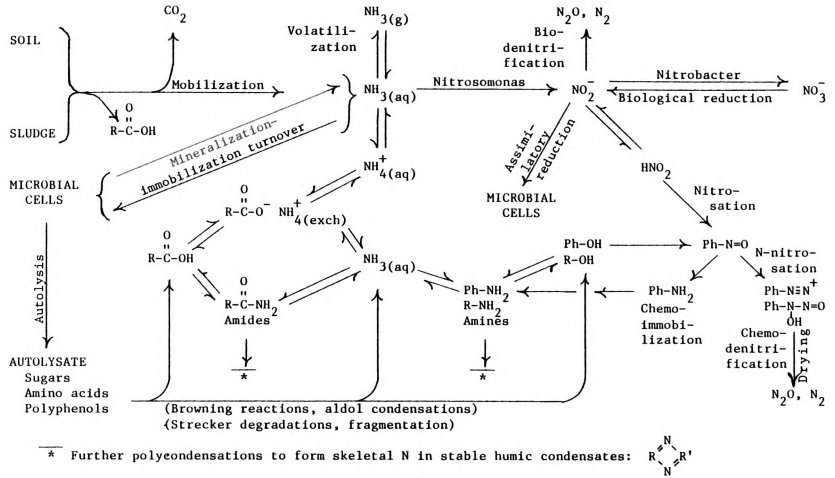

Fig. 25. Transformations and equilibria involving N in active phases (extractable fractions) of soil and ludge systems.

NH_3 in the aqueous phase. As shown in Fig. 25, aqueous phase equilibria can lead to losses of NH_3 by volatilization, or to protonation and electrovalent adsorption of NH_4^+ at acid sites in mineral or organic colloids (Nelson, 1982). Condensation of NH_3 with dienols or polyphenols to form alkali labile amides and amines may occur also (Broadbent and Stevenson, 1966; Nommik and Vahtras, 1982). As long as available energy substrates are present, NH_3 will be closely cycled by immobilization in successive heterotrophic populations (referred to by Jansson and Persson, 1982, as mineralization-immobilization turnover, or MIT).

The above processes were effective in preventing losses of NH_3 by volatilization through the first eight weeks of incubation (Fig. 18). Comparison of Fig. 18 with Figs. 13 and 14 indicates that significant volatilization of NH_3 occurred only after energy substrates had been depleted and extensive lysis of microbial cells had occurred.

As noted in Fig. 25, degradative reactions that follow the autocatalytic condensation of amino acids with sugars or with polyphenols would be expected to give rise to NH_3 or NH_4^+ in both diffusable (exchangeable) and alkali-labile condensed forms (Nommik and Vahtras, 1982; Stevenson, 1982). Early sequences in the Maillard browning reactions between amino acids and sugars are reversible (Mortland and Wolcott, 1965). The same is true for aldol condensations between NH_3 and polyphenols (Lindbeck and Young, 1965). The reversibility of these early reaction sequences may have

contributed to the large reciprocal fluctuations between diffusable NH_4^+ and the "amino sugar" fraction observed in all incubated systems (Figs. 15, 16, 17, 19).

The alkali labile amides and amines visualized as early condensation products in Fig. 25, are subject to extensive further polycondensation to form high molecular weight polymers. The skeletal N in these humic condensates would not be released by distillation with NaOH.

Transformations of N were further complicated by interactions involving the autotrophic nitrifying bacteria. A significant aspect of these interactions was their marked sensitivity to removal of ambient NH_3 by trapping (Figs. 21 and 22). Jansson and Persson (1982) note that heterotrophic and autotrophic organisms compete for mineralized NH_3, not only with each other, but also with physical and chemical processes which adsorb or fix NH_3 in combinations that cover a wide range of bonding energies. Equilibria among these forms shift quickly in the direction of more stable, less available combinations. For this reason, newly mineralized NH_3 or NH_4^+ in the close vicinity of the active biomass will be used preferentially.

Data obtained in the first experiment indicate that utilization of N by oat seedlings was adversely affected by dominantly NH_4^+ nutrition during periods when NO_3^- levels were low. Thus, from the standpoint of plant nutrition, the critical biosphere interactions at high rates of sludge

addition involved nitrification on the one hand and processes that removed NO_3^- or NO_2^- on the other.

Net removals of NO_3^- during the first weeks of incubation were most likely due to assimilatory reduction by adaptive heterotrophs. Removal of freshly mineralized NH_3 by acid trapping apparently favored an earlier adaptation to assimilative use of NO_3 as an alternative N source. Dissimilatory reduction to N_2O or N_2 by facultative anaerobes did not appear to have occurred during the flush of microbial activity supported by energy substrates initially present.

At the acid pH of the sludge and the sand/sludge mixture (Table 1), NO_2^- produced by Nitrosomonas was apparently "side-tracked" by non-enzymatic reactions with polyphenols originally present in soil or sludge or released by lysis of microbial cells. These side-tracking reactions involve undissociated nitrous acid (HNO_2) and are, therefore, favored by low pH (Mortland and Wolcott, 1965; Nelson, 1982).

As outlined in Fig. 25, initial products of nitrosation are readily reduced to aromatic amines that are entirely analogous to the labile amines formed in browning reactions or by aldol condensations of NH_3 (Nelson, 1982; Nommik and Vahtras, 1982; Stevenson, 1982). Thus, in acid systems, NO_2^--N may be side-tracked and retained in a cycle involving Nitrosomonas and labile products of chemical fixation of HNO_2. Such a cycle would not depend on organic energy

sources. It would be sustained by the energy derived from oxidation of NH_3 and the catalytic effect of protons produced in the oxidation. This cycle could continue as long as appropriately reactive polyphenols were present to trap NO_2^- and prevent its further oxidation to NO_3^- by Nitrobacter.

This proposition is supported by the very large accumulations of alkali labile N ("amino sugar" fraction) in sludge alone and the associated decreases in pH (cf. Fig. 14 and Table 1). A similar tendency for pH to decline as alkali labile N increased was apparent in sand/sludge but not at the alkaline pH of soil or soil/sludge. Nevertheless, side-tracking reactions could have proceeded in microenvironments closely adjacent to active Nitrosomonas populations and could, therefore, have interfered with normal accumulations of NO_3^- in soil or soil/sludge also.

Side-tracking of NO_2^- can lead also to chemodenitrification (Broadbent and Stevenson, 1966; Mortland and Wolcott, 1965; Nelson, 1982). However, losses of N_2O or N_2 by side-tracking are observed only during cycles of drying. No losses of moisture occurred during incubation. Thus, increases in N not accounted for near the end of incubation (Figs. 13 and 14) do not appear to have been due to chemodenitrification. It is more likely that N in measured forms declined at this time because of increased synthesis of proteins by successional microbial populations,

or because of accelerated polycondensation of alkali-labile amides and amines to form stable residual humic condensates (Fig. 25).

CONCLUSIONS

From the standpoint of theory, two potentially significant concepts are derived from this study:

1. Lysis of microbial cells may be an important aspect of biosphere transformations in soils. The possible significance of lysis in humification has been alluded to (Swaby and Ladd, 1966), but its implications do not appear to have been studied systematically. Data reported here suggest that the availability of N can be influenced dramatically by non-enzymatic reactions among products of autolysis. The probable nature of reactive species leads to the expectation that the availability of other nutrients, including potentially toxic heavy metals, may be influenced also.

2. "Side-tracking" of nitrification at the NO_2^- stage has been recognized as a probable mechanism for chemodenitrification in acid soils. Results of the present study suggest that, in the absence of drying, the side-tracked N may be retained in an active cycle involving Nitrosomonas and labile products of reaction of HNO_2 and polyphenols. This cycle would be sustained by the energy from oxidation of NH_3 and the catalytic effects of protons produced by the Nitrosomonas reaction.

From the standpoint of methodology, several obvious deficiencies in the data should be noted. Microbial proteins were estimated by differences as N not otherwise accounted for. This interpretation was supported by the plotted data. However, confirmation of the indicated autolysis of microbial cells will require specific measurement of proteins or of α-NH_2 after acid hydrolysis of extracts and autoclaved supernatants.

The difference between distillable and diffusable NH_3 is not specific for amino sugars. A more specific determination for amino sugars would have helped to support interpretations based on the probable role of alkali-labile condensates. Chromatographic and spectrophotometric criteria for characterizing postulated humic condensates according to molecular size will be needed in future studies to test the propositions presented here.

It is likely that incubations longer than 16 weeks will be necessary to estimate the length of time before normal net release of NO_3^- might be expected.

Nevertheless, the use of extraction and autoclaving to focus on important components in the active biosphere appears promising. Also, the quick uptake plant assay appears useful to reveal rapidly changing short term nutritional effects associated with decomposition of sludges or other organic amendments.

APPENDIX

APPENDIX

Table 3. Dry weight of oat seedlings after two weeks' contact with previously incubated soil/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Mean for rates
	0	4	8	16	
T/ha	mg/pot				
0	605 ef †	700 de	570 efg	608 ef	621 B †
15	800 bcd	924 ab	855 bc	745 cd	831 A
30	819 bcd	569 efg	516 fg	701 de	651 B
60	748 cd	564 efg	537 fg	834 bcd	671 B
Sludge alone	1000 a	483 fg	462 g	557 fg	625 B

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 4. Dry weight of oat seedlings after 2 weeks' contact with previously incubated sand/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Mean for rates
	0	4	8	16	
T/ha	mg/pot				
0	714 cde †	752 bcd	626 defg	581 efgh	663 A ÷
15	867 b	543 fgh	475 gh	738 bcd	656 AB
30	818 bc	513 fgh	467 h	621 defg	605 AB
60	722 cde	477 gh	541 fgh	637 def	594 B
Sludge alone	1000 a	483 gh	462 h	557 fgh	625 AB

÷ a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 5. Nitrogen concentration of oat seedlings after 2 weeks' contact with previously incubated soil/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Mean for rates
	0	4	8	16	
T/ha	%N				
0	2.45 g †	2.74 efg	2.55 fg	2.59 fg	2.58 D †
15	3.49 bcd	3.49 bcd	3.41 cd	3.28 cd	3.41 B
30	3.18 de	3.01 de	3.15 de	3.17 de	3.13 C
60	3.75 abc	3.04 def	3.29 cd	3.45 bcd	3.38 B
Sludge alone	3.92 ab	3.18 de	3.74 abc	4.00 a	3.71 A

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 6. Nitrogen concentration of oat seedlings after 2 weeks' contact with previously incubated sand/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Mean for rates
	0	4	8	16	
T/ha	---------- %N ----------				
0	1.32 hi †	1.18 i	1.48 hi	1.75 h	1.48 D †
15	2.77 fg	2.76 fg	2.95 efg	2.81 efg	2.82 C
30	3.06 defg	2.71 g	2.67 g	3.14 defg	2.89 C
60	3.10 defg	3.28 cdef	3.32 cde	3.49 bcd	3.29 B
Sludge alone	3.92 ab	3.18 defg	3.74 abc	4.00 a	3.71 A

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 7. Nitrogen uptake by oat seedlings after 2 weeks' contact with previously incubated soil/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Mean for rates
	0	4	8	16	
T/ha	---------- lc/pot ----------				
0	14.8 h †	16.1 fgh	14.5 h	15.6 h	15.7 D
15	28.0 bcd	31.7 b	29.3 bc	24.3 cde	28.3 A
30	25.7 cde	17.0 gh	16.4 h	22.7 def	20.5 C
60	27.9 bcd	17.1 gh	17.7 fgh	28.7 bc	22.8 B
pure sludge	39.1 a	15.3 h	17.2 gh	22.2 efg	23.5 B

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 8. Nitrogen uptake by oat seedlings after 2 weeks' contact with previously incubated sand/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Mean for rates
	0	4	8	16	
T/ha	mg/pot				
0	9.4 ij †	8.8 j	9.1 ij	10.1 hij	9.4 C †
15	24.1 bc	14.8 efghi	14.0 fghij	20.7 bcde	18.4 B
30	25.1 b	13.8 fghij	12.4 ghij	19.4 bcdef	17.7 B
60	22.7 bcd	15.7 efgh	18.4 cdefg	22.2 bcd	19.7 B
pure sludge	39.1 a	15.3 efgh	17.2 defg	22.2 bcd	23.5 A

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 9. Ammonium-N in sludge alone.

Extractants	Incubation time in weeks				Mean for Extractants
	0	4	8	16	
	μgN/g				
2N KCl	196 h †	1973 e	2697 b	2289 c	1781 B †
Saturated $CaSO_4$	267 h	213 d	2259 b	3039 a	1924 A
autoclaved 2N KCl	528 g	1396 f	1945 e	2132 d	1500 C

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 10. Amino sugar-N fractions in sludge alone.

Extractants	Incubation time in weeks				Means for Extractants
	0	4	8	16	
	μgN/g				
2N KCl	77 e	1858 c	2665 b	5696 a	2574 A
Saturated $CaSO_4$	65 e	469 d	197 c	5624 a	2033 B
autoclaved 2N KCl	523 d	2943 b	1865 c	1897 c	1807 C

† a,b,c...;A,B,C...mean values accompanied by the same letter are not significantly different at P(05).

Table 11. Directly extractable NH_4^+-N in soil/sludge mixtures.

Extractant	Sludge rate	Incubation time in weeks 0	4	8	16	Means for rates
	T/ha	µgN/g ±				
2N KCl	0	2.7 n ≠	4.1 m	5.4 l	2.7 n	3.6 D ≠
	15	43.5 i	49.3 h	59.6 g	13.5 k	36.2 C
	30	84.3 f	241 d	395 c	27.2 j	121 B
	60	128 e	496 b	549 a	51.7 h	205 A
Saturated $CaSO_4$	0	4.3*	8.6*	16.9*	4.3*	7.2*
	15	45.2	142 *	171*	16.9*	65.6*
	30	56.7*	285 *	373*	34.1*	119
	60	116	513	802*	98.5*	262 *

± geometric means (antilog of mean log transform)

≠ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

* means for saturated $CaSO_4$ extract are significantly different from corresponding means for 2N KCl extract.

Table 12. Directly extractable NH_4^+-N in sand/sludge mixtures.

Extractant	Sludge rate	Incubation time in weeks 0	4	8	16	Means for rates
	T/ha	---------- µgN/g † ----------				
	0 ≐	0	0	0	0	
2N KCl	15	15.5 k §	194 e	140 j	81.6 h	67.6 C §
	30	36.7 j	365 bc	381 c	177 f	173 B
	60	44.9 i	412 b	478 a	326 d	231 A
	0	0	0	0	0	
Saturated $CaSO_4$	15	25.7*	276 *	298 *	59.9*	106*
	30	38.4	360	458 *	176	183*
	60	59.9*	608 *	720 *	248 *	284 *

† geometric means (antilog of mean log transform)

≐ Values for the rate zero are not included in ANOVA.

§ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

* means for saturated $CaSO_4$ extract are significantly different from corresponding means for 2N KCL extract.

Table 13. Directly extractable amino sugar-N in soil/sludge mixtures.

Extractant	Sludge rate	Incubation time in weeks 0	4	8	16	Means for rates
	T/ha	µgN/g †				
2N KCl	0	16.9 ef ≠	16.3 ef	17.6 ef	10.9 g	15.4 C ≠
	15	5.2 h	19.5 de	38.9 c	13.3 fg	15.1 C
	30	13.1 fg	65.8 b	83.2 b	18.9 e	34.1 B
	60	25.9 d	193 a	189 a	21.5 de	67.1 A
Saturated $CaSO_4$	0	4.3*	4.3*	42.4*	7.8*	8.8*
	15	5.8	17.5	24.9	10.0	12.6
	30	24.6*	83.0	390 *	24.9	66.7*
	60	38.8	74.2*	170	64.2*	74.9

† geometric means (antilog of mean log transform)

≠ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

* means for saturated $CaSO_4$ extract are significantly different from corresponding means for 2N KCl extract.

Table 14. Directly extractable amino sugar-N in sand/sludge mixtures.

Extractant	Sludge rate	Incubation time in weeks 0	4	8	16	Means for rates
	T/ha	µgN/g †				
2N KCl	0 ≐	0	0	0	0	
	15	13.6 h §	24.5 fg	66.3 c	16.0 h	24.4 C §
	30	22.3 g	45.0 d	127 b	29.7 ef	44.2 B
	60	36.6 de	110 b	331 a	109 b	110 A
Saturated $CaSO_4$	0	0	0	0	0	
	15	4.3*	31.4	49.2*	98.3*	28.4*
	30	17.5	311 *	343 *	72.6*	108 *
	60	12.8*	321 *	300	124	111

† geometric means (antilog of mean log transform)

≐ Values for the rate zero not included in ANOVA.

§ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

* means for saturated $CaSO_4$ extract are significantly different from corresponding means for 2N KCl extract.

Table 15. Ammonium-N released by autoclaving from soil/sludge mixtures.

Sludge rate	Incubation time in weeks				Means for rates
	0	4	8	16	
T/ha	μgN/g †				
0	57.0 k ≠	111 i	59.2 k	93.9 j	77.0 D ≠
15	95.2 i	260 e	149 gh	115 i	143 C
30	107 i	363 c	321 d	159 fg	211 B
60	136 h	433 b	634 a	174 f	284 A

† geometric means (antilog of mean log transform)

≠ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

Table 16. Ammonium-N released by autoclaving from sand/sludge mixtures.

Sludge rate	Incubation time in weeks				Means for rates
	0	4	8	16	
T/ha	μ gN/g †				
0	0 ≠	0	0	0	
15	36.7 j §	97.5 f	93.4 f	25.7 k	54.1 C §
30	54.4 h	186 d	224 c	46.9 i	101 B
60	64.3 g	426 b	493 a	120 e	200 A

† geometric means (antilog of mean log transform)

≠ Values for the rate zero not included in ANOVA.

§ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

Table 17. Amino sugar-N released by autoclaving from soil/sludge mixtures.

Sludge rate	Incubation time in weeks				Means for rates
	0	4	8	16	
T/ha	---------- µgN/g † ----------				
0	26.7 fg ≠	18.9 g	26.4 fg	26.2 fg	24.4 D ≠
15	29.8 efg	40.8 def	48.3 cde	31.5 efg	36.9 C
30	39.0 ef	151 b	67.5 cd	25.2 fg	56.3 B
60	37.3 ef	643 a	72.1 c	40.6 def	91.6 A

† geometric means (antilog of mean log transform)

≠ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

Table 18. Amino sugar-N released by autoclaving from sand/sludge mixtures.

Sludge rate	Incubation time in weeks				Means for rates
	0	4	8	16	
T/ha	---------- µgN/g † ----------				
0	0 ≠	0	0	0	0
15	1.9 d §	27.4 b	7.2 c	20.5 b	9.4 C §
30	17.9 b	62.2 a	16.9 b	18.5 b	24.3 B
60	20.6 b	95.0 a	79.9 a	31.9 b	47.3 A

† geometric means (antilog of mean log transform)

≠ Values for the rate zero not included in ANOVA.

§ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

Table 19. Nitrogen as NO_3^- + NO_2^- in soil/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks				Means for rates
	0	4	8	16	
T/ha	---------- µgN/g † ----------				
0	43.4 ef ‡	58.1 e	86.9 d	98.1 d	68.1 B ‡
15	35.4 f	308 b	295 bc	190 c	153 A
30	37.5 f	2.6 k	4.2 ij	296 b	11.6 D
60	30.9 f	3.5 j	5.4 hi	563 a	23.9 C
Sludge only	5.0 hij	4.7 hij	6.2 h	13.2 g	6.68 E

† geometric means (antilog of mean log transform)

‡ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

Table 20. Nitrogen as NO_3^- + NO_2^- in sand/sludge mixtures and sludge alone.

Sludge rate	Incubation time in weeks 0	4	8	16	Means for rates
T/ha	---------- µgN/g † ----------				
0	0 ≒	0	0	0	
15	0.86 b §	1.68 gh	2.06 gh	1.31 gh	1.47 D §
30	0.94 h	2.61 efg	3.86 de	1.29 gh	2.18 C
60	0.71 h	3.49 def	5.85 bc	3.64 de	3.42 B
Sludge only	5.02 bcd	4.73 cd	6.30 b	13.3 a	7.33 A

† geometric means (antilog of mean log transform)

≒ Values for the rate zero not included in ANOVA.

§ a,b,c...;A,B,C...mean values accompanied with the same letter are not significantly different at P(05).

Table 21. Nitrogen recoveries for soil alone (trapped samples).

Forms of nitrogen	Time of incubation in weeks								
	0	2	4	6	8	10	12	14	16
	μgN/g								
Extracted in 2NKCl	19.7	25.9	25.1	25.6	17.9	35.1	73.9	89.3	85.5
Released by autoclave	78.8	51.7	39.4	57.3	89.7	132	133	98.9	115
Nitrate (NO_3^--N)	57.1	10.2	23.8	17.5	22.2	17.1	17.1	19.3	61.2
Nitrite (NO_2^--N)	0.17	0.25	3.06	1.83	1.49	4.48	0.12	0.17	0.26
Volatilized NH_3-N	--	0.30	0.75	1.34	1.61	3.20	8.21	13.06	20.64
Residual N †	927	988	846	923	908	846	923	954	919
Total accounted for	1083	1077	938	1027	1041	1038	1155	1175	1202
Percent recovered ǂ	95	95	82	90	91	91	101	103	106

† = Residual N = Total Kjeldahl nitrogen after autoclaving.

ǂ = Total-N accounted for as percent of 1138.6 μg/g Kjeldahl-N calculated as input.

Table 22. Nitrogen recoveries for soil/sludge mixture (trapped samples).

Forms of nitrogen	Incubation time in weeks								
	0	2	4	6	8	10	12	14	16
	µgN/g								
Extracted in 2NKCl	49.3	255	261	433	616	722	795	510	546
Released by autoclave	177	159	140	183	197	350	325	225	319
Nitrate (NO_3^--N)	50.1	3.29	45.9	8.90	16.9	6.23	1.55	147	1.80
Nitrite (NO_2^--N)	0.29	0.24	1.25	4.41	6.72	0.41	0.10	0.18	0.27
Volatilized NH_3-N	--	6.10	14.20	34.5	59.9	216	220	269	308
Residual N †	2227	1796	1231	1239	1323	1169	1239	1292	1285
Total accounted for	2504	2220	1694	1902	2220	2464	2581	2444	2460
Percent recovered ǂ	92	81	62	70	81	90	95	90	90

† = Residual N = Total Kjeldahl nitrogen after autoclaving.

ǂ = Total-N accounted for as percent of 2724.8 µg/g Kjeldahl-N calculated as input.

Table 23. Nitrogen recoveries for sand/sludge mixture (trapped samples).

Forms of nitrogen	Time of incubation in weeks								
	0	2	4	6	8	10	12	14	16
	µgN/g								
Extracted in 2NKCl	29.6	261	372	520	876	828	574	666	482
Released by autoclaving	88.7	94.9	70.5	89.0	155	200	245	139	225
Nitrate (NO_3^--N)	0.48	2.77	1.84	0.95	1.50	1.65	1.38	0.86	1.13
Nitrite (NO_2^--N)	0.01	0.18	0.22	0.35	0.25	0.39	1.05	1.07	0.20
Volatilized NH_3-N	--	4.2	9.5	18.8	21.3	91.9	134.7	164.7	175.7
Residual N †	1374	1054	900	692	515	477	500	615	554
Total accounted for	1493	1417	1354	1321	1570	1599	1456	1586	1263
Percent recovered ǂ	92	88	84	82	97	99	90	98	78

† = Residual N = Total Kjeldahl nitrogen after autoclaving.

ǂ = Total-N accounted for as percent of 1616.0 µg/g Kjeldahl-N calculated as input.

Table 24. Nitrogen recoveries for sludge alone (trapped samples).

Forms of nitrogen	Time of incubation in weeks								
	0	2	4	6	8	10	12	14	16
	μgN/g								
Extracted in 2NKCl	246	1198	1466	1777	3712	5192	5781	5513	5380
Released by autoclaving	1222	1154	1076	1648	2158	2957	3253	3089	3052
Nitrate (NO_3^--N)	6.70	7.10	5.60	4.90	6.50	3.70	4.80	4.10	3.70
Nitrite (NO_2^--N)	0.28	0.35	0.43	0.54	0.44	0.59	0.63	0.39	0.51
Volatilized NH_3-N	--	0.22	0.60	0.77	0.86	1.34	1.81	2.08	2.32
Residual N †	57596	57088	57537	55763	51116	51133	52114	52214	50987
Total accounted for	59072	59447	60086	59194	56993	59288	61154	60823	59425
Percent recovered ‡	95	96	97	95	92	96	99	98	96

† = Residual N = Total Kjeldahl nitrogen after autoclaving.

‡ = Total-N accounted for as percent of 62000.0 μg/g Kjeldahl-N calculated as input.

Table 25. Directly extractable amino sugar fraction in soil alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	4.9	11.1 cd ǂ	6.47 d	7.49 cd	9.18 cd	10.5 cd	16.5 bc	21.9 b	30.8 a	14.3
Not trapped	4.9	6.16	10.7	9.58	14.4	7.52	13.1	6.59*	6.10*	9.26*

† = Values for time zero were not included in ANOVA.

ǂ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 26. Amino sugar fractions released by autoclaving from soil alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	9.8	17.3 c ‡	18.2 c	25.6 c	51.2 b	76.4 a	81.2 a	29.8 c	69.9 a	46.2
Not trapped	9.8	14.8	24.7	29.9	70.0*	68.9	68.9	49.5*	77.1	50.5

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 27. Directly extractable amino sugar fraction from soil/sludge and sand/sludge mixtures.

Trapped vs. not trapped	Medium	Incubation time in weeks									Means for trapped and not trapped
		0†	2	4	6	8	10	12	14	16	
		µgN/g									
Trapped	Soil/sludge	9.9	34.5 lm	205 efghijk	169 ghijk	289 def	482 ab	411 abc‡	123 jklm	279 efg	249
	Sand/sludge	1.27	49.3 lm	297 de	135 ijklm	442 ab	505 a	312 cde	254 efgh	214 efghijk	276
Not trapped	Soil/sludge	9.9	27.1	51.2*	237	431*	405	184*	144	298*	222
	Sand/sludge	1.27	49.3	52.1*	112	392	479	227*	254	255	228*

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 28, Amino sugar fraction released by autoclaving from soil/sludge and sand/sludge mixtures.

Trapped vs. not trapped	Medium	Incubation time in weeks									Means for trapped and not trapped
		0 †	2	4	6	8	10	12	14	16	
		μgN/g									
Trapped	Soil/sludge	29.6	68.6 efgh‡	83.8 def	82.9 def	71.8 efg	116 bcd	148 b	71.3 efg	139 bc	97.8 A
	Sand/sludge	9.9	14.8 i	21.2 i	35.1 ghi	65.5 efgh	101 cde	119 bcd	30.7 hi	119 bcd	63.2 B
Not trapped	Soil/sludge	29.6	17.3*	35.3*	43.3*	118*	122	143	92.8	190*	94.9
	Sand/sludge	9.9	14.8	28.5	28.2	75.5	193*	85.6	44.2	117	73.4

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...; A,B,...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 29. Directly extractable amino sugar fraction from sludge alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	76.5	350 i ‡	924 h	867 h	2161 g	3599 c	3861 ab	2933 e	2917 e	2202
Not trapped	76.5	355	810	920	2312	4030*	3708	3215*	2566	2239

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 30. Amino sugar fraction released by autoclaving from sludge alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	455	329 f ‡	533 def	777 d	1309 c	1860 b	2315 a	1980 ab	1995 ab	1387
Not trapped §	455	323	370	711	1385	2077	2323	2013	1953	1394

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

§ = Values for not trapped are not different from values for trapped.

Table 31. Directly extractable NH_4^+ from soil alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	14.8	14.8 cd ‡	21.2 b	17.6 bc	10.8 de	10.5 de	27.8 a	30.9 a	27.9 a	20.2
Not trapped	14.8	14.8	10.4*	14.1	10.8	10.5	7.52*	4.49*	5.59*	9.77*

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 32. Ammonium-N released by autoclaving from soil alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	69.0	39.4 de ǂ	21.2 h	31.7 fg	38.6 def	55.1 b	51.7 bc	71.7 a	45.1 cd	44.3
Not trapped	69.0	38.2	21.2	28.2	34.1	52.6	50.2	66.2	31.7*	40.3*

† = Values for time zero were not included in ANOVA.

ǂ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 33. Directly extractable NH_4^+ from soil/sludge and sand/sludge mixtures.

Trapped vs. not trapped	Medium	Incubation time in weeks									Means for trapped and not trapped
		0 †	2	4	6	8	10	12	14	16	
		μgN/g									
Trapped	Soil/sludge	39.4	191 k ‡	56.3 l	264 hijk	327 efghi	240 hijk	385 cdefg	412 bcde	268 hijk	268 B
	Sand/sludge	28.3	212 jk	49.4 l	384 cdefg	434 abcd	323 efghi	434 abcd	412 bcde	318 efghi	321 A
Not trapped	Soil/sludge	39.4	192	191*	221	388	212	512*	410	318	306*
	Sand/sludge	28.3	214	324*	343	527	283	449	390	302	354*

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...;A,B...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 34. Ammonium-N released by autoclaving from soil/sludge and sand/sludge mixtures.

Trapped vs. not trapped	Medium	Incubation time in weeks									Means for trapped and not trapped
		0 †	2	4	6	8	10	12	14	16	
		μgN/g									
Trapped	Soil/sludge	148	159 fghij ‡	141 hij	182 defg	197 cde	351 a	325 a	225 bc	319 a	237 A
	Sand/sludge	78.8	94.9 klt	70.5 lmn	89.0 lm	155 ghij	200 cd	245 b	139 hij	225 bc	152 B
Not trapped	Soil/sludge	148	86.2*	52.9*	79.4*	85.3*	168*	193*	196	129*	124*
	Sand/sludge	78.8	81.3	49.4	56.4	70.0*	124*	167*	141	162*	106*

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...;A,B...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 36. Ammonium-N released by autoclaving from sludge alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	μgN/g									
Trapped	767	825 cde ≠	542 f	871 cd	848 cde	1097 a	937 bc	1110 a	1057 ab	911
Not trapped	767	829	733*	721*	754	1054	846	1061	1057	882*

† = Values for time zero were not included in ANOVA.

≠ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 37. Volatilized NH_3 for soil alone and sludge alone.

Medium	Time of incubation in weeks									Means for media
	0	2	4	6	8	10	12	14	16	
	μgN/g									
Soil alone	--	0.29 k†	0.74 i	1.33 h	1.60 g	3.19 d	8.18 c	13.0 b	20.6 a	2.74 A
Sludge alone	--	0.20 l	0.59 j	0.75 i	0.85 i	1.32 h	1.81 fg	2.08 ef	2.32 e	0.98 B

† = a,b,c...;A,B...means accompanied by the same letter are not significantly different at P(05).

Table 38. Volatilized NH_3 for soil/sludge and sand/sludge mixtures.

Medium	Time of incubation in weeks									Means for media
	0	2	4	6	8	10	12	14	16	
	μgN/g									
Soil/sludge	--	6.11 m †	14.2 k	34.5 h	59.9 g	216 c	219 c	268 b	308 a	71.7 A
Sand/sludge	--	4.27 n	9.54 l	18.4 j	21.2 i	91.9 f	134 e	165 d	175 d	39.4 B

† = a,b,c...;A,B...means accompanied by the same letter are not significantly different at P(05).

Table 39. Nitrate plus nitrite for soil alone and soil/sludge mixture.

Trapped vs. not trapped	Medium	Incubation time in weeks									Means for trapped and not trapped
		0 †	2	4	6	8	10	12	14	16	
		μgN/g ǂ									
Trapped	Soil alone	57.1	7.5 h §	22.9 efg	17.4 fg	21.4 efg	16.6 g	16.9 fg	19.0 fg	37.1 defg	18.3 A
	Soil/sludge	50.1	3.3 hijk	45.7 cde	3.4 hijk	5.5 hi	2.7 ijk	1.5 k	144 b	1.7 jk	6.4 B
Not trapped	Soil alone	57.1	88.4*	61.4*	56.3*	88.3*	77.7*	67.3*	78.1*	88.4*	74.7*
	Soil/sludge	50.1	2.8	22.7	39.4*	24.4*	3.9	3.6*	513*	3.9	14.3*

† = Values for time zero were not included in ANOVA.

ǂ = Geometric means (anti log of mean log transforms)

§ = a,b,c...:A,B...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 40. Nitrate plus nitrite for sand/sludge mixture and sludge alone.

Trapped vs. not trapped	Medium	Incubation time in weeks									Means for trapped and not trapped
		0 †	2	4	6	8	10	12	14	16	
		μgN/g ‡									
Trapped	Sand/sludge	0.48	2.76 jklmn§	1.76 mnop	0.97 p	1.62 nop	1.67 mnop	1.35 op	0.90 p	1.14 op	1.52 B
	Sludge alone	6.70	7.07 c	5.58 de	4.92 efg	6.53 cd	3.65 ghijk	4.79 efgh	3.98 fghij	3.63 ghijk	5.02 A
Not trapped	Sand/sludge	0.48	3.03	3.68*	2.28*	3.17*	4.17*	3.16*	2.93*	2.39	3.09*
	Sludge alone	6.70	7.71	8.68*	7.61*	10.95*	4.99*	4.58	3.59	4.47	6.57*

† = Values for time zero were not included in ANOVA.

‡ = Geometric means (anti log of mean log transforms)

§ = a,b,c...;A,B...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 41. Residual N after autoclaving for soil and mixtures of sludge with soil or sand.

Trapped vs. not trapped	Medium	Time of incubation in weeks									Means for trapped and not trapped
		0 †	2	4	6	8	10	12	14	16	
		μgN/g									
Trapped	Soil only	926	979 efg ‡	846 hij	926 fghi	907 fghi	846 hij	923 fghi	953 fgh	919 fghi	912 B
	Soil/sludge	2226	1796 a	1230 bc	1238 bc	1323 b	1169 cd	1238 bc	1292 b	1284 b	1321 A
	Sand/sludge	1373	978 efg	880 ghij	692 kl	515 nop	476 op	500 op	615 lmn	553 mno	651 C
Not trapped	Soil only	926	861*	765	876	849	815	846	923	907	855*
	Soil/sludge	2226	1715	1207	1215	1292	1084	1161	1076*	1169*	1240*
	Sand/sludge	1373	1011	638*	580	492	411	492	553	476	582*

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...:A,B,C...means accompanied with the same letter are not significantly different at P(05).

* = Means for not trapped are significantly different from corresponding means for trapped at P(05).

Table 42. Residual N after autoclaving for sludge alone.

Trapped vs. not trapped	Incubation time in weeks									Means for trapped and not trapped
	0 †	2	4	6	8	10	12	14	16	
	%N									
Trapped	5.76	5.71 ab ‡	5.76 a	5.58 abc	5.11 ef	5.11 ef	5.21 cdef	5.22 cdef	5.10 ef	5.35
Not trapped §	5.76	5.57	5.74	5.49	5.18	5.19	5.24	5.37	4.98	5.35

† = Values for time zero were not included in ANOVA.

‡ = a,b,c...means accompanied with the same letter are not significantly different at P(05).

§ = Values for not trapped are not different from values for trapped.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aleem, M.I.H., M. Alexander. 1960. Nutrition and physiology of Nitrobacter agilis. Applied Microbiol. 8:80-84.

Alexander, M. 1961. Introduction to Soil Microbiology. p. 272-308. John Wiley & Sons, New York.

Allison, F.E. 1963. Losses of gaseous nitrogen from soils by chemical mechanisms involving nitrous acid and nitrites. Soil Sci. 96:404-409.

Allison, F.E. 1965. Evaluation of incoming and outgoing processes that affect soil nitrogen. In W.V. Bartholomew and F.E. Clark (ed.) Soil Nitrogen. Agronomy 10:573-606. Amer. Soc. of Agron., Madison, Wisconsin.

Ashida, J. 1965. Adaptation of fungi to metal toxicants. Annu. Rev. Phytopathol. 3:153-174.

Barrow, V.L. 1955. Use of activated sewage sludge as a fertilizer. World Crops 7:435-437.

Bear, F.E., and A.L. Prince. 1947. Agriculture value of sewage sludge. New Jersey Agr. Exp. Sta. Bull. No. 733, New Brunswick, N.J. 12p.

Beauchamp, E.G., G.E. Kidd, and G. Thurtell. 1978. Ammonia volatilization from sewage sludge applied in the field. J. Environ. Qual. 7:141-146.

Berrow, M.D., and J. Webber. 1972. Trace elements in sludges. J. Sci. Fed. Agr. 23:93-100.

Bloomfield, C., W. I. Kelso, and G. Pruden. 1976. Reactions between metals and humified organic matter. J. Soil Sci. 27:16-31.

Bloomfield, C., and G. Purden. 1975. The effects of aerobic and anaerobic incubation on the extractability of heavy metals in digested sewage sludge. Environ. Pollut. 8:217-232.

Bollag, J.M., and G. Tung. 1972. Nitrous oxide release by soil fungi. Soil Biol. Biochem. 4:271-276.

Boswell, F.C. 1975. Municipal sewage sludge and selected element application to soil: Effect on soil and fescue. J. Environ. Qual. 4:267-273.

Bremner, J.M. 1965a. Total nitrogen. In C.A. Black (ed.) Methods of soil analysis. Agronomy 9, Part II: 1149-1178. Amer. Soc. of Agron., Madison, Wisconsin.

Bremner, J.M. 1965b. Inorganic forms of nitrogen. In C.A. Black (ed.) Methods of soil analysis. Agronomy 9, Part II: 1179-1237. Amer. Soc. of Agron., Madison, Wisconsin.

Bremner, J.M. 1965c. Organic forms of nitrogen. In C.A. Black (ed.) Methods of soil analysis. Agronomy 9, Part II: 1238-1255. Amer. Soc. of Agron., Madison, Wisconsin.

Bremner, J.M., and A.M. Blackmer. 1978. Nitrous oxide: emissions from soils during nitrification of fertilizer nitrogen. Science 199:295-296.

Bremner, J.M.. and L.A. Douglas. 1971. Use of plastic films for aeration in soil incubation experiments. Soil Biol. Biochem. 3:289-296.

Bremner, J.M., and F. Fuhr. 1966. Tracer studies of the reaction of soil organic matter with nitrite, p. 337-346. In the use of isotopes in soil organic matter studies. Pergamon Press, Inc., Elmsford, N.Y.

Bremner, J.M., and K. Shaw. 1955. Determination of ammonia and nitrite in soil. J. Agr. Sci. 46: 320-328.

Bremner, J.M., and K. Shaw. 1958. Denitrification in soils II. Factors affecting denitrification. J. Agr. Sci. 51:40-52.

Broadbent, F.E. 1973. Organics. p. 97-101. In Recycling municipal sludges and effluents on land. National Assoc. of State University and Land-Grant Colleges. Library of Congress Catalog no. 73-88570.

Broadbent, F.E., and F. Clark. 1965. Denitrification In W.V. Bartholomew and F. Clark (ed.) Soil Nitrogen. Agronomy 10:344-359. Amer. Soc. Agron. Madison, Wisconsin.

Broadbent, F.E., and F.J. Stevenson. 1966. Organic matter interactions. p. 169-187. In H.N. McVickar et al. (ed.) Agricultural Anhydrous Ammonia. Technology and Use. Proc. Symp. St. Louis, Mo., 29-30. Sep. 1965. Agric. Ammonia Inst., Memphis, Tenn., Am. Soc. of Agron., and Soil Sci. Soc. of Am., Madison, Wisconsin.

Bunting, A.H. 1963. Experiments on organic manures, 1942-49. J. Agri. Sci. 60:121-140.

Bunzl, K., W. Schmidt, and B. Sansonik. 1976. Kinetics of ion exchange in soil organic matter: IV. Adsorption and desorption of Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, and Ca^{2+} by peat. J. Soil Sci. 27:32-41.

Cady, F.B., and W.V. Bartholomew. 1960. Sequential products of anaerobic denitrification in Norfolk soil material. Soil Sci. Soc. Am. Proc. 24:477-482.

Chaney, R.L. 1973. Crop and food chain effects of toxic elements in sludge and effluents. p. 129-144. In Proc. of the Joint Conf. on Recycling Municipal Sludges and Effluents on Land. Champaigne-Urbana, Ill.

Coker, E.G. 1966a. The value of liquid digested sewage sludge: I. The effect of liquid sewage sludge on growth and composition of grass-clover swards in southeast England. J. Agric. Sci. 67:91-97.

Coker, E.G. 1966b. The value of liquid digested sewage sludge: III. The results of an experiment on barley. J. Agric. Sci. 67:105-107.

Craswell, E.T. 1978. Some factors influencing denitrification and nitrogen immobilization in clay soils. Soil Biol. Biochem. 10:241-245.

Cunningham, J.D., D.R. Keeney, and J.A. Ryan. 1975. Yield and metal composition of corn and rye grown on sewage sludge amended soil. J. Environ. Qual. 4:448-454.

Day, H.D., and T.C. Tucker. 1958. Production of small grains pasture forage using sewage effluent as a source of irrigation and plant nutrients. Agron. J. 51:569-572.

Day, A.D., T.C. Tucker, and M.G. Vavich. 1962a. City sewage for irrigation and plant nutrients. Crops and Soils 14(8):7-9.

Day, A.D., T.C. Tucker, and M.G. Vavich. 1962b. Effects of city sewage effluent on the yield and quality of grain from barley, oats, and wheat. Agron. J. 54: 133-135.

Day, A.D., and R.M. Kirkpatrick. 1973. Effects of treated municipal wastewater on oat forage and grain. J. Environ. Qual. 2:282-284.

Dement, J.D., G. Stanford, and C.M. Hunt. 1959. A method for measuring short term nutrient absorption by plants: III. Nitrogen. Soil Sci. Soc. Amer. Proc. 23:371-374.

Dowdy, R.H., and W.E. Larson. 1975. Metal uptake by barley seedlings grown on soils amended with sewage sludge. J. Environ. Qual. 4:229-233.

Duggan, J.C., and C. Wiles. 1976. Effects of municipal compost and N fertilizer on selected soils and plants. Compost Sci. 17:24-31.

Eno, C.F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci. Soc. Amer. Proc. 24:277-279.

Epstein, E., D.B. Keane, J.J. Meisinger, and J.O. Legg. 1978. Mineralization of nitrogen from sewage sludge and sludge compost. J. Environ. Qual. 7:217-221.

Evans, J.D. 1968. Using sewage sludge on farmland. Compost Sci. 9(2):16-17.

Feigin, A., S. Feigenbaum, and H. Limoni. 1981. Utilization efficiency from sewage effluent and fertilizer applied to corn plants growing in a clay soil. J. Environ. Qual. 10:284-287.

Fewson, C.A., and D.J.D. Nicholas. 1961. Utilization of nitrate by microorganisms. Nature (London) 190:2-7.

Firestone, M.K. 1982. Biological denitrification In F.J. Stevenson (ed.) Nitrogen in Agricultural Soils. Agronomy 22:289-326.

Fraps, G. 1932. The composition and fertilizer value of sewage sludge. Texas Agr. Exp. Sta. Bull. No. 445, College Station, Tex. 23 p.

Gadd, G.M., and A.J. Griffiths. 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. 4:303-317.

Garner, H.V. 1962. Experiments with farmyard manure, sewage sludges, and two refuses on microplots at schools, 1940-1949. Empire J. Exp. Agr. 30(120): 295-304.

Garner, H.V. 1966. Experiments on the direct, cumulative and residual effects of town refuse, manures, and sewage sludge at Rothamsted and other centres, 1940-47. J. Agr. Sci. 67:223-233.

Guenzi, W.D., W.E. Beard, F.S. Watanabe, S.R. Olsen, and L.K. Porter. 1978. Nitrification and denitrification in cattle manure-amended soil. J. Environ. Qual. 7:196-202.

Haghiri, F. 1973. Cadmium uptake by plants. J. Environ. Qual. 2:93-96.

Hartman, L.M. 1974. A preliminary report: Fungal flora of the soil as conditioned by varying concentrations of heavy metals. Am. J. Bot. 61:23.

Hashimoto, A.G., and D.C. Ludington. 1971. Ammonia desorption from concentrated chicken manure slurries in livestock waste management and pollution abatement. Proc. Intl. Symp. Livestock Waste. Am. Soc. Eng., Columbus, Ohio.

Hinesly, T.D., R.L. Jones, and E.L. Ziegler. 1972. Effect on corn of application of heat-dried anaerobically digested sludge. Compost Sci. 13:26-30.

Hinesly, T.D., E.L. Ziegler, and G.L. Barrett. 1979. Residual effects of irrigating corn with digested sewage sludge. J. Environ. Qual. 8:35-38.

Hoagland, D.R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.

Hortenstine, C.C., and D.F. Rothwell. 1968. Garbage compost as a source of plant nutrients for oats and radishes. Compost Sci. 9(2):23-25.

Hsieh, Y.P., L.A. Douglas, and H.L. Motto. 1981. Modeling sewage sludge decomposition in soil: II. Nitrogen transformation. J. Environ. Qual. 10:59-64.

Jackson, M.L. 1960. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Jansson, S.L. 1958. Tracer studies on nitrogen transformations in soil with special attention to mineralization-immobilization relationship. Kungliga Lantbrushogskolans Annalar. 25:101-361.

Jansson, S.L. 1971. Use of ^{15}N in studies of soil nitrogen. p. 129-166. In A.D. McLaren and J. Skujins (ed.) Soil biochemistry II. Mercel Dekker Inc., New York.

Jansson, S.L., and J. Persson. 1982. Mineralization and immobilization of soil nitrogen. In F.J. Stevenson (ed.) Nitrogen in Agricultural Soils. Agronomy 22:229-252.

John, M.K., H.H. Chuah and C.J. VanLaerhoven. 1972. Cadmium contamination of soil and its uptake by oats. Environ. Sci. Technol. 6:555-557.

John, M.K., and C.J. VanLaerhoven. 1976. Effect of sewage sludge composition, application rate, and lime regime on plant availability of heavy metals. J. Environ. Qual. 5:246-251.

Keeney, D.R., K.W. Lee, and L.M. Walsh. 1975. Guideline for the application of wastewater sludge to agricultural land in Wisconsin, Wis. Dep. of Natural Res. Tech. Bull. No 88, Madison, Wis.

Kelling, K.A., A.E. Peterson, L.M. Walsh, J.A. Ryan, and D.R. Keeney. 1977a. A field study of agricultural use of sewage sludge: I. Effect on crop yield and uptake of N and P. J. Environ. Qual. 6:339-345.

Kelling, K.A., L.M. Walsh, D.R. Keeney, J.A. Ryan, and A.E. Peterson. 1977b. A field study of the agricultural use of sewage sludge: II. Effect on soil N and P. J. Environ. Qual. 6:345-351.

King, L.D. 1973. Mineralization and gaseous loss of nitrogen in soil-applied liquid sludge. J. Environ. Qual. 2:356-358.

King, L.D. 1981. Effect of swine manure lagoon sludge and municipal sewage sludge on growth, nitrogen recovery, and heavy metal content of fescue grass. J. Environ. Qual. 10:465-472.

King, L.D., A.J. Leyshon, and L.R. Webber. 1977. Application of municipal refuse and liquid sewage sludge to agricultural land: II Lysimeter Study. J. Environ. Qual. 6:-67-71.

King, L.D., and H.D. Morris. 1972a. Land disposal of liquid sewage sludge: I. The effect on yield, in vivo digestibility, and chemical composition of coastal bermuda grass (Cynodon dactylon L. Pers) J. Environ. Qual. 1:325-329.

King, L.D., and H.D. Morris. 1972b. Land disposal of liquid sewage sludge: II. The effect on soil pH, manganese, zinc, and growth and chemical composition of rye (Secale cereale L.). J. Environ. Qual. 1:425-429.

Kissel, D.E., and S.J. Smith. 1978. Fate of fertilizer nitrate applied to coastal bermuda grass on a swelling clay soil. Soil Sci. Soc. Am. J. 42:77-80.

Koelliker, J.K, and J.R. Miner. 1973. Desorption of ammonia from anaerobic lagoons. Trans. Am. Soc. Agric. Eng. 16:143-151.

Kowalenko, C.G. 1978. Nitrogen transformation and transport over 17 months in field fallow microplots using N. Can. J. Soil Sci. 58:69-76.

Lance, J.C. 1972. Nitrogen removal by soil mechanisms. J. Water Pollut. Control Fed. 44: 1352-1361.

Lauer, D.A., D.R. Bouldin, and S.D. Kalusner. 1976. Ammonia volatilization from dairy manure spread on the soil surface. J. Environ. Qual. 5:134-141.

Liang, C.N., and M.A. Tabatabai. 1977. Effects of trace elements on nitrogen mineralization. Environ. Pollut. 12:141-147.

Liang, C.N., and M.A. Tabatabai. 1978. Effect of trace elements on nitrification in soils. J. Environ. Qual. 7:291-293.

Lindbeck, M.R., and J.L. Young. 1965. Polarography of intermediates in the fixation of nitrogen by p-quinone-aqueous ammonia systems. Anal. Chim. Acta 32:73-80.

Little, T.M., and F.J. Hills. 1978. Agricultural experimentation design and analysis. John Wiley & Sons, Inc., New York.

Lunt, H.H. 1953. The case of sludge as a soil improver. Water Sewage Works 100:295-301.

Magdoff, F.R., and J.F. Amadon. 1980. Nitrogen availability from sewage sludge. J. Environ. Qual. 9:451-455.

Magdoff, F.R., and F.W. Chromec. 1977. Nitrogen mineralization from sewage sludge. Environ. Sci. Health, A12:191-201.

Mahler, R.J., F.T. Bingham, and A.L. Page. 1978. Cadmium-enriched sewage sludge application to acid and calcareous soils: Effect on yield and cadmium uptake by lettuce and chard. J. Environ. Qual. 7:274-280.

Mays, D.A., G.L. Terman, and J.C. Duggan. 1973. Municipal compost: Effects on crop yields and soil properties. J. Environ. Qual. 2:89-92.

McGarity, J.W., and J.A. Rajaratnam. 1973. Apparatus for the measurement of losses of nitrogen as gas from the field and simulated field environments. Soil Biol. Biochem. 5:121-131.

Merz, R.C. 1959. Utilization of liquid sludge. Water Sewage Works. 106:489-493.

Miller, R.H. 1974. Factor affecting the decomposition of an anaerobically digested sewage sludge in soil. J. Environ. Qual. 3:376-380.

Milne, R.A., and D.N. Graveland. 1972. Sewage sludge as a fertilizer. Can J. Soil Sci. 52:270-273.

Mitchell, G.A., F.T. Bingham, and A.L. Page. 1978a. Yield and metal composition of lettuce and wheat grown on soils amended with sewage sludge enriched with cadmium, copper, nickel, and zinc. J. Environ. Qual. 7:165-170.

Mitchell, M.J., R. Hortenstine, B.L. Swift, E.F. Neuhauser, B.T. Abrams, R.M. Mulligan, B.A. Brown, D. Craig, and D. Kaplan. 1978b. Effects of different sewage sludges on some chemical and biological characteristics of soil. J. Environ. Qual. 7:551-559.

Mortland, M.M. 1965. Nitric oxide adsorption by clay minerals. Soil Sci. Am. Proc. 29: 514-519.

Mortland, M.M., and A.R. Wolcott. 1965. Sorption of inorganic nitrogen compounds by soil materials. In W.V. Bartholomew and F.E. Clark (ed.) Soil nitrogen. Agronomy 10:150-197. Am. Soc. of Agron., Madison, Wis.

Mortvedt, J.J., and P.M. Giordano. 1975. Response of corn to zinc and chromium in municipal wastes applied to soil. J. Environ. Qual. 4:170-174.

Mosier, A.R., G.L. Hutchinson, B.R. Sabey, and J. Baxter. 1982. Nitrous oxide emissions from barley plots treated with ammonium nitrate or sewage sludge. J. Environ. Qual. 11:78-81.

Muller, J.F. 1929. The value of raw sewage sludge as fertilizer. Soil Sci. 28:423.

Nelson, D.W. 1982. Gaseous losses of nitrogen other than through denitrification. In F.J. Stevenson (ed.) Nitrogen in Agricultural Soils. Agronomy 22:327-364.

Nelson, D.W., and J.M. Bremner. 1969. Factors affecting chemical transformation of nitrite in soils. Soil Biol. Biochem. 1:229-238.

Nelson, D.W., and J.M. Bremner. 1970. Role of soil minerals and metallic cations in nitrite decomposition and chemodenitrification in soil. Soil Biol. Biochem. 2:1-8.

Nishita, H., B.W. Kowalewsky, and K.H. Larson. 1956. Influence of organic matter on mineral uptake by barley. Soil Sci. 82:307-318.

Noer, O.J. 1926. Activated sludge: Its composition and value as a fertilizer. J. Amer. Soc. of Agron. 18:953.

Nommik, H. 1956. Investigations on denitrification in soil. Acta Agric. Scand. V. 12:195-228.

Nommik, H., and K. Vahtras. 1982. Retention and fixation of ammonium and ammonia in soils. In F.J. Stevenson (ed.) Nitrogen in Agricultural Soils. Agronomy 22:123-171.

Page, A.L. 1974. Fate and effects of trace elements in sewage sludge when applied to agricultural lands. A literature review study. USEPA-670/2-74-005. 96p.

Permi, P.R., and A.H. Cornfield. 1969. Incubation study of nitrification of digested sewage sludge added to soil. Soil Biol. Biochem. 1:1-4.

Permi, P.R., and H. Cornfield. 1971. Incubation study of nitrogen mineralization in soil treated with dry sewage sludge. Environ. Pollut. 2:1-5.

Peterson, J.R., C. Lue-Hing, and D.R. Zenz. 1973. Chemical and biological quality of municipal sludge. p. 26-37. In W.E. Sopper and L.T. Kardos (ed.) Recycling Treated Municipal Wastewater and Sludge Through Forest and Cropland. Penn. State Univ. Press, University Park, Penn.

Pratt, P.F., F.E. Broadbent, and J.P. Martin. 1973. Using organic wastes as nitrogen fertilizer. Calif. Agric. 29(6)L:10-13.

Quraishi, M.S.I., and A.H. Cornfield. 1973. Incubation study of nitrogen mineralization and nitrification in relation to soil pH and level of copper (II) addition. Environ. Pollut. 4:139-163.

Reuss, J.O., and R.L. Smith. 1965. Chemical reactions of nitrites in acid soils. Soil Sci. Soc. Amer. Proc. 29:267-270.

Ritchie, G.A.F., and D.J.D. Nicholas. 1972. Identification of the sources of nitrous oxide produced by oxidative and reductive processes in Nitrosomonas europaea. Biochem. J. 126:1181-1191.

Rolston, D.E., F.E. Broadbent, and D.A. Goldhamer. 1979. Field measurement of denitrification: II. Mass balance and sampling uncertainty. Soil Sci. Soc. Am. J. 43:703-708.

Rolston, D.E., M. Fried, and D.A. Goldhamer. 1976. Denitrification measured directly from nitrogen and nitrous oxide gas fluxes. Soil Sci. Soc. Am. J. 40:259-266.

Ryan, J.A., D.R. Keeney, and L.M. Walsh. 1973. Nitrogen transformations and availability of an anaerobically digested sewage sludge in soil. J. Environ. Qual. 2:489-492.

Ryan, J.A., and D.R. Keeney. 1975. Ammonia volatilization from surface applied sewage sludge. J. Water Pollut. Control Fed. 47:386-393.

Ryden, J.C., and L.J. Lund. 1980. Nitrous oxide evolution from irrigated land. J. Environ. Qual. 9:387-393.

Sabey, B.R., N.N. Agbim, and D.C. Markstrom. 1975. Land applications of sewage sludge: III. Nitrate accumulation and wheat growth resulting from addition of sewage sludge and wood wastes to soils. J. Environ. Qual. 4:338-393.

Sabey, B.R., N.N. Agbim, and D.C. Markstrom. 1977. Land application of sewage sludge: IV. Wheat growth, N content, N fertilizer value, and N use efficiency as influenced by sewage sludge and wood waste mixtures. J. Environ. Qual. 6:52-58.

Sabey, B.R., and W.E. Hart. 1975. Land application of sewage sludge: I. Effect on growth and chemical composition of plants. J. Environ. Qual. 4:252-256.

Schwing, J.E., and J.L. Puntenney. 1974. Denver plan: Recycle sludge to feed forms. Water Wastes Eng. 11:24-27.

Sheaffer, C.C., A.M. Decker, R.L. Chaney, and L.W. Douglass. 1979. Soil temperature and sewage sludge effects on corn yield and macronutrient content. J. Environ. Qual. 8:450-454.

Sikora, L.J., C.F. Tester, J.M. Taylor, and J.F. Parr. 1980. Fescue yield response to sewage sludge compost amendments. Agronomy Journal 72:79-84.

Silveira, D.J., and L.E. Sommers. 1977. Extractability of copper, zinc, cadmium, and lead in soils incubated with sewage sludge. J. Environ. Qual. 6:47-52.

Sinha, M.K., S.K. Dhillon, and S. Dyamond. 1978. Solubility relationships of iron, manganese, copper and zinc in alkaline and calcarous soils. Aust. J. Soil Res. 16:19-26.

Smith, C.J., and P.M. Chalk. 1980. Fixation and loss of nitrogen during transformations of nitrite in soil. Soil Sci. Am. J. 44:288-291.

Smith, D.H., and F.E. Clark. 1960. Volatile losses of nitrogen from acid or neutral soils or solutions containing nitrite and ammonium ions. Soil Sci. 90:86-92.

Sommers, L.E. 1977. Chemical composition of sewage sludges and analysis of their potential use as fertilizers. J. Environ. Qual. 6:225-232.

Sommers, L.E., D.W. Nelson, and K.J. Yost. 1976. Variable nature of chemical composition of sewage sludge. J. Environ. Qual. 5:303-306.

Sommers, L.E., D.W. Nelson, and D.J. Silveira. 1979. Transformation of carbon, nitrogen, and metals in soils treated with waste materials. J. Environ. Qual. 8:287-294.

Stanford, G. 1969. Extraction of soil organic nitrogen by autoclaving in water: 2. Soil Sci. 107:323-328.

Stanford, G. 1982. Assessment of soil nitrogen availability. In F.J. Stevenson (ed.) Nitrogen in Agricultural Soils. Agronomy 22:651-688.

Stanford, G., and W.H. DeMar. 1969. Extraction of soil organic nitrogen by autoclaving in water: 1. Soil Sci. 107:203-205.

Stanford, G., and W.H. DeMar. 1970. Extraction of soil organic nitrogen by autoclaving in water: 3. Diffusable ammonia, an index of soil nitrogen availability. Soil Sci. 109:190-196.

Stanford, G., S. Dzienia, and R.A. VanderPol. 1975. Effect of temperature on denitrification rate in soils. Soil Sci. Soc. Am. Proc. 39:867-870.

Stark, S.A., and C.E. Clapp. 1980. Residual nitrogen availability from soils treated with sewage sludge in a field experiment. J. Environ. Qual. 9:505-512.

Stephenson, R.J. 1955. Availability of nitrogen in sewage sludges. Sewage Ind. Wastes. 27:34-39.

Stevenson, F.J. 1965. Amino sugars. In C.A. Black (ed.) Methods of soil analysis. Agronomy 9, Part II: 1429-1436. Amer. Soc. Agron., Madison, Wisconsin.

Stevenson, F.J. 1982. Organic forms of soil nitrogen. In F.J. Stevenson (ed.) Nitrogen in Agricultural Soils. Agronomy 22:67-122.

Stewart, N.E., C.T. Corke, E.G. Beauchamp, and L.B. Webber. 1975. Nitrification of sewage sludge using miscible displacement and perfusion techniques. Can. J. Soil Sci. 55:467-472.

Stucky, D.J., and T.S. Newman. 1977. Effect of dried anaerobically digested sewage sludge on yield and element accumulation in tall fescue and alfalfa. J. Environ. Qual. 6:271-274.

Swaby, R.J., and J.N. Ladd. 1966. Stability and origin of soil humus. In The Use of Isotopes in Soil Organic Matter Studies. (Rep. FAO/IAEA Technical Meeting, Brunswick-Volkenrode, 1963), Spec. Suppl. J. Appl. Radiat. Isotopes, pp. 153-154.

Talburt, D.E., and G.T. Johnson. 1967. Some effects of rare earth elements and yttrium on microbial growth. Mycologia 59:493-503.

Terman, G.L., J.M. Soileau, and S.E. Allen. 1973. Municipal compost: effects on crop yields and nutrient content in greenhouse pot experiments. J. Environ. Qual. 2:84-89.

Terry, R.E., D.W. Nelson, and L.E. Sommers. 1979. Carbon cycling during sewage sludge decomposition in soils. Soil Sci. Soc. Amer. J. 43:494-499.

Terry, R.E., D.W. Nelson, and L.E. Sommers. 1981. Nitrogen transformation in sewage sludge-amended soils as affected by soil environmental factors. Soil Sci. Soc. Am. J. 45:506-513.

Tester, C.F., L.J. Sikora, J.M. Taylor, and J.F. Parr. 1977. Decomposition of sewage sludge compost in soil: 1. Carbon and nitrogen transformation. J. Environ. Qual. 6:459-463.

Tester, C.F., L.J. Sikora, J.M. Taylor, and J.F. Parr. 1979. Decomposition of sewage sludge compost in soil: III. Carbon, nitrogen, and phosphorus transformation in different sized fractions. J. Environ. Qual. 8:79-82.

Touchton, J.T., L.D. King, H. Bell, and H.D. Morris. 1976. Residual effect of liquid sewage sludge on coastal bermuda grass and soil chemical properties. J. Environ. Qual. 5:161-164.

Varanka, M.W., Z.M. Zablocki, and T.D. Hinesly. 1976. The effect of digested sludge on soil biological activity. J. Water Pollut. Control Fed. 48(7):1728-1740.

Vlamis, J., and D.E. Williams. 1961. Test of sewage sludge for fertility and toxicity in soils. Compost Sci. 2(1)26-30.

Volz, M.G., and J.L. Starr. 1977. Nitrate dissimilation and population dynamics of denitrificating bacteria during short-term continuous flow. Soil Sci. Soc. Am. Proc. 41:891-896.

Webber, J. 1972. Effects of toxic metals in sewage on crops. Water Pollut. Contr. 71:404-413.

Wijler, J., and C.C. Delwiche. 1954. Investigations on the denitrifying process in soil. Plant Soil 5:155-169.

Wilson, D.O. 1977. Nitrification in soil treated with domestic and industrial sewage sludge. Environ. Pollut. 12:73-82.

Woldendrop, J.W. 1963. The influence of living plants on denitrification. Meded. Landbouwhogesch. Wageningen 63:1-100.

Woldendrop, J.W. 1968. Losses of soil nitrogen. Stikstof 12:32-46.

Wullstein, L.H., and C.M. Gilmour. 1964. Non-enzymatic gaseous loss of nitrite from clay and soil systems. Soil Sci. 97:426-430.

Yoneyama, T., and T. Yoshida. 1978. Nitrogen mineralization of sewage sludge in soil. Soil Sci. Plant Nutr. 24:139-144.

Yoshida, T., and M. Alexander. 1970. Nitrous oxide formation by Nitrosomonas europaea and heterotrophic microorganisms. Soil Sci. Soc. Am. Proc. 34:880-882.