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AMMONIA AND NITRATE NITROGEN IN THE SOIL PROFILE AND ITS RELATION TO VARIOUS NITROGEN TREATMENTS ON DRY-LAND WINTER WHEAT

by

Abraham E. Van Luik

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Soil Science

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1975

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Abraham E. Van Luik

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ABSTRACT

Ammonia and Nitrate Nitrogen in the Soil Profile and

Its Relation to Various Nitrogen Treatments

On Dry-land Winter Wheat

by

Abraham E. Van Luik, Master of Science

Utah State University, 1974

Major Professor: Dr. Raymond W. Miller
Department: Soil Science and Biometeorology

In a dry-land winter wheat field, patterns of mineral nitrogen distributions were investigated before and after fertilizer additions.

Large differences in the added mineral nitrogen recoverable three weeks after treatment were found to be specific to nitrogen source and treatment within source.

Initial losses averaged 50 percent for urea treatments, 40 percent for calcium nitrate treatments, and varied from a loss of 18 percent to a gain of 22 percent for ammonium nitrate treatments. Ammonium sulfate proved the most variable with a 36 percent average loss for the before-planting treatment and a 61 percent gain for the after-planting treatment.

This initial gain and loss behavior correlated at the 2 percent level of significance with the subsequent grain yield ($r = 0.774$, 8 df), and was still discernable in soil test results of late April, where total mineral nitrogen depletion since before treatment correlated positively at the 10 percent level of significance with the nitrogen loss found 3 weeks after treatment.

For a small sample of nine plots, a late July sampling revealed that depletions of mineral nitrogen since April were much more predictive of grain yields than were the actual April-N levels (r^2 = 0.787 versus r^2 = 0.460). This result confirms the large role played by differential moisture stress regimes in the field, since depletions during the drying season of late spring and early summer depend on the availability of moisture.

Initial fertilizer behavior, determining fertilizer losses before the onset of crop usage, and a favorable later moisture regime were seen as the two largest determinants of yield under the conditions of this experiment. Since the latter factor is largely beyond further control, the former is the only factor open to manipulation. Generally, after planting treatments were lower in initial losses of mineral nitrogen, and also generally provided somewhat higher surface mineral nitrogen levels in early spring, which was found to be weakly correlated with yield. Surface accumulations in early spring can only be beneficial if sufficient spring moisture is available for downward transport into the root zone, however, and a drier spring than prevailed during this experiment could forseeably reverse this relationship by keeping such surface nitrogen accumulations from becoming available to the plant in spring.

(164 pages)

INTRODUCTION

Practically all the nitrogen taken up by a crop is in the water soluble, mineral forms of ammonium (NH_4^+) and nitrate (NO_3^-) salts. The sources for these mineral nitrogen forms, in non-legume crops, are mainly that which naturally occurs in the soils and that which has been chemically fixed and added by man.

The naturally occurring nitrogen in most agricultural soils has been largely the result of the operation of the nitrogen cycle over many years, setting up an equilibrium between nitrogen input and outflow before cultivation began.

Dry-land farming areas of the intermountain region, in their natural states, were generally sparsely vegetated, with a low density animal population, mainly due to low precipitation levels or unfavorable annual precipitation patterns. As such, it is obvious that great organic nitrogen reserves were not built up in these soils, as compared with their more heavily vegetated counterparts in such areas as the North Central United States (Pauli, 1967).

As discussed by Stevenson (1964), these organic nitrogen reserves are in a complex equilibrium with soil mineral nitrogen forms both available and unavailable to plants. This equilibrium may be illustrated in the simplified formula:

Stabilized organic -N $\rightleftarrows$ "fresh" organic -N $\rightleftarrows$ mineral N.

At steady state, generally less than 0.1 percent of the total soil nitrogen exists in the available mineral forms (Stevenson, 1964).

With a large reserve of soil organic nitrogen, the amount of mineral nitrogen available to the plant at any given point in time may be more than adequate, without amendment, for a few cropping years. After a few seasons, however, the relative nitrogen content of the soils will begin to drop, and the equilibrium concentration of the available mineral nitrogen will become less and less adequate (Sauchelli, 1964).

In those dry-land areas where moisture is the major limiting factor in biomass production, natural organic nitrogen reserves are lower than in areas where moisture stress is less acute. Moreover, the kinetics of chemical and microbiological reactions involved in nitrogen mineralization are affected by moisture stress. Drying a soil may temporarily increase the mineralization rate, but the overall effect on a crop must take into account increased volatilization losses of ammoniacal nitrogen, decreased mobility of the mineralized nitrogen in the soil solution, and a loss of plant mobility as a consequence of slowed root and shoot growth (Baligar, 1971; Brouwer, 1966). Rewetting, as by a timely shower, results in increased nitrogen uptake by the plant which in turn increases the overall demand on the mineral nitrogen in the system. Thus, moisture and nitrogen take turns as limiting factors in a natural dry-land farming system that has been cropped for some time.

The value of adding mineral nitrogen to such a system is immediately apparent: the organic nitrogen reserve depletion is slowed, and the larger mineral nitrogen reserve allows a more immediate and efficient response to any incoming water (Neidig and Sneider, 1924).

Much work has been done, in Utah and elsewhere, investigating the effects of the rate, time, and source of nitrogen fertilizers on

crop-yields. Reviewing many of these studies, the conclusion is inescapable that rate, time, and source of nitrogen affects crop yields only insofar as it affects the timeliness of the availability of nitrogen to the plant.

Factors which determine the timing of fertilizer-nitrogen availability include soil and climatic characteristics as they interact with properties of the individual fertilizer material. The main factors which determine the nature of this interaction are: (1) the fertilizer material's susceptibility to leaching, (2) its absorptive character, (3) its tendency to volatilize, and (4) its ability to induce soil physical or chemical property changes.

In view of these observations and general principles, this investigation was undertaken to gain more insight into these availability factors by studying the fate of selected solid nitrogen fertilizers under a dry-land winter wheat crop.

REVIEW OF LITERATURE

Sources of Mineral Nitrogen in the Soil

Since mineral nitrogen is the only important form used by a growing crop, as noted above, an in-depth look at mineral nitrogen sources and their dynamics follows.

Mineralization

The amount of organic nitrogen present in the soil, and the rate of its mineralization, determine the availability of nitrogen to the crop plant. Mineralization is a microbiological process, consisting of ammonification and usually followed by nitrification, producing ammonia from the organic nitrogen and subsequently oxidizing the ammonia to nitrate by way of nitrite (Stevenson, 1964).

Mineralization rates

Climate determines overall mineralization rate fluctuations (Campbell and Biederbeck, 1972). Harmsen and Van Schreven (1955) described the annual changes in mineralization rates in the temperate zone. Typically, winter seems to have a sterilizing effect, with no significant mineralization taking place. In spring optimum mineralization rates occur, tapering off towards summer and slowing markedly by fall. Coincidentally, mineral nitrogen in the soil is at its highest level in spring, decreasing thereafter until, under a crop, very little mineral nitrogen is found in mid-summer. A slight increase usually occurs again in fall as the crop demands decrease.

The two main components of climate, temperature, and moisture, are responsible for this observed annual cycle. Temperature increases generally enhance all microbiological activity, so that both ammonification and nitrification are stimulated (Baligar, 1971).

Moisture levels, as reported by many workers (Miller and Johnson, 1964; Alexander and Clark, 1965), have a differential effect on mineralization, with ammonification being much less retarded by high moisture tensions than nitrification. As a consequence, during the wetness of spring, as maximum nitrification is encouraged, nitrate may be expected to dominate the mineral nitrogen fraction in the soil. As the soil dries in the transition from spring to summer, the nitrification is inhibited more than ammonification, the ammonia concentration fraction may be expected to rise as the nitrate fraction drops; however the total mineral nitrogen in the soil is decreasing at this same time.

Immobilization and mineralization

Adding a carbonaceous residue to the soil, if favorable conditions exist, will stimulate substantial increases in microbial activity and population size. As these heterotrophic microorganisms proliferate, soil nitrogen is drawn upon to provide for protein and tissue needs in the increasing biomass. Depending on the amount of nitrogen in the soil, and in the added materials, this competition between microorganism and plant may be quite serious in terms of plant growth.

As the carbon source is degraded, and supports less and less microbial life, the ratio of carbon to nitrogen in the residue is lowered, and a point will be reached where residue-nitrogen is enough to meet the needs of remaining microorganisms. Beyond this point, an

excess of nitrogen will exist in relation to the remaining carbon, and as the degradation continues, this surplus nitrogen will be ammonified. In addition, the dying back of the microbial population provides a food source for other groups of heterotrophic organisms. This decaying microbial matter has a low C/N ratio, and much of its nitrogen is also mineralized. Thus, immobilization is the reverse of mineralization, and is a first step in adding to the reserve pool of soil organic nitrogen. In any natural system in the temperate zone, both immobilization and mineralization will be taking place throughout the season, but there will be net mineralization during spring and early summer, while net immobilization will take place in late summer and fall (Harmsen and Van Schreven, 1955).

Immobilization and mineralization in dryland wheat culture. Under conditions typical of dryland wheat farming, as in this study, wheat straw is the main carbonaceous material regularly incorporated into the soil. The immediate effect of such incorporation is net nitrogen immobilization. Measurements by Black and Reitz (1972), showed that approximately 1 kg of NO_3-N was immobilized by every 100 kg of incorporated wheat straw. The typical high yield wheat crop, according to these authors, produces about 14 times as much straw as grain. Under stubble mulching as practiced in this study, however, only about sixty percent of the straw is initially brought into close contact with the soil, or minimally incorporated. At times during the fallow year, this surface mixing is redone so that at the planting of the following crop there is very little straw left and that which is left is already partially decomposed. Immobilization is, therefore, not a significant competitor for nitrogen with the young wheat plants, and the straw has

served to increase water storage during the fallow season while it has been slowly decomposing on and in the soil surface, contributing eventually to the soil mineral and organic nitrogen pools.

The time involved in wheat-straw decomposition for this climatic region has been studied by Brown and Dickey (1970). Their results show that wheat straw decomposition during the winter months was negligible. In spring, decomposition began, and by late summer the wheat straw had reached its maximum nitrogen content. This nitrogen maximum ranged from one to six times the original nitrogen content of 0.26 percent, depending on the stage of decomposition, which in turn depended on the location of the straw in relation to the soil: Straw suspended above the soil surface showed no change in nitrogen content; straw laid on the soil surface showed doubled and tripled nitrogen contents; and straws incorporated into the soil had a six-fold increase in nitrogen content over the time span of spring to late summer. Thirty percent of the surface applied straw was decomposed after eighteen months, while greater than ninety percent of the incorporated straw was lost during this same period. These authors also estimated from their data that 30 percent of the wheat straw carbon could be accounted for in the humus fraction of the soil organic matter some time after decomposition.

This 30 percent estimate is close to figures presented by Stewart (1961), who attempted to correlate decreases in mineral nitrogen with increases in certain organic nitrogen fractions. About 75 percent of the immobilized nitrogen was located in the amino acid fraction of the soil hydrolysate. That fraction constitutes approximately half of the total soil organic matter. About 25 percent of the immobilized nitrogen was

found in the less easily degraded fraction of the soil organic matter, which becomes progressively more humus-like through continual biological transformations. Black and Reitz (1972) used the 30 percent retention value for humification to estimate the long term nitrogen immobilization losses due to wheat straw decomposition. Using for humus an average carbon to nitrogen ratio of 10, they calculated that 1100 kg of decomposing wheat straw would cause a loss of 7.7 kg of nitrogen to the humic nitrogen reserve, from which it is only very slowly re-released. This 7.7 kg-N includes 2.9 kg-N contributed by the straw itself, so that only 4.8 kg-N per 1100 kg straw is taken from other soil sources. When compared to the 11.0 kg of soil-N for 1100 kg straw which these same authors indicated would be immobilized initially by decomposition, this shows that over half of the initially immobilized nitrogen is released either by mineralization into the mineral nitrogen fraction or by microbial assimilations into the more labile organic nitrogen fractions. Full incorporation of the straw and ideal conditions for decomposition is assumed in these figures.

Fertilization

The second source of mineral nitrogen in the soil is fertilizers, either indirectly from added N-bearing organic materials, or directly from mineral-N compounds. Since this study involves a dry-land grain, crop rotations with legumes are impractical and will therefore not be discussed as a means of adding nitrogen to the soil.

Organic nitrogen fertilization

Millar et al. (1966) report that straw has an approximate C/N ratio of 80; and that only manures and legumes approach the C/N ratio of 20 which

is most desirable. In experiments conducted by Niazi et al. (1968), in Pakistan, manures proved unable to provide nitrogen in sufficient quantities at the peak demand times of the wheat plant, whereas no such stresses were observed in the same experiments where mineral-N sources were applied.

Inorganic nitrogen fertilization

Since increasing mineral nitrogen in the soil through inorganic nitrogen fertilization is the main topic of the present experiment, it will now be considered in detail.

Mineral nitrogen fertilizers and mineralization. Niazi et al. (1968) report that added ammoniacal-N from $(NH_4)_2SO_4$ was nitrified according to the same seasonal rate distributions as observed for the mineralization of soil organic nitrogen previously discussed. They observed little or no nitrification until spring, when the rate increased rapidly. NO_3^- content of the soil peaked within a month, and remained steady thereafter for the remainder of the season. Peak nitrification slightly preceded peak crop demands, allowing a short lived but fortunate nitrate build-up from a crop-need standpoint. As will be discussed at greater length later, nitrification of ammonia increases the mineral nitrogen mobility and hence its general availability to the plant roots, if proper moisture conditions exist.

It is often reported that added fertilizer-nitrogen stimulates the extraction of soil nitrogen by the plant. This phenomenon has been attributed to different effects by different workers, some of which effects will be discussed later in this study. For the present, Broadbent (1965) confirming the phenomenon, attributes it in part to stimulated mineralization of soil organic nitrogen. Using tagged organic nitrogen,

and adding untagged increments of $(NH_4)_2SO_4$, resulted in tagged mineral N increases highly correlated with rates of applied $(NH_4)_2SO_4$. Nitrate additions provided no such correlations, however, and since this priming effect is also observed in experiments using nitrate sources only, increased mineralization is only a partial explanation of this commonly observed increase in soil nitrogen uptake when fertilizer nitrogen is added.

Another effect of added ammonia in the soil is the inhibition of nitrification. Stojanovic and Alexander (1958) and Aleem and Alexander (1960), report that at high ammonium levels there is a significant buildup of nitrite, probably because of an inhibition of the Nitrobacter microorganism responsible for the nitrite to nitrate oxidation. They also noted a general decrease of ammonia oxidation to nitrite.

Harada and Kai (1968) studied this retardation of ammonia oxidation due to high ammonia concentrations and found the critical NH_4^+-N level for such retardation at over 200 ppm in their culture media. Such levels may be reached in the soil when ammoniacal nitrogen forms are added, but under moderate rates of application such concentrations should occur only in small areas of the immediate placement zone, with nitrification inhibition local to these small areas only, and unaffected in the soil at large.

Increases in mineral nitrogen levels due to pooling effects. Jansson (1971) suggests that such localized pools of mineral nitrogen will accrete to themselves fresh locally mineralized nitrogen and discourage, temporarily, its biological refixation. Ammonium pools, from the above noted resistance to oxidation, would be expected to cause more such

pooling of mineral nitrogen than nitrate nitrogen pools. Such nitrate pools are not colonized by the soil microflora to the same extent as ammonium pools, because these microorganisms much prefer the ammonium form of nitrogen for their own use. (However, this same author found nitrate nitrogen to be acceptable to microorganisms when net immobilization created an ammonia shortage.) Since nitrate fertilizers provide less of a priming effect, and since this priming effect is less in nitrifying than in non-nitrifying soils, Jansson (1971) hypothesizes that the above described pooling effect with added ammonium fertilizers causes increased soil-N levels, which in turn makes possible increased soil-N uptake by the plant. The fact that non-cropped soils with added fertilizer nitrogen also show increases in available soil mineral nitrogen led Jansson (1971) to reject outright the hypothesis that increased soil nitrogen uptake was due primarily to increased plant root and shoot growth (Alecksic et al., 1968).

Legg and Stanford (1967) tried quantifying the priming effect by measuring the increase in mineral nitrogen from the soil and the corresponding amount of fertilizer nitrogen immobilized. They found, qualitatively, that increased fertilization rates resulted in increased soil mineral nitrogen levels, as is to be expected if the above noted pooling effect is a true explanation of the mechanism involved. Quantitatively, in an experiment using twelve different soils in pots, the authors found that at fertilizer addition rates of 200 ppm-N, there was 5 mg of fertilizer nitrogen immobilized for every mg of soil nitrogen released.

Possible Loss Mechanisms of Soil Mineral Nitrogen

Loss mechanisms of mineral nitrogen will here be defined as being those processes which render the mineral nitrogen permanently unavailable to the plant.

Denitrification

Reduction of oxidized forms of nitrogen may result in gaseous losses of nitrogen from the soil. Facultative anaerobic microorganisms use the NO_3^- and NO_2^- forms of nitrogen as an oxygen source when the O_2 percentage in the soil is reduced to less than one, generally (Skujins, 1974). Such oxygen tensions can result from aeration problems caused by water logging, poor soil structure, or an unusually high rate of organic matter decay (Hausenbuiller, 1970).

An experiment by Loewenstein et al. (1957) sought to quantify the magnitude of denitrification of some acid soils. On uncropped field soils losses of 35 percent of applied ammonium sulfate were found at pH 5.5, while 72 percent of the applied N was lost at pH 6.5. Identical treatments on plots cropped with oats, showed losses of only 7 and 8 percent, respectively. The differences were attributable to the reduced level of nitrified NO_3^--N mainly due to crop uptake.

A similar experience on four neutral to slightly alkaline soils, by Stefanson (1972), showed that under wheat, generally, high water content favored denitrification. At low soil water, NO_3^--N was more available to the plant and little denitrification occurred. At high soil water, NH_4^+-N in the soil was preferable to NO_3^--N, as it was less liable to become denitrified and hence lost to the plant.

Broadbent and Clark (1965) studied denitrification losses in the field under conditions not unlike those of this present study. Ten to fifteen percent of the total annual nitrogen input was found to be lost by denitrification.

A loss of such small magnitude is in itself not economically intolerable.

Volatilization of ammonia

Hydrolysis of exchangeable NH_4^+-N results in an equilibrium concentration of NH_3 in the soil solution which will equilibrate with NH_3 gas into the soil atmosphere and, if at a shallow enough depth in the soil, with the air above the soil. There is, therefore, a constant outflow of NH_3 gas from the soil, directly proportional to the ammonia concentration in the soil solution and the partial pressures of H_2O and NH_3 in the contiguous atmosphere (Jewitt, 1942). The magnitude of this outflow can be appreciable if the ammonia is in the immediate soil surface, and if moisture and pH are high enough in the immediate area to hydrolyze and not neutralize the ammonium hydroxide formed (Hausenbuiller, 1970).

Decreasing the access of the ammonia in the soil solution to the atmosphere above the soil is the key to minimizing volatilization losses. Using a sandy loam, Wahhab (1957) determined that at a placement depth of only 3 cm, $(NH_4)_2SO_4$ volatilization losses, as compared to a similar surface treatment, were cut by two-thirds. Percentage-wise, 12.9 percent of the surface applied nitrogen was lost, as compared to 4.3 percent at the 3 cm depth.

These results agree fairly well with an earlier study on surface fertilized soils by Martin and Chapman (1951). In the two sandy loams

with pH above 7, heavy rates of nitrogen fertilizers were applied to the soil surface and subjected to four wetting and drying cycles in the laboratory. The soils were analyzed after seventy days and comparative volatilization loss rates computed. Approximate average loss values for the two soils were: $(NH_4)_2SO_4$ lost about 20 percent; urea, 15 percent; NH_4NO_3, 7 percent; and $NaNO_3$, 0 percent.

These figures are roughly of the same magnitude as the estimates of Broadbent and Clark (1965) of microbiological denitrification losses. These loss percentages also approach the estimates of Dinchev and Badzhov (1969), who presumed biological and physical volatilization losses in their field trials with wheat and maize reached 27-29 percent when maize followed wheat in the cropping sequence.

Fixation of ammonia

Ammonia, as a cation, is adsorbed by negatively charged clay particles and by organic matter. The organic matter fixation results in nitrogen incorporation into the high molecular weight humus-like organic fraction rather quickly (Broadbent, 1968). Since this is not a permanent removal, but only long term, it will be further considered in the section on residual fertilizer effects.

Clay fixation, on the other hand, can remove ammonium from the available nitrogen pool quite permanently. Preul (1965) reports that clay adsorption of ammonium was time dependent, taking a few hours to establish equilibrium, whereafter it was essentially irreversible.

In 1965, Broadbent found that clay-fixed ammonium is not involved in exchange with other sources. Even though exchangeable ammonium declined to very low levels, clay fixed ammonium was not available to

nitrifying bacteria or to heterotrophic organisms using nitrogen for decomposition of organic matter. This fixation process is presumed to be the result of ammonium replacing other cations in the expanded lattices of some clay minerals, resulting in strong lattice bonding due to the optimum physical size of the ammonia ion (Stevenson, 1964).

Raza and Muhammed (1971) used two Pakistani soils and measured some of the characteristics of this clay fixation of ammonium. Increased amounts of ammonium, added as $(NH_4)_2SO_4$, resulted in increased absolute amounts of clay-fixed ammonium, but the percentage of clay-fixed ammonium decreased. Amounts fixed increased with time, but fixation rate decreased with time. Net fixation was positively correlated with clay content.

The effect of heat on ammonium fixation was examined by Peterburgskie and Korchagina (1965). The rate of clay fixation of ammonium was increased by heating to 70°C for 24 hours. Ammonium thus fixed was found to be unavailable to plants planted subsequently, for the duration of their active growth season in the greenhouse.

Fertilization practices and clay fixation of ammonium. Most normal fertilizer practices on soils capable of fixing ammonium will result in little economic loss due to ammonium fixation, according to Legg and Allison (1959). In comparisons of similar treatments on ammonium-fixing and non-ammonium-fixing soils, no significant yield differences were found. The ammonium-N loss attributable to clay fixation at a fertilizer application rate of 56 kg-N/ha amounted to less than 2 kg-N/ha. The authors explain that the usual laboratory evaluation of ammonium fixation includes saturation of the soil solution with ammonium salts

and subsequent removal of surplus ammonium salts by washing, which leaves only the fixed ammonium. In most fields, the ammonium is more likely to be surface adsorbed on the particles, rather than in the interstitial sites, and hence is mostly in an exchangeable form rather than a clay-fixed form, depending on clay type, clay content, and relative ammonia status of the soil in question (Raza and Muhammed, 1971).

Non-normal fertilizer practices can aggravate the permanent clay fixation of ammonium. Shilova (1966) reports that normal additions of an N-P-K fertilizer, supplying tagged $(^{15}NH_4)_2SO_4$, resulted in availability of this tagged ammonium, with the naturally fixed ammonium in the soil remaining unavailable. K applied at three times the normal rate, however, resulted in a significant portion of the tagged ammonia becoming unavailable due to clay fixation. K, applied twelve days before the NP fertilizer had no effect on N availability, but K applied twelve days after the NP addition caused a marked increase in ammonium fixation. That in-the-field ammonium fixation by clay is normally a slow process is borne out by the report of these authors that a soil systematically fertilized for 30 years was still able to fix ammonium.

The marked effect of potassium on clay fixation of ammonium is held to be due to a blocking effect by the K^+ ion. This blocking effect is the result of further clay lattice contraction when a K^+ ion enters, successfully trapping an NH_4^+ ions at or near the edge of an interstice (Tisdale and Nelson, 1966).

These phenomena suggest three positions of a NH_4^+ ion on a clay particle: adsorbed on the surface in exchangeable form; at or near the open end of an interstitial opening in a slowly or semi-exchangeable

form; and fixed well inside a clay interlayer in a non-exchangeable form. Potassium, by its lattice contracting properties, serves to trap the semi-exchangeable form and render it non-exchangeable until such time as a lattice expanding cation such as calcium or magnesium again exchanges with the potassium (Tisdale and Nelson, 1966).

An experiment by Shilovah and Smirnov (1968) illustrated the importance of this second mode of fixation, the semi-exchangeable form. Using a potted soil, known to be able to fix ammonia, tagged ammonium sulfate and tagged calcium nitrate were added to very young oat plants. Uptake from $Ca(NO_3)_2$ was rapid and practically ceased by the shooting stage, after which soil nitrogen was drawn upon. Uptake from $(NH_4)_2SO_4$ was considerably lower, with soil nitrogen supplementing this form throughout the season. Fixed, tagged ammonium was slowly released when exchangeable levels became very low at the tillering stage. Very little of this fixed, tagged ammonium was left at the end of plant growth. Plant weight and total uptake of nitrogen were the same for both treatments, but the utilization of soil nitrogen was increased more by the $(NH_4)_2SO_4$ than by the $Ca(NO_3)_2$.

Leaching of nitrogen

The mobility of nitrogen forms in the soil will be closely examined below in the discussion of nitrogen availability. Rather than discussing mechanisms of leaching losses here, the incidence and magnitude of leaching losses for soil and climatic conditions similar to those of this present study will be investigated.

Krause and Batsch (1968) confirmed the seriousness of leaching in a high rainfall area on a sandy soil. September applied NH_4NO_3 was

found 88 percent removed by leaching in December, aided by nitrification continuing even when outside air temperatures fell below freezing. Obviously, fall applied nitrogen could not be expected to remain in the soil until spring under such soil and climatic conditions.

Devine and Holmes (1964) describe a large scale experiment wherein ten locations were staked for fertilizer and application time comparisons. The ten locations represented a wide diversity in annual rainfall patterns and amounts. Sources used were $(NH_4)_2SO_4$; NH_4NO_3; and $Ca(NO_3)_2$, all at the rate of 67 kg-N/ha and either applied all in autumn, one-third in autumn and two-thirds in spring, or all in spring. Similar treatments gave similar results under similar rainfall conditions. In high rainfall areas, fall and split applications were less effective than similar spring applications, due to leaching losses. In the autumn applications, yields were highest with $(NH_4)_2SO_4$, intermediate with NH_4NO_3, and lowest with $Ca(NO_3)_2$, presumably because of differences in leaching susceptibility. In low rainfall areas, autumn applications yielded almost as high as spring applications. The authors measured rainfall amounts for November through March for each area. This rainfall, in inches, was compared with the yield increase from the fall-N application expressed as a percentage of the yield increase due to the corresponding spring-N application. The resulting relationship was quite linear, and predicts that for a rainfall of less than 7 inches (the rainfall during this period for the present experiment was 6.75 inches) fall applications should yield 90 percent of the corresponding spring applications. This prediction is a realistic estimate: Watson et al. (1963) report a 10 percent loss in nitrogen uptake when fall

applications were compared with identical spring applications in a year with 12.5 inch rainfall for this same period.

Studies conducted in the arid regions of the western United States indicate that rainfall is indeed the determining factor in leaching magnitudes.

Peterson (1952) found that in Northern Utah spring applications of nitrogen were superior to fall applications during a period of above normal precipitation. In a more arid area, at the same time, fall and spring applications proved equal in producing grain yields. Differences in the yields at the sites with higher precipitation are probably due to the leaching caused by snow-melt after an unusually wet winter.

Spring precipitation does not seem to be responsible for any significant leaching losses, since in experiments by Nielson and VanEpps (1955, 1960, 1966), at various times, timely spring rains seemed inevitably to boost yields.

Additionally, Nielson and Banks (1960) reported on a study conducted north of Logan, Utah, wherein nitrate movement in irrigated soil was studied. Forty inches of water were applied to one plot by the furrow method, and twenty-seven inches were applied to another plot using sprinklers. No appreciable nitrate movement was observed below twenty-four inches depth in the soil.

The foregoing suggests that for this geographical area, under a normal precipitation regime, losses of nitrate by leaching should not assume any great proportions.

Peterson (1952) does note, however, that wheat crops in shallow soils do not respond well to nitrogen, for reasons other than nitrogen deficiency. A nitrogen deficiency problem on shallow soils with a slowly

permeable subsoil may in part be due to nitrate leaching, as suggested by Painter et al. (1964), in Idaho. Winter and early spring soil water movements over the compacted layer will carry nitrates laterally downslope, according to the authors.

Such a shallow soil condition was found to exist in a portion of the field used in this study, and lateral, downslope leaching will be a possibility deserving some analysis in the data.

Availability of Mineral Nitrogen in the Soil

The concentration of mineral nitrogen in the soil is a function of the difference between the rate of nitrogen genesis and the rate of nitrogen removal by the source and loss mechanisms discussed previously, and also by the rate of removal by the plants comprising the crop. Also, mobility of the growing plant root system and of the nitrogen in the soil are involved, as they determine the coordination between the plant's peak demand times and the peak availability times of the nitrogen in the soil.

Mobility of mineral nitrogen in the soil

The preceding discussions on nitrate leaching and on ammonium fixation by clays and organic matter shed some light on the question of nitrogen mobility in the soil. Generally nitrate is considered to be quite mobile, while ammonium is not.

Availability of these nitrogen forms depends in part on their ability to migrate to nearby root zones. Wheat, in particular, is not greatly affected by the form of nitrogen being supplied, ammonium or nitrate. Yields are somewhat better if more than one source is

available, as will usually be the case in any non-sterile soil (Hutchinson and Miller, 1909; Thelin and Beaumont, 1934).

Under conditions of no water stress, Spratt and Gasser (1970) found more dry matter and grain produced from a $Ca(NO_3)_2$ fertilization than from $(NH_4)_2SO_4$ added with a nitrification inhibitor. These increased yields with nitrate-N could well be caused by the greater mobility of this nitrogen form. The authors also report that in the ammonium treatments, leaves and stems during the extension to flowering stages of growth contained half the nitrate nitrogen of the same parts in the nitrate treatments. It is suggested by Spratt and Gasser (1970), that the nitrification inhibitor contributed to this lack of nitrate, and also tended to lengthen the duration of physical immobilization. In other words, the nitrification inhibitor accomplished its purpose in keeping the ammonium in the soil longer than it would normally. Apparently, however, the ammonium was also rendered less available to the plant, due to a more prolonged immobility, which caused the plant in turn to rely more heavily on existing soil nitrogen as was the experience of Shilovah and Smirnov (1968).

Spratt and Gasser (1970) repeated their experiment under water stressed conditions and reported yields to be equal from both sources, although yields were depressed for both sources as compared to the above experiment with plenty of water. Nielson and VanEpps (1955, 1960, 1966) and others who have worked in arid climates (Peterson, 1952; Painter and Baker, 1960) all emphasize the possibility of no response to nitrogen under water deficient conditions. The explanations given for these depressed responses may not be the result of plant physiology changes under drought, as suggested, but may be only water stress having

become the limiting factor of the plant's growth. Otherwise the conclusion must be drawn that ammonium is relatively more available than nitrate under low moisture conditions, which is not consistent with known nitrogen behavior in soils.

On the contrary, Krantz et al. (1943) report that ammonium nitrogen was immobile in his field experiments while nitrate nitrogen moved downward with rainfall, and afterward moved upward again with the water during soil drying. Other workers similarly report the immobility of ammonium. Ray et al. (1957), for example, report on the movement of ammonia in soils and describes the process as dependent on soil texture, organic matter content, cation exchange capacity, and water movements. Only on coarse sands was there any appreciable movement of ammonia with water flow.

Tyler et al. (1958) placed a variety of nitrogen sources at a four-inch depth in four types of dry soil, watered to field capacity, and analyzed for distributions after two weeks. Ammonia nitrogen did not move downward to any significant degree except in the coarser-textured soils where small amounts moved downward to about four inches. Had there been no nitrification, the authors report, no nitrogen would have been available outside the immediate areas of placement.

In a study by Overrein (1963), ammonium from the hydrolysis of urea tended to move upward significantly more than downward. In the experiment of Tyler et al. (1958) this was found to be the case for both the urea and NH_4OH ammonium. This phenomenon illustrates the necessity alluded to in the section on ammonia volatilization losses above, for deeper placement of ammonium sources. As these experiments indicate, urea, and ammonium hydroxide are more prone to such upward movement and

consequent losses that are the usual fertilizer ammonium salts. This greater vulnerability may be related to the hydrolysis mechanism, which produces ammonium carbonate, which in turn is easily hydrolyzed to the more volatile ammonium hydroxide form (Hausenbuiller, 1970).

Of interest, also, especially in arid-zone agriculture, is the experiment of Stewart and Eck (1958) showing the necessity of rain for moving nitrate nitrogen into the root zone of the crop. On a silt loam, with no rain, surface applied nitrate did not move over three inches into the profile at a moisture content of 9 percent; 77 percent of the nitrate did not move over a half inch into the profile, the rest was found in amounts decreasing with depth down to three inches.

Nielson and Banks (1960) report the very prominent upward movement of nitrates in a soil which is drying. In other experiments, Nielson and VanEpps (1955, 1960, 1966) observe that a timely spring or early spring rain may, in dry-land culture, decide the success or failure of a given crop. The authors suggest that the spectacular influence of such timely storms is due to their waters reaching the root zone with the nitrate nitrogen accumulated on the surface during the preceding drying cycle.

Wheat plant growth interactions with nitrogen availability factors

Perhaps this section is best introduced with this admonition that for best results, nitrogen fertilizers should be applied close to crop usage times (Pack, 1957).

Time of application as an availability factor. Early spring applications on dry-land winter wheat are timed to give a boost to the available nitrogen level as the crop's peak demand period approaches. Fall

applications by this time may have been rendered unavailable to some extent by the loss and mobility factors previously discussed. Therefore, under some circumstances, spring applications prove superior in terms of grain yield over fall applications, while under other circumstances spring applications have no advantage or may actually prove disadvantageous (Watson, 1939; Peterson, 1952; Nielson and Van Epps, 1955, 1960, 1966; Devine and Holmes, 1964). In all these citations moisture stress was responsible for the lack of superiority of the spring application as compared with the fall application.

As discussed by Nielson and Van Epps (1966), spring applications in the climatic region of the present study, i.e., of Northern Utah, need to be early since precipitation prospects decrease as late spring approaches. If application is later, lack of precipitation may prevent the applied nitrogen from reaching the root zone. In contrast, fall applied nitrogen would already be in the rooting zone in some appreciable, even if somewhat diminished, amount. Forseeably, as roots and shoots extend downward seeking new moisture, fall applied nitrogen would be extracted along the whole root penetration path.

Such a circumstance would result in superior performance from fall applications. Marginal downward movement of spring applied nitrogen, due to some but not plentiful spring moisture, could thus cause equal responses, with no real advantage being apparent from either time of fertilization.

A special case, where spring applied nitrogen has actually depressed yields, was characterized by Nielson and Van Epps (1955). Early benefits from spring nitrogen may be apparent soon after fertilization, but may be nullified by a lack of moisture later in the season.

In some of these cases, the early nitrogen may have stimulated excessive vegetative growth to a point where this becomes a burden to the plant under conditions of extreme drought, with a subsequent depression of yields.

Much work has been done to further understanding of the principles involved in this interaction between nitrogen and water availability.

Ternan et al. (1969) observe that with adequate moisture it is hard to put on too much nitrogen. Although much of this nitrogen will go to produce straw rather than grain (Hutcheon and Paul, 1966), if nitrogen is absorbed in excess of these increased vegetative needs, grain yield and protein content will increase (Ternan et al., 1969).

A general rule, as proposed by many observers (Neidig and Sneider, 1924; Hutcheon and Paul, 1966; Ternan et al., 1969) is that adequate moisture and adequate nitrogen will result in increased grain yield and increased protein content. Adequate moisture and inadequate nitrogen will especially depress protein accumulation because vegetative nitrogen demands will not be fully met. Low moisture and adequate nitrogen will usually result in lower yield and increased protein content.

The latter observation led Nielson and Van Epps (1955) to suggest that even in a dry year, nitrogen fertilization may pay off by increasing the protein yield per acre. Even if the grain yield per acre is depressed, the increased economic value of the crop may more than compensate for the fertilization cost.

Experiments carried out under no water or nitrogen stress are instructive in pointing out the wheat plant's behavior under ideal conditions.

Nitrogen-wheat relations without moisture stress. Pavlov (1973) found that in a pot experiment with sufficient nitrogen the entire growth period as much as half of the grain protein was synthesized during kernel ripening at the expense of soil nitrogen. In a second experiment, where nitrogen stress was induced after the flowering stage, with adequate moisture still, wheat kernels were fully formed and grain yields were the same as in the first experiment, but the grain protein was synthesized wholly at the expense of nitrogen accumulated in vegetative organs. Hence the protein content in this case was much lower than in the first case where vegetative nitrogen redistribution contributed only half the protein nitrogen. In a field experiment by the same authors, it was found that the main portion of kernel protein was produced from the reusing of nitrogen accumulated by vegetative organs. This outflux was highest from leaves, then roots, thirdly from stems, and lastly from ear scales.

Pavlov's (1973) observation concerning uptake during kernel ripening reflects a rather late development in wheat research concerning the ability of the plant to extract nutrients from the soil late in its life cycle.

Earlier research had assumed and found a virtual cessation of soil nitrogen uptake by the time of ear emergence (Watson, 1939). Thorne (1962) effectively demonstrated that if the nitrogen was readily available after ear emergence, it would be taken up with a resultant delay in the senescence of vegetative parts.

Fittingly, Watson, Thorne, and French (1958, 1963) studied the problem in some detail and found that in spring barley, winter wheat, and spring wheat, only 70 percent of the maximum nitrogen content of

these grains had been absorbed by the time of ear emergence; i.e., late May or even early June in the case of this present experiment.

Thorne (1962) explains the difference in early research results as compared to these later ones as being mainly due to lack of field experimentation. For example, in this experiment, the lack of grain yield response to late nitrogen applications in pots was found to be related to stimulation of new unproductive tiller growth. In the field, to the contrary, no new growth occurred and late nitrogen uptake went into existing vegetative parts, enriching them and enhancing their contribution to the grain nitrogen when these parts senesced.

Balba et al. (1972), in a pot experiment, checked the effect of application time on wheat yield by placing fertilizer at seeding time, tillering, and ear emergence. Uptake efficiency of the tillering application was measured to be double that of seeding time uptake efficiency. Yield response was nearly the same for the first two application times and lower for the ear emergence application. The authors recommend a tillering application, basing their recommendation on the above results. Since this was a pot experiment, such a recommendation is not warranted. Nearly equal yields from seeding or tillering applications, as reported in this same study, suggest that during the entire growth season roughly the same nitrogen amounts were available, and lower fertilizer nitrogen uptake efficiency only suggests increased soil nitrogen uptake efficiency. A field trial, as suggested by Thorne (1962), may well have responded differently to the ear emergence application. Direct field implementation of pot experiment findings is not sound practice.

Nitrogen-wheat relations under moisture stressed conditions. Non-ideal water and nitrogen conditions are more nearly the norm for the climate and soils of Utah dry-land wheat areas (Peterson, 1952).

The onset of moisture stress with plentiful nitrogen will result in high accumulations of nitrate nitrogen in the plant (Baker and Tucker, 1971; Spratt and Gasser, 1970).

Spratt and Gasser (1970) further observed a rapid decrease in this accumulation with timely rewetting, presumably, according to these authors, this indicates that the wheat plant's nitrate reductase system function is highly dependent on an adequate water supply.

As a consequence, a spring storm after a period of drought may not only bring the benefit of surface accumulated nitrogen into the root zone (Nielson and Banks, 1960) but may further affect a rapid plant response by making immediately available nitrates stored in the plant itself during the dry period.

In spite of such rapid recoveries from the effects of short-term spring droughts, dry-land wheat yields from low rainfall areas are characteristically low, as compared with nearby irrigated areas (McNeal et al., 1968).

An early Utah study (Stewart and Hirst, 1912) declared dry-land wheat from this area to be characteristically lower in moisture and higher in protein content than similar crops grown under irrigation.

Improved practices, including proper fertilization, have reversed this protein content characteristic. McNeal et al. (1968) report that under irrigation grain-nitrogen contents were either nearly equal or higher than under nearby dry-land conditions. From detailed analyses of plant part nitrogen contents at successive stages of development,

these authors determined that the reason for the superiority of the irrigated wheat was mainly due to the greater amount of top growth, and the continuation of nitrogen uptake from the soil into the later stages of growth. In addition, the nitrogen content of the dry land wheat top growth was lower than that of the irrigated wheat. Translocation of top growth-nitrogen accounted for 75 percent of the dry-land wheat grain nitrogen, as compared with 66 percent for the irrigated wheat. As so often happens in dry-land culture, soil moisture was limiting during the later stages of plant growth, and nitrogen uptake presumably ceased at that time.

The wheat-fallow rotation cropping sequence used in this present experiment has been shown to be very effective in storing necessary moisture for the succeeding crop (Leggett and Nelson, 1960). Nevertheless, fertilizer response has proved to be significantly correlated only with spring precipitation, and not with the biennial precipitation amounts for both the fallow and cropping year (Peterson, 1952). The biennial precipitation levels were positively correlated with yields from the unfertilized plots, however, and the dramatic differences in yields between fallow and continuous cropping systems for dry-land wheat in a low rainfall area have been conclusively demonstrated by Leggett and Nelson (1960).

This whole section has served mainly to emphasize the fact that moisture, in the dry-land areas of Utah, is usually the limiting factor to plant growth and grain yields. Nitrogen availability is vital in the periods of adequate moisture while the plant is still growing or capable of absorbing nutrients.

Dynamics of nitrogen uptake and utilization in the wheat plant. To gain an appreciation of the fact that the wheat plant is a growing organism in need of constant nourishment, especially in the earlier growth stages, the following discussion is presented on the dynamics of nitrogen uptake and utilization by the growing plant.

Turchin et al. (1956) studied labeled ammonia nitrogen uptake in wheat under various conditions. In the roots, ammonia nitrogen taken up was utilized by amino acid synthesis within 15 minutes. A study of oats and rye by Turchin et al. (1953) revealed that the newly synthesized amino acids were used in synthesis of constitutional proteins within four hours. These constitutional proteins are constantly renewed and the older ones transformed into the more slowly recycled reserve proteins. Turchin et al. (1956) further report that in young plants virtually all protein in the leaves is renewed every 90-100 hours, and in another study (1957) that the pyrrole nucleus of chlorophyll in oats was renewed within every 72 hours in a young plant.

Grain yield predictions from quantitative assessments of nitrogen availability

Although Jansson (1971) notes that not much lasting success has been had in quantifying the nitrogen immobilization and mineralization cycle, many useful studies have appeared which have taken one parameter, such as mineral nitrogen level for example, and successfully related it to grain yield or response.

These studies tend to be quite regional in scope and in application since the regional parameters of climate and soil characteristics are

very often yield-determining with the nitrogen parameter being more an interacting than a main effect. Within a relatively homogeneous zone, however, yield-nitrogen correlations may be usefully and directly made because variations due to climate and soil may be assumed to be absorbed in the field-error term of the analysis of variance.

Eck and Tucker (1968) attempted to find an equation that would predict the dry-land winter wheat yield given the nitrogen level, moisture at seeding, precipitation during the growing season, moisture in spring, temperatures at selected times, and organic matter content of the soil. This ambitious attempt at a general yield response formula failed because correlations, although significant for moisture, nitrogen level, and yield, were not high enough to allow meaningful predictive equations to be based on any factors, alone or in combination.

Wright (1969) attempted similar correlations, using as factors the time of application, time of seeding, class of soil, and seasonal differences. The relative importance of these factors was found to be in decreasing order as listed. Correlation results were highly specific to conditions at hand, and no generalized predictions were attempted by the author.

State agencies have traditionally, in bulletins and circulars, published fertilizer recommendations geared to local conditions. Some diversity of recommendations is noted among the western states: for example, Nevada soils seem to need phosphorus as much as nitrogen (Spencer and Goodale, 1955), while in Utah no response to phosphorus was noted earlier (Peterson, 1952). More recently some Utah sites have shown a response to phosphorus fertilization (Nielson and Van Epps, 1966).

These diversities and changes with time show the need for continuous regional re-evaluations of conditions, and occasional updatings of the recommendations.

Several regional predictive attempts for dry-land grains have been outstandingly successful.

Canadian researchers have been particularly prolific in this endeavor. Cook et al. (1957), in Saskatchewan, determined a nitrate accumulation capacity for 31 stubble field soils both in the field and in the greenhouse, and also for 30 fallow field soils. The field accumulation figures were highly significantly correlated with field grain yields, $r = 0.846$ and 0.830, for the stubble and fallow fields, respectively. The greenhouse incubations gave a 0.874 correlation coefficient when related to plant nitrogen uptake. The incubation period for the field soils was 14 days, and the nitrate levels after 14 days were used as the index of nitrate accumulation capacity. The greenhouse test of mineralization versus nitrogen uptake lasted 30 days. Nitrogen uptake versus nitrogen accumulation level gave a linear regression line. For the field studies, accumulation capacity of nitrate nitrogen, in the top six inches of soil, yielded a curvilinear regression line when correlated with yield. This relation was generally linear between 10 and 45 ppm nitrate, and after curving leveled out again, giving the impression that yields were maximized after the 60 ppm nitrate accumulation level.

Soper et al. (1971), in Manitoba, found that the amount of easily extractable nitrogen in the soil at the time of seeding was a very good test for predicting cereal responses to nitrogen, and is an indicator of how much nitrogen the soil is capable of supplying a crop. The best results were with a nitrate nitrogen determination to 61 cm of depth,

which, when curvilinearly correlated with crop (barley) nitrogen uptake gave a coefficient of determination, R^2, of 0.84. Plant nitrogen uptake was exponentially correlated with crop yield, and gave an r^2 of 0.86. A direct correlation of nitrate content of the soil to the 61 cm optimum level correlated with grain yield, however, gave an r^2 of 0.59 only. At the 65 degrees of freedom level this is still significant at the 1 percent level. Curiously, when a linear function, with a lower r^2 for nitrate versus plant uptake, was substituted into the yield predicting equation, the R^2 value was raised to 0.69. Thus for practical purposes, a linear relation between nitrate level and yield is recommended, as it is by other researchers also (Ternan and Brown, 1968).

Nuttall (1973), in Saskatchewan, added a new twist to the research pattern of the above researchers by taking into account both ammonium and nitrate nitrogen, and by relating them to barley yield increases over non-fertilized plot yields. Results indicate that both the ammonium and the nitrate nitrogen in the soil are significantly related to grain yields. Supposedly grain yield increase, rather than just grain yield magnitude, gives a relation relatively more independent of soil and climatic variations. R^2 values ranged mostly in the 0.60's, significant at the 1 percent level for 22 degrees of freedom. As can be expected from such a low coefficient of determination, however, estimated yields from the predictive equations were quite variable. Confidence intervals constructed at the 90 percent level gave the yield increases standard variations of from $\pm$ 50 to $\pm$ 90 percent and higher in some cases. These reflect expected variations from the predicted yield increases due to climatic and soil variations, and are not unlike deviations from the mean yield increases that were encountered in the actual experimental data,

due to field and climatic variations between sites used for the same treatments. More uniform conditions would wipe out this large variability, according to the authors, and the methods of this experiment are recommended to those who determine local fertilizer recommendations.

Some investigators use only NO_3^--N, and others use both NH_4^+-N and NO_3^--N at the time of seeding as an indication of total available nitrogen. Superiority of one method over the other is a seemingly local phenomena. In the above studies by Soper (1971), and Nuttall (1973), in different Canadian provinces, one found it best to use only nitrate-N, the other found it important to use both mineral forms. An Australian research team, Storrier et al. (1971), reports trying both approaches. With the combined ammonia and nitrate test at seeding, the R^2 value was only 0.185 when nitrogen level was correlated with wheat grain yield. Nitrate level alone, correlated with wheat yield, in these same trials gave an R^2 of 0.36, almost twice as good, but still much below the levels of correlation reported in the above studies by Soper (1971) and Nuttall (1973).

These types of results point out a common problem in the reporting of statistical results. In none of the studies so far mentioned in this section was there express and clear mention made of the degrees of freedom for each regression test.

Since there is no basis for comparing R's or R^2's without knowing the degrees of freedom, the R^2 of 0.38 for Storrier (1971), and the R^2 of 0.86 of Soper (1971), cannot be legitimately compared. The degrees of freedom for the latter is twenty, so that from the tables it is obvious that the 0.86 is significant at the 1 percent level (Gore, 1952). From the same tables it appears that 0.38 is also significant at the 1 percent

level for any degree of freedom above 14. Since nowhere appears the number of experimental trials, however, we cannot tell whether this regression was run over the whole range of experimental units, or whether it was run on treatment averages consisting of a certain number of replications each. In the first instance field variability could have contributed to the point scatter, while in the second case this would have been sharply reduced, and the results would be indeed sadly lacking in correlation.

Perhaps one of the more ambitious projects on yield prediction of wheat in a low rainfall dry land culture was carried out by Leggett (1959) in the State of Washington. Spring soil moisture, spring through summer precipitation, and nitrate nitrogen at seeding time, were correlated separately and together with grain yields. Over the whole range of experiments, with 60 degrees of freedom, the correlation for soil moisture combined with precipitation over the growing season proved the best moisture-based yield predictor, with an R of 0.87. Nitrate level at seeding time (fall), added to the amount of fertilizer nitrogen added, gave the best correlations of nitrogen with grain yields with an R of 0.74 at 58 degrees of freedom. (Degrees of freedom obtained by counting points on regression graphs.) When other than linear equations were fitted to the data, R values decreased, therefore moisture-yield, and nitrogen-yield relationships over the ranges encountered in these experiments were found to be linear.

Fertilizer recommendations based on grain yield predictive equations. Although Leggett (1959) explains that the equations given are specific to, and therefore best used in, the dry-lands of eastern Washington,

some of the general relationships between moisture, nitrogen, and yield should be applicable to other dry-land areas. For example, it was calculated that 4 inches of water were required to allow the crop to reach the heading stage. Each additional inch of water increased yields approximately 6 bushels per acre. Moreover, 3 pounds of nitrogen per acre were required to increase the wheat yield one bushel per acre, over the range where nitrogen, not water, was the limiting growth factor.

Other investigators have made fertilizer recommendations based on fertilizer rate, time, and source experiments, which are valid in their soil and climatic regions.

The fall nitrogen fertilizer recommendations for areas of Idaho, close to the northern Utah site of this present study, have been variously given as 30 lbs-N/acre (Roylance and Klages, 1959), and as 20 lbs-N/acre ± 20 lbs depending on whether moisture prospects are subnormal or above normal for the year (Painter and Baker, 1960).

Recommendations for northern Utah have been given as ranging from 30 to 50 lbs-N per acre (Nielson and Van Epps, 1966) in recent years, and twenty years ago as ranging from 40 to 60 lbs/acre, unless moisture and stand were not encouraged when a rate of less than 40 lbs/acre would be more practical (Peterson, 1952).

Leggett (1959) and Nielson and Banks (1960) both observed that of the nitrogen not recovered by the crop one year, a small part is usually recovered by the following crop if the moisture level is not again limiting.

Long-term consequences of nitrogen fertilization

Peterson (1952) applied 125 lbs and 250 lbs $NaNO_3$-N/acre and noted that after two seasons of such applications a definite deterioration of the soil structure occurred. Painter and Singleton (1960) used four nitrogen sources and measured changes in soil physical properties including pH, pHs, ECe, and soluble Ca^{++} and Mg^{++}. With normal application rates and practices no appreciable alterations were noted.

With an exception for repeated use of sodium containing nitrogen carriers then, soil structure will probably be unaffected by normal nitrogen fertilization practices.

Increases in the soil organic nitrogen level. It has been noted in the preceding section that in dry-land wheat culture some of the nitrogen not used in one season would probably be used by the following crop (Leggett, 1959). To gain insight into this carryover effect, the study of Cheng (1961) is instructive. After two annual fertilizer treatments, added tagged nitrogen was characterized as to amount and position. Some fertilizer nitrogen was still in its original form in the soil surface, but this amounted to only 2 percent of the amounts added. Of the transformed nitrogen, over 90 percent was found in acid hydrolyzable forms such as amino sugars, amides, and amino acids, and in plant residues and some clay-fixed forms. The remaining tagged nitrogen (less than 10 percent) was in non-acid hydrolyzable clay-fixed and organic-residue forms. Therefore, in this experiment, nearly all the transformed nitrogen in organic form was still considered relatively available after two years.

Other investigators, such as Black and Reitz (1972), estimate that, typically, closer to 30 percent of added nitrogen is rendered unavailable through himification after a cropping year. Stewart (1961) measured the release rates of the humified, or near humified non-acid hydrolyzable fraction of the soil organic nitrogen added after a fertilization. After four cropping seasons, less than half this fraction was rereleased and used by the crops. Even though this equilibrium release rate was quite slow, a surprisingly active immobilization-mineralization cycle was indicated by the substantial interchange found between the fertilizer and the soil nitrogen.

Naturally, in order to increase the total nitrogen content of a cropped soil, more nitrogen must be added over a period of time than is taken out by crops and other loss mechanisms (Tisdale and Nelson, 1966). A common observation is that soil organic matter, as well as soil nitrogen levels are increased with adequate nitrogen fertilization and crop residue return (Tisdale and Nelson, 1966).

Soil organic matter level and soil nitrogen level. Scharf (1968) convincingly demonstrates, however, that organic matter increases are no guarantee of nitrogen level increases. In a 14-year fertilizer trial, increments of nitrogen were added up to 135 kg-N/ha/year. At this high level, the 14-year increase of organic carbon in the soil reached 0.113 percent. The nitrogen level in the soil, on the other hand, dropped by 6.5 mg-N per 100 g soil over the 14-year period under this high rate of nitrogen fertilization. At lower rates, below 75 kg-N/ha/year, the 14-year nitrogen drop ranged from 11 to 15 mg-N/100 g soil. Note that these soils were quite high in nitrogen before the onset of the 14-year experiment.

Soil characteristics and nitrogen level changes under fertilization. The initial soil nitrogen level seems to be an important factor in determining whether a soil will gain or lose total nitrogen under fertilization rates reasonably near crop demand rates. In a study by Legg and Stanford (1967), twelve soils were analyzed for nitrogen availability using the method and formula of Fried and Dean (1952), which assesses the availability of soil nitrogen by the empirical formula:

$$A = B \left(\frac{1 - y}{y}\right)$$

where A is the nitrogen made available to the crop, and is referred to as the availability index of the soil; B is the amount of fertilizer nitrogen added; and y is the fraction of plant nitrogen derived from B compared to the total plant nitrogen.

From the formula it is easily seen that any value less than 0.5 indicates that more soil nitrogen was taken up by the plant from soil forms than was taken up from fertilizer nitrogen, and, therefore, especially if B is high, the availability index will be high.

For the twelve soils of Legg and Stanford (1967), standardized A values were computed for each soil. It was found that soils with high A values, which would be soils high in nitrogen available to a crop during a season, would lose total soil nitrogen with fertilization and cropping, even when reasonably high amounts of nitrogen were applied. Soils with low A values, on the other hand, gained in total nitrogen content with applications adequate to cover crop removal. Even when applications did not cover the nitrogen taken up by a crop when higher levels of fertilization were used, the yields were reduced from these

higher levels so that the additions were in fact adequate for crop needs, and total nitrogen was still increased in these soils.

Since Utah's low rainfall, dry-land soils are characteristically low in organic matter (Pauli, 1967) and in nitrogen (Peterson, 1952), such increases in total soil nitrogen are a very real possibility for the field used in this present study, and the residual effect observed by Leggett (1959) and Nielson and Banks (1966) could be more dramatic than those observed by Jansson (1971) and Stewart (1961) because less extensive humification as characteristic in drier soils (Pauli, 1967) would result in fractionally more of the immobilized nitrogen being in the more readily available hydrolyzable form (Cheng, 1961).

Jansson (1971) observes that typically up to half the added fertilizer nitrogen may remain in the soil due to biomass incorporation and crop residue incorporation. This is a good estimate in the case of wheat, judging from grain to straw ratio (Percival, 1921) and the straw nitrogen content (Peterson, 1965), and studies on the activity of such residual nitrogen after cropping are revealing.

Humification and re-mineralization dynamics. Allen (1971) studied fertilizer fates through five crop years using nitrogen-15. The labeled nitrogen was originally incorporated into the soil biomass as protein and cell wall materials, and then slowly underwent humification with only 1 percent being in a mineralized form at any given time. The rate of mineralization of the original tagged-N decreased with time over the experimental period, indicating that the humification process makes the nitrogen progressively more stable, and progressively less available to the crop plants. Approximately one-fourth of the labeled nitrogen

was recovered after the first cropping year, while about one-eighth remained after five years, illustrating this decreasing availability.

Jansson (1963) studied these perennial effects of tagged nitrogen fertilizers and calculated a half life of 35 to 45 years for tagged nitrogen in the soil humic matter. It appeared, however, that fertilizer nitrogen in this humified state was twice as active in the mineralization-immobilization cycle as compared to the residual soil nitrogen. Even after nine years this higher rate of activity was still apparent.

Judging from pot experiment results, Jansson (1971) estimates that an arable soil in the temperate region which has received 50 kg-N/ha/year for fifty years should have as much as 40 percent, or 600 kg/ha, of this added nitrogen in immobilized, organic forms. With moderate rates of mineralization, such a reserve could deliver 10-15 kg N/acre/year to a growing crop.

Obviously, if such dynamics could be known for a given region on a somewhat more precise quantitative basis, fertilization patterns could be adjusted to maximize crop yields, as is presently the main concern, and also to optimize the soil organic nitrogen level so that in time a steady state will be reached between soil and fertilizer nitrogen delivery to the crop, and crop nitrogen removal.

MATERIALS AND METHODS

The Experimental Field and Its Soil

The experimental field is located in an area of eastern Box Elder County, Utah, known as Blue Creek. The soil of the experimental site has been classified as a Parleys silt loam (fine-silty, mixed, mesic) with 6 to 10 percent slopes. General characteristics of this series are that they occur in upland elevations from 4200 to 5600 feet, are generally well drained with a moderate erosion hazard, and are in a 15-18 inch annual precipitation area with a frost free period of 110 to 130 days. Primary use is for non-irrigated small grains, with a non-irrigated capability unit designated as IIIe.

Ten-year precipitation records (Nielson, 1974) reveal a 14-inch annual average for our field site, with our experimental year being above average with 18.52 inches of precipitation recorded.

Water relations for this soil were characterized in the soil survey. Permeability was found to be moderately slow, with slow to moderate intake rate. The available water-holding capacity to a depth of 150 cm is 25 to 30 cm of water. Roots may penetrate beyond 150 cm. Usually strong lime accumulations are found at about the 85 cm depth.

The Experimental Design

This investigation was designed to study the fate of added ammonium and nitrate nitrogen under winter wheat. The experimental design used was the randomized complete block design with four replications. This

design lends itself readily to statistical analysis by two-way analysis of variance, with follow-up investigations of significant treatment effects using Duncan's Multiple Range Test. Each treatment received 56 kg of nitrogen per hectare. The field design and treatment distributions are detailed in Figure 1.

Data collection procedures

The main source of data for this experiment was soil sampling. Each sample representing a plot consisted of cores obtained from six places within that plot for the 15 cm or 30 cm sampling depths. Deeper depths were sampled from two places in each plot, and samples consisted of these two cores, combined and homogenized in the field as much as was practicable. The September and April samplings consisted of separating the cores for the following depth increments: 0-3 cm, 3-15 cm, 15-45 cm, 45-75 cm, and 75-120 cm. The October sampling consisted of the 0-3 cm, 3-15 cm, and 15-30 cm depth increments. Finally, the July sampling included 0-45 cm, 45-75 cm, and 75-120 cm increments.

Soil sampling dates were (1) September 19, 1972, (2) October 20, 1972, (3) April 28, 1973, and (4) July 31, 1973. These samplings are referred to as the September, October, April, or July samplings, respectively.

Other data were also collected, consisting of bulk density, plant, and grain samplings.

The bulk density determinations served only as a guide for estimating the whole-field bulk densities at levels representative of the sampling depth increments. The actual determination was very limited, consisting of two pits wherein two samples were taken from each depth increment, resulting in four samples per soil sampling depth increment.

ROWS:	COLUMNS: I	II	V	III	IV
A	1	10		9	8
B	2	5		6	3
C	3*	11	3	1	7
D	4	2		11	5
E	6	9		7	2
F	10	3		4	11
G	7	1		8	6
H	8	4	2	3	10
I	5	6		2	1
J	9	7		5	4
K	11	8		10	9

Key to treatment numbers; application rates in kilograms nitrogen per hectare:

No.	Material used	Application time and method	NH_4-N Applied	NO_3-N Applied
1	None (Control)		0	0
2	Ammonium nitrate	at seeding, with drill	28	28
3	Ammonium nitrate	before seeding, with Gandy spreader	15	41
4	Ammonium nitrate	after seeding, with Gandy spreader	15	41
5	Ammonium nitrate	in Spring, hand broadcast	15	41
6	Calcium nitrate	before seeding, with Gandy spreader	0	56
7	Calcium nitrate	after seeding, with Gandy spreader	0	56
8	Ammonium sulfate	before seeding, with Gandy spreader	56	0
9	Ammonium sulfate	after seeding, with Gandy spreader	56	0
10	Urea	before seeding, with Gandy spreader	56	0
11	Urea	after seeding, with Gandy spreader	56	0

*Column I, row C, received double the intended amount of fertilizer, and has been replaced by Column V, row C.

†Plot dimensions are 3.36 m by 4.57 m.

Figure 1. Experimental plot layout and treatment detail.

The averaged values for each increment were used as best estimates of the actual bulk densities for each level in the calculations converting parts-per-million (ppm) nitrogen levels to kg/ha nitrogen levels at each increment.

Plant sampling was very limited, and consisted only of a few stems and leaves from a few selected plots to spot-check total and nitrate nitrogen contents of the vegetative parts towards plant maturity.

Grain yields were estimated at harvest. Five squares were measured off inside each plot, a square meter each, and the grain was harvested, threshed, and weighed for each square. The average of each of these five yield determinations was taken as the plot-yield value, and recorded in kg of grain per hectare.

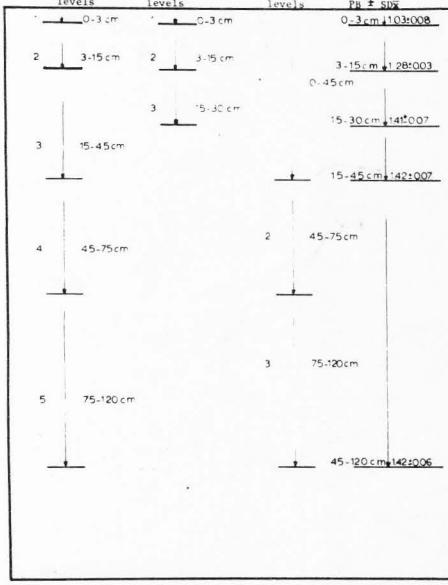
Soil description at each sampling-depth increment

From the soil survey description of the soil profile by horizons, the soil at each sampling depth increment may be characterized. These horizon descriptions are tabulated together with bulk density figures and sampling depth increments in Figure 2.

Pre-treatment and Storage of Samples

Soil samples from the September sampling were air dried in the laboratory and subsequently transferred to the freezer. All other samplings were frozen immediately after reaching the laboratory. The pre-treatment and storage of the soil samples is discussed at length in Appendix A.

Plant and grain samples were stored in closed polyethylene bags until the time for their analysis.



Horizon Descriptions from Soil Survey

Horizon indexes and levels	Colors: air dry	Colors: field moist	Texture	Structure and other descriptions	Roots	Pores	Boundary	Alkalinity and pH
Ap-0 0-15 cm	grayish brown	very dark brown	silt loam	weak, fine, granular. soft, very friable, non-sticky and slightly plastic	few fine roots	few fine pores	clear and smooth	mildly alkaline 7.6
A-1 15-30 cm	grayish brown	very dark brown	silt loam	weak, fine, and medium subangular blocky. slightly hard, friable, slightly sticky and slightly plastic	few fine roots	common fine pores	abrupt and smooth	mildly alkaline 7.4
B 21t 30-50 cm	brown	dark brown	silty clay loam	weak, medium prismatic parting to moderate, fine, and medium subangular blocky. Very hard, firm, sticky and plastic. Common thin clay films on ped faces	few fine roots	few fine pores	gradual and wavy	mildly alkaline 7.6
B 22t 50-85 cm	brown	brown	silty clay loam	same as B21t, except with many moderately thick clay films on ped faces	few fine roots	few fine pores	gradual and wavy	mildly alkaline 8.0
B 3ca 85-120 cm	pale brown	dark yellowish brown	light silty clay loam	medium subangular blocky few thin clay films on ped faces moderately calcareous with lime in veins paste when moist	very few fine roots	few fine pores	gradual and wavy	moderately alkaline 8.4
C ca 120-150 cm	brown	brown	heavy loam	massive, hard, friable slightly sticky and slightly plastic moderately calcareous with lime disseminated	--	few fine pores	--	strongly alkaline 8.6

Figure 2. Soil profile descriptions and sampling depth increments.

Analytical Procedures

Determination of available mineral nitrogen in the soil samples

Soil samples were analyzed according to the procedure outlined by Bremner (1965). This included extractions with 2N KCl by shaking for one hour, filtration of the extractants using Eaton-Dikeman No. 512 fluted filter paper in 60° long stem funnels, and refrigeration of these filtrates until the time of analysis.

Analyses were done using reagents and apparatus as described by Bremner (1965) with the single exception of the indicator solution, which is described in detail in the section on plant analysis, below. The nitrate nitrogen was determined using Devarda's alloy in the magnesium oxide distillation procedure, and by subtracting the results of a parallel distillation using magnesium oxide only. Bremner's (1965) procedure was followed despite some initial problems that were encountered, because these problems were mainly in data processing and interpretation, and not in the procedural aspects of the analyses. A discussion of these problems, and of the accuracy and precision of the MgO-Devarda alloy distillation procedure as used in this laboratory is the subject of Appendix B.

For comparative purposes, nitrate nitrogen in a selected group of soils was also determined using the phenoldisulfonic acid colorimetric procedure, as described by Bremner (1965).

Nitrite nitrogen was assumed negligible in all these analyses, and not accounted for.

Analysis of the plant and grain samples

Total organic nitrogen, including any ammonium nitrogen present, was determined in both the grain and plant samples using the Macro-Kjeldahl procedure as revised and outlined by Lamborn (1961) for use in the Soils Laboratory at Utah State University.

A description of the method follows:

(1) Apparatus: 800 ml Macro-Kjeldahl flasks and standard Macro-Kjeldahl digestion stand and distillation apparatus.

(2) Reagents:

(a) Concentrated, reagent grade, H_2SO_4

(b) Concentrated NaOH solution, 40-45 percent NaOH by weight

(c) Digestion mixture consisting of 500 g Na_2SO_4, anhydrous powder; 78 g $CuSO_4 \cdot 5H_2O$; 5 g powdered selenium metal

(d) Indicator solution of 350 mg. bromocresol green in 10 ml 95 percent ethanol added to 1 ml of 0.5 N NaOH and 200 ml distilled water. To this mixture is added 22.1 ml of an aqueous 1 percent solution of new Coccine, and then is added 750 mg of p-nitrophenol which has been dissolved in a few ml of 95 percent ethanol. The total mixture is made up to 250 ml, and adjusted to where it will have a light grey color in a pH 4.6 buffer solution, either by

additions of new Coccine or bromocresol green solutions. Use 100 ml of indicator for 18 liters of 2 percent boric acid solution.

(e) Boric acid solution, 2 percent by weight

(f) 0.0715 N standard H_2SO_4 for titrations

(g) Zinc metal, granular, size not critical but 20 mesh is satisfactory.

(3) Procedure: Perform analyses in duplicate. Weigh 1 gram of plant material into Kjeldahl flasks, add a teaspoon of digestion mixture, and soak these contents with distilled water. Add 25 ml concentrated sulfuric acid, and digest. Cool after complete digestion, when solution has been clear about 15 minutes, add 400 ml distilled water. Cool again. Add about a gram of zinc and immediately pour 75 ml of concentrated sodium hydroxide down the side of the flask to allow it to run under the solution. Place on distillation stand and distill 150-200 ml into 50 ml of the boric acid-indicator solution, then titrate. Subtract the value of a blank determination.

Results of the 46 duplicated analyses of the grain samples provided the following information on the reliability of the results: (1) mean difference between duplicates was 0.58 percent protein, (2) standard deviation of this mean difference was 0.06 percent protein, and (3) the standard deviation of the 46 differences between duplicates was 0.39 percent protein.

From these data, it may be seen that the average difference between duplicates that can be expected from this procedure may range from 0.52 to 0.64 percent protein. In the case of this particular analysis, since the mean of two values was used to represent the protein percent

assigned to a plot, a reasonable range of precision for these protein percentages is ± 0.1 to ± 0.5 percent protein, with an average precision of ± 0.3 percent protein.

Raw nitrogen percentages calculated from the data provided by this Macro-Kjeldahl procedure were converted to protein percentages by using conversion factors of 5.7 for grain and of 6.25 for vegetative parts, as recommended by the A.O.A.C. (Horwitz, 1970).

The determination of nitrate nitrogen in the plant material was accomplished using the procedures of Lamborn (1972), as used at the Soils Laboratory at Utah State University.

Briefly, the method consists of taking 0.1 g of the finely ground plant sample and extracting the nitrate from it by using 25 ml of silver sulphate (3.5 g Ag_2SO_4/liter). Extraction is accomplished by shaking for a few minutes, adding a scoop of Darco-G-60 Carbon, shaking a few more minutes, then adding a scoop of calcium hydroxide, shaking again, adding a scoop of magnesium carbonate, shaking once more for a few minutes and then filtering through fluted filter paper. A 10 ml aliquot is then taken to dryness on a steam bath, 3 ml of phenoldisulfonic acid are added and the residue is brought into solution, 25 ml distilled water is added and the solution is allowed to cool. When cool, 15 ml of an NaOH + Edta solution (360 g NaOH + 15 g disodium Edta in 100 ml H_2O) is added and the solution is analyzed colorimetrically at 420 mμ after it has cooled again. Blanks and standards are run to allow conversion of absorption readings into ppm of nitrate nitrogen using a standard curve.

RESULTS AND DISCUSSION

Condition of the field in September

Uniformity of nitrogen distribution before treatment was assessed for the field using the kilograms of nitrogen per hectare data for each depth increment in each plot. This procedure was used to determine ammonium-N (NH_4-N) and nitrate-N (NO_3-N) uniformity at each depth layer and cumulative for all depth levels of the profile. The resulting eighteen distribution charts were analyzed by two-way analysis of variance to determine any significant differences in nitrogen content due to position in the field.

The result, as illustrated in Table 1, was that for NH_4-N there were some high spots in the profile, but the cumulative (0-120 cm) values over the entire field did not vary significantly.

Nitrate-N levels in the field showed a significant difference between the two separated portions of the field. Columns 1 and 2, which were adjacent blocks, were significantly lower in nitrate nitrogen than columns 3 and 4, which are at some distance away. These NO_3-N variations occur at the shallower depths of 3-15 and 15-45 cm, as well as at the cumulative depths including the 0-120 cm profile as also shown in Table 1.

The field at the time of treatment

In eight plots not receiving a fall fertilizer treatment, change from September 19 to October 20 was determined for NH_4-N and for NO_3-N. This change in nitrogen was found to be very predictable given the September levels of NH_4-N and NO_3-N. See Table 2.

Table 1. Differences in HN_4-N and NO_3-N levels in the field before treatments. F-value from two-way analysis of variance were run for each separate depth increment and for each cumulative depth profile

Depth increment in cm	F Values		High or low spots, locations and values for NH_4-N; kg/ha	Depth increment in cm	F Values		High or low spots, locations and values for NO_3-N; kg/ha
	Rows	Columns			Rows	Columns	
0-3	1.19	3.28*	Columns I & II less than III & IV (0 vs 1 averages)	0-3	1.24	<1	None of significance
3-15	<1	1.63	None of significance	3-15	1.04	2.86†	Column II less than other 3 (13 vs 18 average)
15-45	<1	1.36	None of significance	15-45	1.09	2.85†	Columns I & II lower than III & IV (18 vs 26 average)
45-75	<1	4.46#	Column III higher than rest (21 vs about 15 avg)	45-75	<1	<1	None of significance
75-120	<1	<1	None of significance	75-120	<1	1.44	None of significance
0-15	<1	1.79	None of significance	0-15	<1	2.98*	Column II less than other 3 (14 vs 20 avg)
0-45	1	1.74	None of significance	0-45	1.28	4.90**	Columns I & II less than III & IV (35 vs 46 average)
0-75	1.11	1.77	None of significance	0-75	1.02	2.98*	Same as above (55 vs 70 average)

Table 1. Continued

Depth increment in cm	F Values		High or low spots, locations and values for NH_4-N; kg/ha	Depth increment in cm	F Values		High or low spots, locations and values for NO_3-N; kg/ha
	Rows	Columns			Rows	Columns	
0-120	1.35	1.41	None of significance	0-120	1.03	3.67#	Same as above (75 vs 97 average)

**Significant at 1% level.
#Significant at 2.5% level.
*Significant at 5% level.
†Significant at 10% level.

Table 2. Changes in NH_4-N and in NO_3-N from September 19 to October 20, 1972, for control plots by depth increments of 0-3 cm; 3-15 cm; and 15-30 cm. The ability to predict October sample values from September sample values is tested by correlation analysis and given as "r" values. Significant r-value indicates that October samples are predictable from September values

Plot code number	September NH_4^+-N level and the change by October 20; values in kg-N/ha						September NO_3^--N level and the change by October 20; values in kg-N/ha					
	0-3 cm		3-15 cm		15-30cm		0-3 cm		3-15 cm		15-30cm	
IV-D	0	+4	3	+6	21	-19	1	0	18	-9	6	-2
IV-I	0	+1	3	+5	6	+2	1	0	18	-11	12	+1
I-A	0	+2	8	+1	4	+9	1	+8	14	-3	4	+11
I-I	0	+2	6	-1	13	+4	1	-1	28	-25	6	+7
II-G	0	+1	6	-1	27	-14	1	0	14	-5	15	0
II-B	1	+1	6	+6	3	+8	1	0	18	-10	9	+2
III-C	1	+1	2	+13	6	0	1	+1	18	-9	18	-10
III-J	0	+1	2	&7	18	+3	1	0	18	-9	18	-10
	No test possible		r^2 = .5785 r = .7605*		r^2 = .6495 r = .8059#		No test possible		r^2 = .963 r = .981**		r^2 = .651 r = .807#	
	Avg. Sept. Value = 4 Avg. Change = +4				12 -1		Avg. Sept. Value = 18 Avg. Change = 10				10 +2	

**Significant at 1% level.
#Significant at 2% level.
*Significant at 5% level.

This predictability was used to estimate the NH_4 and NO_3-N levels for all plots at the time of treatment, for the cumulative 0-30 cm depth increment. In Table 3 is shown that the regression equations from the eight control plots, which are used as the reference, were more reliable when based on the 0-30 cm values than when the smaller, separate depth increments were used as was shown in Table 2.

From these regression equations, the September to October changes in NH_4-N and NO_3-N levels were calculated for each plot. Since the time span from the September sampling to treatment time was one-third the time span from the September sampling to the October sampling, one-third of the computed changes were taken as a best estimate of changes in the September nitrogen level up to the time of treatment. These estimated changes were algebraically added to the September 0-30 cm cumulative nitrogen level values, and served to estimate NH_4-N and NO_3-N levels at the time of treatment.

The resulting values, in their relative field positions, reveal that at treatment time the field was more nearly homogeneous in NH_4-N and NO_3-N levels, in the 0-30 cm increment, than it was at the Semptember sampling.

It is to be noted, however, that Column 3 was still somewhat higher in NH_4-N than Columns 1, 2, and 4. Also, the nitrate levels of Columns 1 and 2 were slightly lower than those of Columns 3 and 4, thus preserving the patterns found in the September sampling.

Since actual measured variance is now altered by adding smoothing interpolations, analysis of variance cannot realistically be applied to these estimated figures.

A comparison of the row and column averages from the September sampling time with these estimated figures for nitrogen forms at treatment

Table 3. Changes in NH_4-N and NO_3-N from Sept. 19, 1972, to Oct. 20, 1972, in plots not receiving fall fertilizer additions. Cumulative 0-30 cm depth increment. Highly significant correlations indicate that the regression equations describing these two change patterns may be used reliably as a tool for predicting October levels given September levels

Plot	Sept NH_4-N kg/ha; 0-30 cm	Oct NH_4-N kg/ha; 0-30 cm	ΔNH_4-N	Sept NO_3-N kg/ha; 0-30 cm	Oct NO_3-N kg/ha; 0-30 cm	ΔNO_3-N
I-A	12	24	+12	19	26	+7
I-I	19	25	+5	35	16	-19
II-B	10	24	+14	28	19	-9
II-G	34	18	-16	30	25	-5
III-C	9	23	+14	25	22	-3
III-J	20	32	+12	38	19	-19
IV-D	24	15	-9	26	14	-12
IV-I	9	17	+8	30	20	-10

Average level of NH_4-N = 17 kg N/ha in Sept.

Average change of NH_4-N = +5 kg N/ha Sept to Oct.

Coefficient of determination = r^2 = .7873.

Coefficient of regression = r = .887** (significant at 1%).

The NO_3-N = 29 kg-N/ha in Sept.

The NO_3-N = -9 kg-N/ha Sept to Oct.

r^2 = .8169.

r = .9038** (significant at 1%).

time illustrates the apparent increase in homogeneity quite strikingly, as in Table 4.

Table 4. Column averages for NH_4-N and NO_3-N levels compared with the corresponding averages estimated for the time of treatment for untreated plots. The narrowed ranges illustrate an increase of homogeneity with time

	NH_4-N in kg/ha		NO_3-N in kg/ha	
Column	September 19	September 29	September 19	September 29
I	14	17	27	25
II	10	14	24	24
III	20	21	32	27
IV	15	16	34	29

Ranges for column averages:

NH_4-N in kg/ha		NO_3-N in kg/ha	
September 19	September 29	September 19	September 29
10-20 ΔN=10	14-21 ΔN=7	24-34 ΔN=10	24-29 ΔN=5

NH_4-N and NO_3-N transformations after fertilizer placement

Large changes occurred in the NH_4-N and NO_3-N levels from the time of treatment to the time of the October sampling, a space of three weeks.

The soil at placement time was warm and dry at the surface. The 1971-1972 water year at the experimental site had been below normal, with 10 inches for this site.

After placement, only a trace of precipitation fell the first few days; but over half an inch fell by the end of the first week.

During the next week and a half a few small showers fell with a large storm towards the end of the third week which deposited 1.40 inches of rain in two days. It was shortly after this latter storm that the October 20 sampling was made.

Thus, nearly a week of warm dry weather followed by a wetter period, of 2 inches of rain in 2 weeks, characterized the time between treatment and the October sampling.

The first part of this period (moist surface and high evaporation) favored ammonia evolution losses from the surface; the second wetter period favored microbiologically-induced losses and transformations.

Performing analysis of variance on the changes in NH_4-N, NO_3-N, and the total (NH_4+NO_3)-N levels during this period showed NH_4-N transformations to be highly treatment dependent. NO_3-N changes were not significantly treatment dependent, and the total (NH_4+NO_3)-N changes, dominated by the highly dependent NH_4-N changes, were found to be significantly treatment related. The F-test values are tabulated below in Table 5. Field column position effect was tested also, to subtract out the variance due to column effects and to determine if this effect had a significant influence on the rate of mineral nitrogen transformations. Field position of columns did not appear to have a significant effect.

Since there is a significant treatment effect for the NH_4-N and (NH_4+NO_3)-N transformations, individual treatments were statistically evaluated for differences in mean NH_4-N changes. Student's t-test, and Duncan's Multiple Range (DMR) tests were employed, but neither were found to be satisfactory discriminators between treatments involving the same fertilizer source, and the tests lacked sensitivity over the whole range of means generally. For example, only one case was found where the

Table 5. Two way analysis of variance results on changes in kg-N/ha levels from fertilizer placement time, September 29, to the time of the October 20 sampling. Nine fall treatments, each with one replication in each column. Significant F values indicate N-level change is dependent on the tested effect (treatment applied or column position).

NH_4-N changes due to effect of:		NO_3-N changes due to effect of:		(NH_4+NO_3)-N changes due to effect of:	
Treatments	Column position	Treatments	Column position	Treatments	Column position
F = 5.66**	F = 1.00	F = 1.82	F = 0.43	F = 1.98†	F = 1.27

**Significant at 1% level.
† Significant at 10% level.

two application modes for the same source yielded significantly different mean results for the NH_4-N level changes.

A valid distribution-free statistical test for this experimental data is Wilcoxon's Rank-sum Test. The tables given for this test list the critical values as that value which has its cumulative probability smaller than the listed significance level, so that the usual parametric-test significance levels may be used and a convenient comparison may be made at, say, the 5 percent significance level, instead of the true significance level of 2.8 percent.

The following small table lists the true significance levels for the critical values of a two-tailed four element by four element rank-test comparison.

Table 6. Computed significance levels for Wilcoxon's Rank Sum Test comparing two treatments with four values each. Critical values given for lower tail of a two tailed test.

α = true significance level:	0.028	0.057	0.114
for a value $\leq$ critical value:	10	11	13

An application of this test to the foregoing nitrogen-level change data shows its sensitivity. The resulting significance levels for differences in treatments using the same fertilizer source is reported in Table 7.

Table 7. Wilcoxon's Rank Sum Test applied to treatments involving the same source. Two-tailed test. Changes in NH_4-N from time of treatment until the October sampling date. Reported in kg-N/ha. Four replications in each mean.

Source	Treatment and change in mean NH_4-N level			Significance level
	with seed	before seeding	after seeding	
NH_4NO_3	+14	+9		No significant difference*
NH_4NO_3	+14		+24	α = 0.114
NH_4NO_3		+9	+24	No significant difference*
$(NH_4)_2SO_4$		-29	+31	α = 0.057
Urea		-42	-24	No significant difference*
$Ca(NO_3)_2$		+2	+15	α = 0.114

*α-levels above 0.114 considered non-significant.

Characterization of treatments

Four treatment parameters have been experimentally determined or estimated from the raw data, namely:

1. Nitrogen levels found in October.
2. Nitrogen levels at the time of, and including, treatment.
3. The differences between these two, or the transformations which were found to be treatment-dependent in the above section.
4. Grain yields and protein contents.

To test the representativeness of these parameters, and to estimate their relative significance, the three nitrogen level parameters were correlated with corresponding grain-yield values.

Two such correlations were computed. The first, a plot by plot comparison of each parameter with yields, using only (NH_4+NO_3)-N figures. The second, taking treatment averages of each parameter only, but additionally, testing the NH_4-N and NO_3-N components separately for their contribution to yield.

The results, reported in Table 8, show the tremendous effects of the plot-to-plot variances in the first correlation as compared with the second correlation using averaged values. On the treatment average correlations, the treatment-time nitrogen levels prove to be a poor determinant of yield in comparison to the October nitrogen levels, suggesting that nitrogen losses between treatment time and October sampling time were rather permanent. The treatment-time to October sampling-time changes, found to be treatment related in the previous section, seem to be unrelated to final grain yields, suggesting that transformations which removed nitrogen from the water-soluble mineral

Table 8. Correlations between three fall-nitrogen related parameters and grain yields, plot by plot for the (NH_4-N + NO_3-N) parameter values, and by treatment averages for the separate NH_4-N, NO_3-N; and (NH_4 + NO_3)-N contributions.

A. Individual plot correlations: 40 data pairs, 38 degrees of freedom

Parameter	Coefficient of determination	Significance level
1. (NH_4 + NO_3)-N level after treatment	r^2 = .1155	Significant at 5% level
2. (NH_4 + NO_3)-N level at October sampling	r^2 = .2296	Significant at 1% level
3. (NH_4 + NO_3)-N change between (1) and (2)	r^2 = .0645	Not significant

B. Treatment average correlations: 10 data pairs, 8 degrees of freedom

Parameter	Coefficient of determination	Significance level
1. NH_4-N level after treatment	r^2 = .0584	Not significant
2. NO_3-N level after treatment	r^2 = .1334	Not significant
3. (NH_4 + NO_3)-N level after treatment	r^2 = .6931	Significant at 1% level
4. NH_4-N level at October sampling	r^2 = .5037	Significant at 5% level
5. NO_3-N level at October sampling	r^2 = .0737	Not significant
6. (NH_4 + NO_3)-N level at October sampling	r^2 = .8345	Significant at 1% level
7. NH_4-N level change from treatment to October 20	r^2 = .0450	Not significant
8. NO_3-N level change from treatment to October 20	r^2 = .0534	Not significant
9. NH_4 + NO_3-N level change from treatment to Oct. 20	r^2 = .0171	Not significant

nitrogen pool, as measured in this experiment, did not render this nitrogen unavailable to the plant for the remainder of the growing season in all cases.

The April sampling

The work so far described sets the stage for the analysis of the April nitrogen data and the grain-yield data. These data show the importance of the field-column effects referred to in both the September and October field evaluations above. For example, analysis of variance on the grain-yield data evaluating the importance of treatment effects and the field column effects resulted in a treatment F-test value which is significant at the 0.5 percent level, but also a field-column effect F-test value significant at the 1 percent level. Column 1 gave yields about 20 percent lower than the rest of the field. That this may be a reflection of water-status differences in the experimental field is strongly suggested by a close look at surface nitrogen levels in the April sampling as they compare with yields at harvest.

Table 9 describes the differences in nitrogen status versus yields in the field columns.

Using the Student's "t" statistic, the nitrogen levels are not found to be significantly different, but the difference between the grain yield of Column I compared with the other three is different at the 95 percent confidence (5 percent significance) level.

Note the lack of correlation between the surface nitrogen levels in Column I as compared to the others, suggesting water-stress as the limiting factor in Column I, at least to a greater extent than in the other columns since all had similar nitrogen contents. Column I was

Table 9. Nitrogen levels for the April 28 sampling in the 0-15 cm surface, correlated with grain-yields, showing columnar differences in nitrogen-yield relations

Column	Average nitrogen level ± the standard error of the mean 0-15 cm $(NH_4 + NO_3)$-N; kg/ha	Average grain yield in kg/ha ± the standard error of the mean	Correlation results: 11 plots per column
I	19 ± 4	1593 ± 106	$R^2 = 0.010$
II	28 ± 7	2036 ± 106	$R^2 = 0.687$**
III	21 ± 6	2038 ± 132	$R^2 = 0.494^{\#}$
IV	21 ± 5	1954 ± 86	$R^2 = 0.228$

**Significant at 1% level.
#Significant at 2% level.

observed to be shallower to restricting layers (cemented parent material) than the others during the samplings, which could be responsible for the water problem in terms of reduced storage. Some plots in this column were shallower than 75 cm to a hardened, calcareous substratum (especially plot I-k).

Further analysis of the April sampling data involves comparisons between this data and the data from the other samplings which appear in a separate section, below.

The July sampling

On July 31, 1973 spot checks of a limited number of plots were made just a week before harvest for the purpose of determining whether all the available nitrogen had been extracted by the growing crop.

The data from this limited sampling revealed no statistically significant differences between the nitrogen levels of the plots sampled, except in the case of one plot where urea was applied in spring. This data proved surprisingly illuminating, however, when comparisons were made between the July and April nitrogen levels, and then compared to the yields in these plots.

For example, correlation between 0-120 cm mineral nitrogen depletion from April to July and the plot yields gave an $r^2 = 0.787$ for nine of the eleven samples taken, which is significant at the 1 percent level.

Regression analysis comparing the 0-120 cm April mineral nitrogen levels with corresponding yields for these same nine plots yielded an r^2 of 0.460, significant at the 5 percent level for this sample size. A similar correlation for the 0-30 cm October mineral nitrogen levels for these plots compared to their respective yields produced an insignificant r^2 of 0.280. This shows that this sampling is representative of the population from which it was drawn, which population as a whole gave an r^2 of 0.23 when October mineral nitrogen was correlated with yield for all 44 plots.

The July sampling being thus representative of the whole field allows a generalization of the finding that nearly 80 percent of the yield variability was a function of nitrogen availability between April and maturity. It may be further hypothesized, that the mineral nitrogen content of the soil in April could have been just as effective a yield predictor as the April-to-maturity depletions, if water could have been adequate over this period of time. Differential drying in the field, causing nitrogen uptake cessation in different locations at

different times, may, therefore, have been responsible for the 30 percent yield variability which was lost when April mineral nitrogen levels were considered without post-maturity nitrogen levels.

The results of a small experiment conducted at the time the crop approached maturity lends credence to the assertion that drying, and subsequent cessation of nitrogen uptake, varied with location in the field. On June 6, 1973 a small sampling of wheat plants was made down the center of the field, taking two samples from each of Columns II, III, and V. These samples were analyzed for nitrate nitrogen, and organic + ammonium nitrogen, so that total nitrogen was known at this time for the leaves and stems. The results of this experiment are tabulated and discussed in Table 10.

Further analysis of the data in Table 10 reveals that there is high correlation ($r^2 = 0.73$, significant at the 5 percent level) between total nitrogen level and grain yield, while there is a negligible relationship between the organic nitrogen level and the grain yield. A conclusion to be drawn from this, based upon the hypotheses of McNeal (1968), is that even though nitrogen uptake had markedly slowed (in all except one case), and even though photosynthesis had almost ceased, in all except this one case in which there was still green pigment in the leaves and stems, inorganic nitrogen was still used in organic synthesis and being translocated to the grain. If the magnitudes of the other r^2 values are meaningful, it may be inferred from them that at the dough stage of growth the nitrogen in the leaves and stems was used more in the grain structural materials than in the grain protein fraction. This is characteristic of intraplant nitrogen

Table 10. Nitrogen levels in some of the wheat plants at the onset of maturity. Diversity in values for nitrates may indicate variability in the time of the onset of maturity, and corresponding cessation of nitrogen uptake, presumably due largely to differential drying patterns in the field. The amount of organic nitrogen may be taken as an indication of the biological activity of the plant.

Field location	Treatment received	Comments	ppm NO_3^--N in leaves and stems	ppm organic + NH_4^+-N in leaves and stems	ppm total-N in leaves and stems	Rank of yields in these 6 plots
North of III-A	None	Stunted (taken from buffer zone between road and field plots)	2	56	58	6
III-A	$(NH_4)_2SO_4$ after planting	Normal	29	140	170	4
V-C	NH_4NO_3 before planting	Normal	28	77	105	3
II-C	Urea after planting	Normal	16	97	113	5
V-E	Urea in spring	Normal	12	146	158	1
II-F	NH_4NO_3 before planting	Only sample taken where plants in the plot were still green in spots	77	87	164	2
Correlation with grain yields (expressed as r^2) =			0.30	0.40	0.73*	
Correlation with protein yields (expressed as r^2) =			0.25	0.32	0.20	

*significant at 5% level.

translocations in the post-dough to ripe stages of growth as described by McNeal (1968).

Grain yield, protein, and moisture-data analysis

Grain yields, in kg-grain/ha were obtained for each soil sampling depth increment in each plot. Two-way analysis of variance revealed treatment effects to be significant at the 0.5 percent level, but, in addition, field column position effects were significant at the 1 percent level. Column I, as previously shown (Table 9), yielded about 20 percent lower than the other three columns. Repeating this analysis without Column 1, in other words on a more uniform field but with only three replications, the column effect was reduced to insignificance, but the treatment effect now could only be considered significant at the 10 percent level. Further analyses of variance, not using Column I, revealed no significant effects due to treatment if only the treatments involving fall applications were used. If this analysis is repeated for fall treatments only, but for all four field column replications, treatment effects are noted significant at the 5 percent level and field column effects register significance at the 10 percent level. Since these subsets were tested after an examination of the data, they may be statistically illegal, but they do serve to point out the thorough confounding of treatment with soil heterogeneity effects.

Protein percentage of the grain for each plot was also analyzed by two-way analysis of variance, with the result that treatment effects were significant at the 2.5 percent level of significance, and field column position effects were non-significant, registering an F-test value at the

25 percent level of significance. Of interest is the fact that the highest yielding field column had the lowest grain protein percentage (Column III). The other three columns, although exhibiting real differences in grain yield, showed no differences in protein content of the grain. If interpreted by the observations of Hutcheon and Paul (1966), this finding would be suggestive of a higher available moisture level in Column III, causing a slight increase in vegetative development at the expense of protein synthesis. From the same standpoint, the nondiffering yields of the control, and the urea after seeding treatment seems to indicate a similar impediment to growth in both cases, namely moisture stress. But when protein percent is compared for these two treatments, it becomes clear that urea after seeding treatment was superior in the providing of nitrogen to the native nitrogen supplying ability of the soil. Even with the same grain yields, the urea after seeding treatment yielded 25 percent more protein, in kg of the protein per hectare, than did the control plot. This is in accord with the observation of Nielson (1955) that even if, in a dry year, yield responses from nitrogen fertilizer are lacking, the increase in the protein yields may well pay for fertilization costs anyway. The chances for a good return from added fertilizer even in a marginal year seem good.

Two-way analysis of variance was run on protein yields, which were obtained by multiplying the grain yield by the protein percent for each experimental plot. Since the protein percent did not change proportionally with the changes in grain yield (correlation coefficient for mean treatment values of grain yield and protein content, $r = 0.0000$), in either direction, these protein yields should reflect much the same treatment and field factor dependencies as the grain yields. F-test

results bore this out, with a 5 percent significance level for treatment effects, and a 10 percent significance level for field column effects.

The lowered significance of the treatment effects, and the higher significance of the field position effects, for these protein yields as compared to the grain yields, again tend to confirm the presence of water stress since under mild water stress nitrogen use for protein synthesis is favored over vegetative production, while under more severe water stress both the vegetative and protein synthesis are depressed. Therefore, nitrogen availability differences due to treatment become moderated, when water and not nitrogen becomes limiting. A corresponding nitrogen availability difference due to field location would become exaggerated because any stressed plots would tend to be concentrated in certain locations in the field.

A summary of the data on which the foregoing discussions are based appears in Table 11, including the results of using Duncan's Multiple Range test to find significantly different subsets for each of the three parameters.

Further analysis on this water stress factor involved a moisture determination on the grain from each plot. It was hoped that the observations of Steward and Hirst (1912) that grain moisture content differs depending on available water, could be quantitatively appraised. This hope was somewhat beclouded by the observation of Dondlinger (1912), that two days of grain exposure to the atmosphere equalized the moisture content of grains formerly ranging from 8 to 13 percent grain moisture. Nevertheless, grain samples from each plot at harvest were sealed in plastic bags and kept sealed until weighing for the moisture determination. Analysis of variance on these moisture percentages for each

Table 11. Grain yield, grain protein percent, and protein yield means with the standard errors of their means for each treatment. Non-significant differences in means calculated using Duncan's Multiple Range Test at the 5 percent level of significance, and these in-distinguishable subsets of means indicated by matching letters: i.e., all A treatment means are not significantly different at the 5 percent significance level, etc. Protein yield calculated by multiplying grain yield by protein content for each plot.

Treatment	Subsets of statistically indistinguishable means at the 5 percent significance level.[a] Grain yield[b]	Protein %[c]	Protein Yield[d]	Mean grain yields, ± standard error of the mean; kg/ha	Mean grain protein contents, ± stand. error of mean, %	Mean protein yield ± stand. error of mean, kg/ha
Control	D	C	C	1531 ± 109	8.8 ± 0.2	136 ± 13
NH_4NO_3, with seed	BCD	A	AB	1839 ± 135	11.4 ± 0.6	208 ± 16
NH_4NO_3, before seeding	ABC	AB	A	2175 ± 91	10.9 ± 0.3	238 ± 13
NH_4NO_3, after seeding	BC	BC	AB	2001 ± 162	10.0 ± 0.2	200 ± 15
NH_4NO_3, in Spring	A	BC	A	2423 ± 137	10.0 ± 0.4	243 ± 17
$(NH_4)_2SO_4$, before seeding	BCD	BC	BC	1887 ± 230	9.5 ± 0.4	177 ± 18
$(NH_4)_2SO_4$, after seeding	AB	BC	AB	2192 ± 150	9.9 ± 0.5	215 ± 10
$Ca(NO_3)_2$, before seeding	BCD	AB	AB	1872 ± 196	10.4 ± 0.5	195 ± 20
$Ca(NO_3)_2$, after seeding	CD	BC	BC	1760 ± 159	10.0 ± 0.4	177 ± 20
Urea, before seeding	BCD	BC	BC	1870 ± 134	9.5 ± 0.2	177 ± 11
Urea, after seeding	D	A	BC	1501 ± 273	11.3 ± 1.0	170 ± 38

[a] A good estimate of the least significant range (LSR) for all these tests is three times the standard error of the mean.
[b] Mean LSR = 421
[c] Mean LSR = 1.5
[d] Mean LSR = 55

block showed the field column effects to be highly significant at the 0.5 percent level.

The field column moisture, protein, protein yield, and grain yield figures are given in Table 12.

Table 12. Summary of field-column position effect due to water stress on the grain yield, grain protein, protein yield, and grain moisture content

Field column	Mean grain yield ± SD of mean (a)	Mean protein percent ± SD of mean (b)	Mean protein yield ± SD of mean (c)	Mean moisture % ± SD of mean (d)
I	1593 ± 106	10.3 ± 0.3	164 ± 12	11.9 ± 0.1
II	2036 ± 106	10.5 ± 0.4	214 ± 13	12.0 ± 0.2
III	2038 ± 132	9.7 ± 0.3	198 ± 16	12.8 ± 0.1
IV	1954 ± 86	10.1 ± 0.4	197 ± 13	12.3 ± 0.1

[a]LSR = 321 at 5% level of significance.
[b]LSR = 1.0 at 5% level of significance.
[c]LSR = 45 at 5% level of significance.
[d]LSR = 0.4 at 5% level of significance.

From the least significant differences, it may be seen that, at the 5 percent level of significance, the only real differences in these figures are (1) the lowest grain yield and the lowest protein yield are in field Column I, and (2) the highest grain moisture was found in Column III. Three-way correlation of grain moisture with yield and protein for the four field columns gave an r of 0.993 at 2 degrees of freedom, which is significant at the 1 percent level, lending greater credence to the water stress hypothesis than is seemingly afforded by just Table 12. Correlation comparing grain moisture with grain yield done inside each block separately for the eleven treatments revealed

a significant (at the 5 percent level) relationship between grain moisture and grain yield only in Column II, with Column I being the least correlated and III and IV being intermediate but still below any useful probability levels. Repeating this experiment for three-way correlation of grain moisture with grain yield and protein, gave a similar result with the effects in Column II being correlated at the 1 percent significance level with $r = 0.866$, and Column I being the lowest at $r = 0.35$, and the other two columns (III and IV) both with r's around 0.40.

The inference to be drawn from these results is that Column II had the most favorable water regime, not especially meaning the most available water, but rather meaning that the timing of the water availability was such that the efficiency of nitrogen and moisture use was higher than in the surrounding field columns.

The role of moisture is thus quite complicated. Column I had lowest yields, and since the plots were found to be relatively shallow to cemented parent material, water stress was thought to be the obvious problem here. Columns II and III yielded equally, but in Column II the grain protein content is higher while the grain moisture content is lower, and the relationship is quite linear between grain moisture, yield, and protein content. Column III has significantly higher grain moisture, and its protein content is the lowest of the four columns, suggesting that field water stress was less here than in other columns and nitrogen use efficiency was down somewhat. And Column IV, with less grain yield than Column III, yielded the same in protein per hectare, showing maybe a little less available moisture during some segment of the growing season with an accompanying increase in nitrogen use efficiency.

It must be kept in mind that these observations on the role of available moisture are hypothetical and not supported by quantitative assessments of moisture in the field, qualitatively they are well supported from the results of the regressions obtained in the above noted correlation analyses.

Mineral nitrogen in the soil profile at the different sampling times

In view of the fact that the experimental field, throughout this investigation, has exhibited some significant heterogeneity, it would be profitable to assess the mineral nitrogen levels at the different sampling times to see if heterogeneities found in the September sampling reappeared some time after the treatments. If so, these differences must then be characteristic of the soils in these local areas of significantly higher or lower mineral nitrogen levels. On the other hand, if no patterns can be established, it may be assumed that different rates of microbial nitrogen cycling within this field, or some locally differing physical properties of the field soil, are not responsible for these high and low mineral nitrogen levels as found in the September sampling of the field. If this happens to be the case, then these September differences are due to transitory effects instead, arising from the recent history of the field, which would naturally change in both magnitude and location with each cropping season.

The October sampling nitrogen levels were dominated by the treatment additions, and the samples without fall treatments have been shown to have changed quite predictably from the September levels toward a more homogeneous common level (in the section describing the field at the time of treatment, above).

The surface to 30 cm depth increment in the April sampling still shows some treatment effects, especially the ammonium nitrate in spring and the two ammonium sulfate treatments in the fall. No significant differences were found outside of these three treatments, and no correlations were significant between September, October or April nitrogen levels for the other eight treatments. The treatment averages for ammonium and nitrate nitrogen levels at the surface to 30 cm depth increment are given for the three main sampling times in Tables 13 and 14.

The September and April 30-120 cm cumulative mineral nitrogen levels were compared plot by plot in an analysis of covariance. These deeper nitrogen levels in April were found to be independent of the corresponding September levels, suggesting that the September mineral nitrogen distribution patterns were not reflections of permanent features of the field structure, but rather reflected the field's recent history only.

It must be noted that when analysis of covariance was run on the 0-30 cm mineral nitrogen levels of September and April, a slight dependency of the April ammonium level on the September ammonium level was noted (at the 5 percent significance level). Reduced analysis of variance on the field column effect in this dependence showed no significant contribution from field column position. Nitrate levels were not found to be dependent using the same test. The explanation for this ammonium nitrogen dependency is simply that little change in ammonium nitrogen levels occurred between September and April, while nitrate nitrogen levels were more highly variable.

Table 13. Treatment average NH_4-N levels at the three main sampling times: Sept. 19, 1972; Oct. 20, 1972; and Apr. 28, 1973. Cumulative N values to 30 cm depth, given in kg-N/ha with standard error of the mean based on a four-plot average

Treatment	September		October		April*	
	NH_4-N kg-N/ha	S.D.$_{\bar{x}}$ kg-N/ha	NH_4-N kg-N/ha	S.D.$_{\bar{x}}$ kg-N/ha	NH_4-N kg-N/ha	S.D.$_{\bar{x}}$ kg-N/ha
Control	16	6	20	2	11	3
NH_4NO_3, with seed	11	2	43	2	12	4
NH_4NO_3, before seeding	18	6	46	5	15	4
NH_4NO_3, after seeding	12	2	55	6	17	6
NH_4NO_3, in Spring	18	3	24	3	29	3
$(NH_4)_2SO_4$, before seeding	16	8	52	6	26	7
$(NH_4)_2SO_4$, after seeding	8	3	100	23	21	7
$Ca(NO_3)_2$, before seeding	14	2	18	2	14	3
$Ca(NO_3)_2$, after seeding	10	5	22	2	12	4
Urea, before seeding	21	9	33	6	14	4
Urea, after seeding	15	4	48	10	17	6

*4 kg NH_4-N/ha for the 0-30 cm depth is the threshold value of the method used; below 4 kg NH_4-N/ha, the method cannot discriminate.

Table 14. Treatment average NO_3-N levels at the three main sampling times: Sept. 19, 1972; Oct. 20, 1972; and Apr. 28, 1973. Cumulative N values to 30 cm depth, given in kg-N/ha with standard error of the mean based on a four-plot average

Treatment	September		October		April	
	NO_3-N kg-N/ha	S.D.$_{\bar{x}}$ kg-N/ha	NO_3-N kg-N/ha	S.D.$_{\bar{x}}$ kg-N/ha	NO_3-N* kg-N/ha	S.D.$_{\bar{x}}$ kg-N/ha
Control	26	3	23	1	9	1
NH_4NO_3, with seed	26	3	54	2	18	9
NH_4NO_3, before seeding	28	3	66	12	12	2
NH_4NO_3, after seeding	28	7	44	4	14	3
NH_4NO_3, in Spring	27	5	17	1	29	9
$(NH_4)_2SO_4$, before seeding	25	5	28	3	17	5
$(NH_4)_2SO_4$, after seeding	21	5	24	5	16	3
$Ca(NO_3)_2$, before seeding	29	10	60	7	8	0
$Ca(NO_3)_2$, after seeding	20	4	58	13	10	1
Urea, before seeding	25	5	31	4	12	1
Urea, after seeding	28	7	21	4	13	5

*8 kg NO_3-N/ha for the 0-30 cm depth is the threshold value of the method used; below 8 kg NO_3-N/ha, the method cannot discriminate.

Decreases in soil mineral nitrogen content reflect the inability of the soil nitrogen immobilization-mineralization cycle to supply the demands of the crop plants to some degree. Even a total lack of available mineral nitrogen cannot be taken as a fool-proof indication of severe nitrogen stress if soil moisture and temperature are conducive to further mineralization and mobilization of soil nitrogen. On the other hand, a significant amount of mineral nitrogen in the soil is no sure indication of it availability to the crop unless moisture is present also. Because of these complications, regressions of the differences in mineral nitrogen levels between the September and April samplings at all depths for all plots with fall treatments compared with plot yields as an indicator of plant nitrogen demands, failed to produce any significant correlation coefficients in almost every instance. The correlation coefficient for the 30-120 cm depth mineral nitrogen depletions, for 39 plots compared with their yields was $r = 0.016$, and for the whole 0-120 cm, nitrogen depletions from September to April run only on the nine treatment means with fall treatments versus treatment-averaged yields gave an $r = 0.236$. Three exceptions to these non-significant regression findings are discussed below.

Some interesting results came from this data. The data in Table 15 serves to introduce these results. This data is especially useful since these differences in the nitrogen levels take into account not only the October and March treatment effects, but also the initial September mineral nitrogen level effects are considered.

Because of the extremely large variances, statistical analysis of these means is not useful since only the largest differences would be significant and these can be picked out easily from a cursory examination

Table 15. Treatment and field column average differences in the soil mineral nitrogen contents between the September and April samplings. Data given for NH_4^+-N, NO_3^--N, and combined (NH_4^+-N + NO_3^--N) in kg-N/ha. Negative signs indicate net depletion from September to April, positive signs indicate gains. The standard deviations of the eleven column or four treatment results are given to indicate the large variations in the magnitudes of the NH_4^+ and NO_3^--N changes within the treatments. Note large field column effects.

Treatment or Field column	0-30 cm Mean nitrogen changes (and standard deviations)			30-120 cm Mean nitrogen changes (and stardard deviations)			0-120 cm Mean nitrogen changes		
	NH_4^+-N	NO_3^--N	Both	NH_4^+-N	NO_3^--N	Both	NH_4^+-N	NO_3^--N	Both
	kg-N/ha			kg-N/ha			kg-N/ha		
Control	-5 (9)	-17 (6)	-22	-14 (10)	-18 (15)	-32	-19	-35	-54
NH_4NO_3 with seed	+1 (6)	-8 (24)	-7	-8 (21)	-20 (25)	-28	-7	-28	-35
NH_4NO_3 before planting	-2 (9)	-16 (9)	-18	-22 (42)	-32 (25)	-54	-24	-48	-72
NH_4NO_3 after planting	+5 (13)	-14 (17)	-9	-33 (28)	-20 (19)	-53	-28	-34	-62
NH_4NO_3 in spring	+10 (5)	+2 (17)	+12	-38 (30)	-28 (15)	-66	-28	-26	-54
$(NH_4)_2SO_4$ before planting	+10 (7)	-8 (7)	+2	-41 (49)	-31 (27)	-72	-31	-39	-70
$(NH_4)_2SO_4$ after planting	+12 (9)	-5 (6)	+7	-15 (7)	-6 (50)	-21	-3	-11	-14
$Ca(NO_3)_2$ before planting	0 (5)	-20 (19)	-20	-22 (17)	-9 (18)	-31	-22	-29	-51
$Ca(NO_3)_2$ after planting	+2 (15)	-10 (6)	-8	-30 (20)	-40 (30)	-70	-28	-50	-78
Urea before planting	-7 (24)	-13 (8)	-20	-34 (49)	-27 (21)	-61	-41	-40	-81
Urea after planting	+2 (12)	-15 (16)	-13	-22 (19)	-18 (20)	-40	-20	-33	-53
I	0 (7)	-13 (15)	-13	-20 (28)	-13 (22)	-33	-20	-26	-46
II	+3 (8)	-9 (12)	-6	-20 (20)	-12 (33)	-32	-17	-21	-38
III	-5 (13)	-17 (8)	-23	-38 (40)	-31 (19)	-69	-43	-48	-91
IV	0 (9)	-20 (13)	-20	-23 (18)	-33 (17)	-56	-23	-53	-76

of this table. For example, treatment effects that are noticeable include the three treatments that gained mineral nitrogen in the 0-30 cm depth (1) NH_4NO_3 in spring, (2) $(NH_4)_2SO_4$ before seeding and (3) $(NH_4)_2SO_4$ after planting. In the first two cases, the mineral nitrogen taken out at the lower depth made up for this net gain in the higher soil depth. But in the case of $(NH_4)_2SO_4$ applied after planting, the treatment with the highest mean yield for all the fall nitrogen applications, there was definitely less difference between the September and April mineral nitrogen levels than is found for any other treatment. A case study was made of this treatment by incremental analysis of the mineral nitrogen levels at each sampling.

The overall pattern for this treatment seems to be large, shallow accumulations of ammonium nitrogen for a few weeks after broadcasting the ammonium sulfate. The large increases in the 0-45 cm depth seem out of proportion to the 56 kg-N/ha added. The replications vary from an increase of 27 kg-N/ha to an increase of 173 kg-N/ha, which is hard to explain except in vague terms including error, and the pooling effect as described by Jansson (1971). It should be noted that the III-A replication at the 3-15 cm depth was rerun twice, so that the 128 kg-NH_4^+-N/ha figure appearing in the figure is an average of three analyses. Such high increases from ammonium nitrogen additions seem to be substantiated by the April sampling finding that for this treatment's replication II-E, there was 96 kg NO_3^--N/ha in the 75-120 cm depth increment. The low increase noted for the first replication, I-J, and its subsequent lower yield, may be due to some unaccounted for surface phenomena. The fact that this replication had one of the higher mineral nitrogen contents of the field in September shows that the plot is not incapable of storing

mineral nitrogen, but it should be recalled from the section discussing the changes in the field mineral nitrogen content from the September sampling to the time of treatment (page 56) that plots high in mineral nitrogen in September would lose some of this mineral nitrogen by the time of treatment. If the regression equation used in Table 3 (page 56) is applied to compute the nitrogen level at the time of treatment, then a net increase of 44 kg-N/ha is noted at the October sampling time, which indicates only a 27 percent loss of ammonium sulfate. This is still no explanation of why this is the only replication that failed to gain mineral nitrogen above the added level in this treatment.

Having analyzed this $(NH_4)_2SO_4$ after-planting treatment in some detail, two outstanding features were found which should be assessed in each of the other fall treatments (1) accumulation of the added fertilizer nitrogen in the surface layers of the soil, and (2) the amount of the available mineral nitrogen three weeks after treatment as a fraction of the amount of nitrogen added.

A treatment by treatment detailing follows in Table 16 in which the net changes in the mineral nitrogen levels from the time of treatment to three weeks after treatment are shown by treatment averages and by field column and grand field averages.

The treatment averages show a general trend toward higher nitrogen levels from NH_4NO_3 and $(NH_4)_2SO_4$ treatments than from urea and $Ca(NO_3)_2$ treatments. There also seems to be a favoring of after-seeding treatments in the cases of ammonium sulfate and urea, possibly due to more concentrated shallow NH_4^+-N accumulations.

Table 16. Increases due to nitrogen fertilizer additions measured for the 0-30 cm depth increment three weeks after treatment. Plot mineral nitrogen contents at the time of treatment estimated from September measurements and known rates of change (see p.). All average increases listed as a percentage of the amount of added material, followed by their standard deviations to show plot to plot variabilities

Treatment or Field column	Mean percent changes from added nitrogen levels followed by their individual plot by plot standard deviations						Mean grain yields given in kg/ha
	ΔNH_4	S.D.	ΔNO_3	S.D.	$\Delta(NH_4+NO_3)$	S.D.	
NH_4NO_3 with seed	+64	25	+100	46	+82	36	1839
NH_4NO_3 before planting	+86	36	+161	82	+125	48	2175
NH_4NO_3 after planting	+121	36	+89	39	+107	32	2000
$(NH_4)_2SO_4$ before planting	+54	21	(net increase)		+66	21	1886
$(NH_4)_2SO_4$ after planting	+138	84	(net increase)		+159	91	2193
$Ca(NO_3)_2$ before planting	(net decrease)		+71	27	+64	29	1872
$Ca(NO_3)_2$ after planting	(net decrease)		+64	45	+64	39	1760
Urea before planting	+20	23	(net increase)		+39	34	1870
Urea after planting	+48	38	(net increase)		+62	48	1501
I	+59	59	+105	100	+75	38	1627
II	+65	59	+73	114	+68	52	2036
III	+109	130	+132	77	+118	71	2038
IV	+53	71	+127	55	+80	43	1953
Whole field	+71	82	+109	91	+86	61	1914

These conclusions are supported by a regression test result of R = 0.774 (significant at the 2 percent level) when the nine treatment total mineral nitrogen accumulations were compared with their mean grain yields. The test suggested that this initial behavior of the fertilizer material accounts for roughly 60 percent of the 83 percent of the variability which can be predicted by the total mineral nitrogen level to this same depth at this point in time, run on these same treatment averages.

The question of the importance of surface accumulations of nitrogen at the time of the October sampling, as suggested by the results of the $(NH_4)_2SO_4$ after-planting treatment characterization will now be treated in some greater detail.

Using the nineteen plots with the highest 0-3 cm mineral nitrogen levels in April, and correlating these levels with the plot grain yields resulted in a highly significant correlation coefficient of r = 0.573 (significant at the 2 percent level). Using the 0-15 cm cumulative nitrogen levels for these same samples, being the addition of the 3-15 cm nitrogen contents to the 0-3 cm contents used above, the correlation coefficient falls to r = 0.339 (not significant).

Doing another regression analysis on the nine highest April 0-3 cm nitrogen level plots versus their yields, however, produced a correlation coefficient of r = 0.282, which is insignificant. These findings illustrate the general nature of the trend illustrated that the surface accumulations of mineral nitrogen (not within reach of the plant roots on dry land) at the beginning or middle of the season will tend to raise yields. What needs to be added is, that if there is no significant down ward movement toward the peak demand time of the crop plants, no yield

response will be seen, as may be interpreted from the poor correlation using only the nine highest April treatments. These great surface accumulations, for the fall treatments, anyway, could be due to a locally greater clay sorption, meaning that the release is very slow and may not be great enough to supply plant needs.

A breakdown of these nineteen out of forty-four highest April surface samples follows by treatments:

1. Ammonium sulfate after planting, all four plots
2. Ammonium sulfate before planting, two plots
3. Ammonium nitrate in spring, three plots
4. Ammonium nitrate after planting, three plots
5. Ammonium nitrate before planting, two plots
6. Ammonium nitrate with seed, two plots
7. Urea before planting, two plots
8. Urea after planting, one plot.

Conspicuously absent are the nitrate treatments, and extra prominent are the ammonium sulfate and ammonium nitrate treatments, each with 75 percent of their plots represented.

SUMMARY AND CONCLUSIONS

Patterns of mineral nitrogen distribution were investigated before and after fertilizer additions.

In the fall, before seeding and fertilizer treatment, nitrate nitrogen levels in the field were double the ammonium levels, with a grand mean of 85 kg/ha of NO_3-N and of 42 kg/ha of NH_4^+-N in the total measured profile of 0-120 cm.

Three weeks after time of treatment, the plots were sampled again and, even though all plots received the same (56 kg-N/ha) amounts of fertilizer nitrogen, highly variable differences in the 0-30 cm mineral nitrogen content were found both between and within treatments.

To subtract from these post-treatment mineral nitrogen levels any variability contributed by pre-treatment mineral nitrogen levels, an estimate was made of the treatment-time mineral nitrogen levels for each plot. This estimate was based on the linear relationship of pre-treatment to post-treatment mineral nitrogen levels as determined on eight plots not receiving a fall treatment. One-third of this 30-day nitrogen level change relationship was taken as a best estimate of the ten-day nitrogen level change between the September sampling and treatment time. To this best estimate was added the amount of nitrogen contributed by the treatment for each plot, and this composite figure served as a treatment time nitrogen-level index in the following analyses.

The unadjusted differences between this treatment-time mineral nitrogen-level and the experimentally determined post-treatment time mineral nitrogen level for each plot was computed, and this change was

averaged for each of the four plots within each treatment as a possible index to treatment effectiveness.

It was statistically determined that the after-seeding treatment using ammonium nitrate had significantly more mineral nitrogen in its 0-30 cm profile than did the same source when applied with the seed. Similarly, ammonium sulfate post-treatment nitrogen levels were significantly higher from treatment after seeding than from treatment before seeding.

The highest loss of added mineral nitrogen was that of the urea before planting treatment, where only about 41 percent of the added mineral nitrogen was recovered. In contrast, the ammonium sulfate after planting treatment gained mineral nitrogen over this same period, resulting in a 160 percent recovery. Two other treatments, both using ammonium nitrate, registered increases over this period, also resulting in recoveries greater than 100 percent. Correlation of these treatments averaged percent recoveries with yields for each treatment shows that about 60 percent of the yield variability may be attributable to this initial fertilizer behavior (see Figure 3).

The later sampling results show not only the behavior of the added materials, but also suggest the influence of differential drying in the field on this behavior. The variability in mineral nitrogen content induced by differential plant uptake confounded attempts to attribute specific characteristics of fertilizer behavior to specific materials and treatments. The reality of this problem was illustrated well by correlating April nitrogen levels across the field columns, negating treatment effects, with average columnar yields.

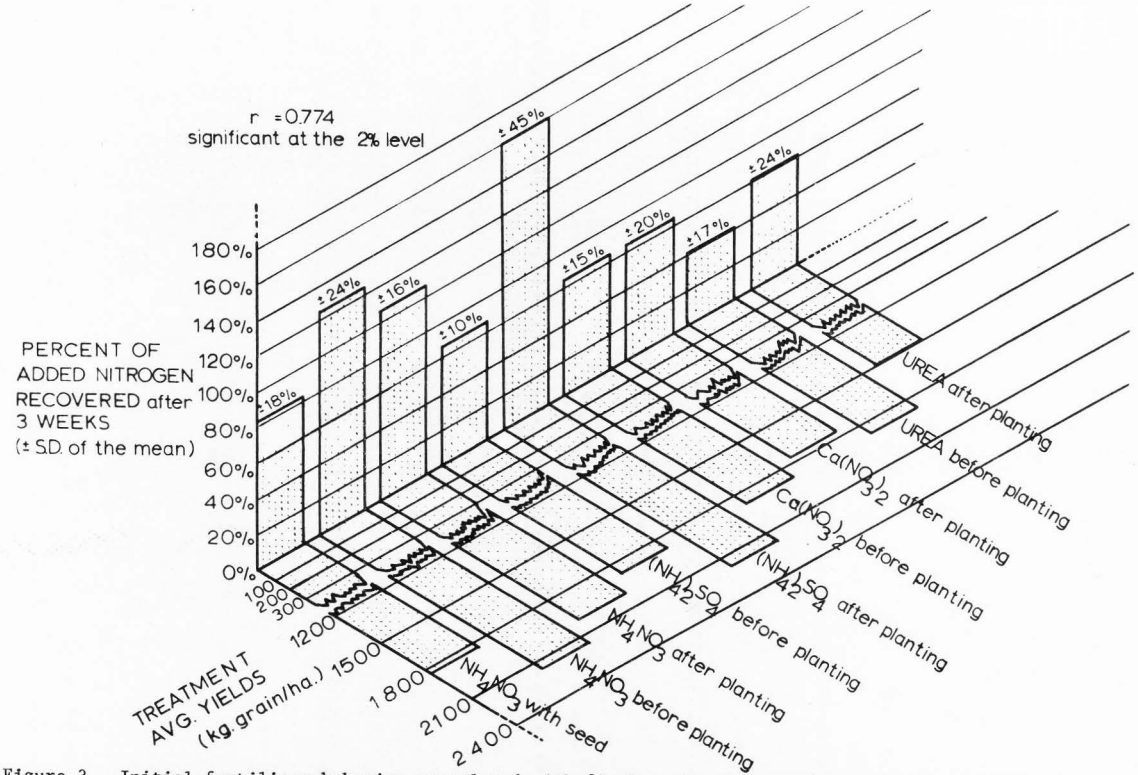


Figure 3. Initial fertilizer behavior correlated with final grain-yields usinng treatment averages.

Only in the center two columns were April nitrogen levels significantly related to subsequent yields, with 50 to 70 percent of the grain yield variability relatable to mineral nitrogen levels. In the other two field columns, in contrast, only one and 23 percent of yield variability were so relatable. Although no field moisture samples were taken, there appeared to be some relatability of protein and grain yield to moisture stress. At the 5 percent level of significance, grain yield and protein yield were found to be higher for the portion of the field with the most favorable moisture content (Column II), as compared with the field area with the least favorable moisture regime (Column I). Moisture adequacy was deduced from grain moisture data and deep, favorable soil areas.

Comparing pre-treatment and early spring mineral N levels for all the treatments, now including one spring treatment added three weeks previous to sampling and having received some precipitation in the meantime, provides no statistically meaningful results except to show a few general and one specific phenomena. Figure 4 is a summary of a previously presented table, and illustrates the dramatic difference between the ammonium sulfate-after sampling and the other ten treatments.

The pattern of initial gains and losses of added mineral nitrogen for each treatment is still detectable in these depletion figures, with highest initial loss rates corresponding to highest depletions by April and also highest initial mineral nitrogen accumulation rates resulting in smaller depletions. Correlation analysis reveals this relationship to be weak, however, at only a 10 percent level of significance, showing that other effects such as leaching and biological transformations have also become important.

Treatments	0-30cm	30-120cm	0-120cm.
Control	-22	-32	-54
NH_4NO_3 with seed	-7	-28	-35
" before seeding	-18	-54	-72
" after seeding	-9	-53	-62
" in spring	+12	-66	-54
$(NH_4)_2SO_4$ beforeseeding	+2	-72	-70
" after seeding	+7	-21	-14
$Ca(NO_3)_2$ before seeding	-20	-31	-51
" after seeding	-8	-70	-78
UREA before seeding	-20	-61	-81
" after seeding	-13	-40	-53

Figure 4. Mineral nitrogen changes in the soil profile from September to April, based on treatment averaged values in kg-N/ha. Note lack of nitrogen depletion for the ammonium sulfate-after planting treatment. The total mineral nitrogen depletions reflect initial fertilizer behavior (see Figure 3) as indicated by a positive Hotelling-Pabst Spearman rank-order correlation coefficient significant at the 10 percent level.

A mid-summer soil N analysis of nine plots, picked as a representative sample of the whole field, suggest that possible differential moisture stress regimes across the field, which would determine the plant's ability to take up mineral nitrogen and therefore the nitrogen depletion, may be increasingly more important in determining yields. These analyses, as shown in Figure 5, may be summarized by saying that April soil N levels accounted for less than half the grain yield variability of these plots, while the April to maturity N depletions, which are in part a summation of the interactions between any percent of this same grain yield variability. Note that this data comes from nine individual plots, and that these are not treatment averages.

The two largest determinants of grain yields in this experiment are predicted to be (1) the initial fertilizer behavior, and (2) the favorability of the moisture regime in the latter stages of growth. Evidence of item 2 was obtained by indirect clues and not from field moisture samples.

Specifically, it appears that ammoniacal surface treatments using an ammonium salt are preferable to either urea or nitrate salt surface treatments. High Yields for the one spring treatment in this particular experiment is not to be taken as evidence of spring treatment superiority over fall treatments. Rather it is believed to reflect a favorable spring moisture regime this particular year, with enough rainfall to carry the mineral nitrogen materials into the root zone.

The success of the ammonium sulfate after planting treatment in comparison to the other fall treatments suggests a desirablilty of having the fertilizer material in a rather concentrated form near the surface. However, in spring the same biological and moisture regimes

1. Oct. N. level	$r^2 = 0.280$	insignificant
(compares with overall	$r^2 = 0.230$)	
2. Apr. N. level	$r^2 = 0.460$	significant (5% level)*
3. July N. level	$r^2 = 0.039$	insignificant*
4. Apr. to July N. depletions	$r^2 = 0.787$	significant (1% level)*

* significance based on General Regression Significance Test results

Figure 5. Comparison of nitrogen level-grain yield correlations at different sampling times for nine representative plots.

that insure the success of a spring treatment, namely accelerated nitrification and a net downward movement would be necessary to induce a fertilizer response. Therefore, with fall application of ammonium fertilizers, the same cautions apply as with a spring treatment regarding climatic variability and risk.

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APPENDIXES

Appendix A

Preservation of the Soil Samples

Soil sample preservation

Ideally, analyses for nitrogen should be made immediately after sampling, according to Ranney and Bartlett (1972), since biological activity can alter nitrogen forms rapidly and may thwart attempts at assessing a specific nitrogen form in its natural concentration.

Realistically, the analyses of large numbers of soil samples takes time, and therefore the samples must be stored, sometimes for extended periods. Additionally, the biological activity that alters nitrogen forms may be manipulated to be minimal in most cases, following proper procedures.

The two procedures most often used are low temperature storage and dry storage, or a combination of the two.

Different soils usually will react differently to a given storage procedure (Selmer-Olsen, et al., 1971), and it would behoove the researcher to study the problem and plan a storage method before the samples are brought in from the field.

Gasser (1961) warns that air dried soils upon rewetting will exhibit a flush in mineralization, after which mineralization rates decrease to fresh soil values. This flush is especially apparent in air-dry soils stored for periods of time less than 12-16 weeks. Ammonium will accumulate during such a flush, making nitrate-only determinations misleading. These results are especially important for researchers contemplating incubation experiments.

Allen and Grimshaw (1962) found that freezing a calcareous soil, a slate soil, a litter soil, and a peat soil, increased the extractable

nitrogen in both wet and air dry samples. For example, the fresh calcareous soil was found to contain 2.5 ppm. extractable ammonium nitrogen. When frozen and re-examined afterwards, the value was 19.0 ppm. Air dried, 3.3 ppm were found, which increased to 14.0 ppm when frozen and analyzed afterwards.

Storrier (1966) suggests that generally sub-zero temperature storage effect the smallest mineral nitrogen changes, and found drying effects to be unpredictable for the soils under study.

Selmer-Olsen et al. (1971) studied nitrate and ammonia nitrogen in field samples stored at room temperature, and at 4°C, in polyethylene. The nitrate levels were slowly increasing during the first 48 hours in the room temperature bags after which the rates increased rapidly. Ammonium nitrogen over the same period increased in some samples and decreased in others, presumably because of nitrification rate fluctuations. At 4°C, the same general results were obtained, but the processes proceeded at a much slower pace.

Quantitatively, Gasser (1958) studied the same type situation, at 2°C, and found a seven ppm mineral nitrogen increase after 28 days. Storage at -10°C for 32 days produced no detectable changes. That care must be taken in storage was also illustrated when a soil was frozen at -10°C for a time, allowed to thaw for 2 days at only 2°C, and refrozen to be analyzed after a total lapse of 32 days. A 12 ppm mineral nitrogen increase was measured after this time. As a consequence, it is not good practice to thaw a soil for an analysis, find there is not enough time, and put it back in the freezer. A better way of handling such a situation is to extract the sample with the extracting solution, and then store it in a refrigerator (Selmer-Olsen et al., 1971).

If absolutely necessary, air drying such a sample overnight would be preferable to refreezing and a subsequent rethawing.

Nelson and Bremner (1972) reported average increases of 4 ppm NH_4^+-N, and 2 ppm NO_3^--N after air drying, and slightly higher increases after oven drying at 55°C. Long term storage (9 months) in air tight bottles resulted in negligible changes thereafter, but paper bag storage, over the same period, resulted in doubled ammonia levels and slight nitrate level increases. These authors recommend field-moist storage in air tight containers at -5°C, since only negligible increases were measured even after 9 months of such storage. Rapid extraction after thawing is also discussed as desirable, although no studies were done to check the effects of reasonably small delays in beginning the sample processing.

It should be understood, however, that the average mineral nitrogen increase figures given by Nelson and Bremner (1972) were determined from air drying ten different soils, three of which showed no significant ammonium nitrogen increase at all, and seven of which showed no real nitrate nitrogen increase after the air drying in the laboratory. It would be helpful to the researcher to set aside a few samples to check thawing, refreezing, and drying effects on the soils being stored, so that proper precautions may be taken to minimize the effects of whichever treatment seems most prone to stimulate mineralization and nitrification.

Storrier (1966) and Bremner (1965) found pretreatments to reduce microbial activity such as toluene to be generally ineffective.

Pretreatment and storage of samples used in this study

The September samples were air dried and stored in polyethylene bags for four to seven months. October, April, and June samples were placed in the deep-freeze, in polyethylene bags, immediately after reaching the laboratory. Thawing and homogenizing was followed by the K Cl extraction procedure as rapidly as practical, usually taking between one and three hours from freezer removal times. Thawing was usually, except in the case of smaller, drier samples, not complete before extraction began. Slightly frozen samples were found to be easier to homogenize by subdividing the semi-solid mass with a hatchet, then were the globs of mud typical of totally thawed surface samples.

Experiments were conducted to check the changes in soil mineral nitrogen levels under conditions of drying, thawing, and both frozen and room temperature storage over prolonged periods.

Tables 17 and 18 describe the first two such experiments. The third such experiment involved a more detailed appraisal of the drying process. Ten samples, from the April sampling, were frozen one month, and then thawed, homogenized, and routinely analyzed. During the analytical process, the remainder of each sample was laid out to dry in a corner of the laboratory. Each successive day for 2 days, these samples were re-analyzed, resulting in analyses for wet samples, analyses after one day of drying, and analyses after two days of drying.

The fourth experiment involved nine samples, widely divergent in available mineral nitrogen content, from the April sampling. These samples were routinely analyzed on various dates in late June and early July,

Table 17. Experiment I. Experimental detail for pre-treatment and storage method assessments on soils from the field used in two investigations. All samples were collected into polyethylene bags which were closed before transportation to the laboratory. All samples kept in such closed bags during freezing and storage. All samples from 0-3 cm depth from untreated sites. Collected 28 Oct 1972

	Pre-treatment, before storage				Storage until analysis		
Sample identification number	In field treatment if any	Period held at room temperature in field-moist condition in closed poly-bag	Period spread out for drying	All samples frozen, time elapsed from collection to freezing	Period kept frozen	Period thawed and kept at room temp.	Final condition at time of analysis
A1	None	3 days	3 days	6 days	3 months		Air-dry
A2	None	3 days	3 days	6 days	1 month	2 months	Air-dry
B1	Few drops toluene	3 days	3 days	6 days	3 months		Air-dry
B2	Few drops toluene	3 days	3 days	6 days	1 month	2 months	Air-dry
C1	None	Not held	3 days	3 days	3 months		Air-dry
C2	None	Not held	3 days	3 days	1 month	2 months	Air-dry
D1	None	Not held	Not dried	0 days	3 months		Field-moist
D2	None	Not held	Not dried	0 days	1 month	2 months	Field-moist

Table 18. Experiment II. Experimental detail for pre-treatment and storage method assessments on soils from the field used in this investigation. All samples collected, frozen, and stored in closed polyethylene bags. Samples taken from untreated sites adjacent to treated sites on 28 Oct 1972, from the 0-3 cm and the 3-15 cm soil depths.

	Pre-treatment before storage				Storage till analysis	
Sample ID number and depth of sample	In field treatment, if any	Period held in closed poly-bag at room temperature in field-moist condition	Period spread out for drying at room temp	All samples frozen, time elapsed from collection to freezing	Period kept frozen	Final condition at time of analysis
A1 0-3 cm	None	3 days	Not dried	3 days	9 months	Field-moist
A2 3-15 cm	None	3 days	Not dried	3 days	9 months	Field-moist
B1 0-3 cm	Few drops toluene	3 days	Not dried	3 days	9 months	Field-moist
B2 3-15 cm	Few drops toluene	3 days	Not dried	3 days	9 months	Field-moist
C1 0-3 cm	None	Not held	3 days	3 days	9 months	Air-dry
C2 3-15 cm	None	Not held	3 days	3 days	9 months	Air-dry
D1 0-3 cm	None	Not held	Not dried	0 days	9 months	Field-moist
D2 3-15 cm	None	Not held	Not dried	0 days	9 months	Field-moist
E1 0-3 cm	Few drops toluene	Not held	Not dried	0 days	9 months	Field-moist
E2 3-15 cm	Few drops toluene	Not held	Not dried	0 days	9 months	Field-moist

then dried in the laboratory at room temperature for three days, and then stored at room temperature in closed polyethylene bags for 3 months before being reanalyzed.

Results and discussion

The first experiment tested the effects of toluene versus no toluene in samples held 3 days in the laboratory at room temperature in closed polyethylene bags, after which they were dried and frozen. Also tested was the desirability of air drying the sample before freezing, and as compared with directly freezing the sample after transport to the laboratory. A sub test on each of these was made by thawing one of each of the frozen samples and keeping it in the lab for 2 months in a closed polyethylene bag. The results of this test appear in Table 19. The second experiment complements the first, in that toluene effects were checked on soils frozen field-moist instead of dry, both immediately after arriving at the laboratory and also after being held at room temperature for 3 days before. Depth-of-sample effects were also brought into the trials since mineral nitrogen levels were found to be higher in the second sampling levels in this field. Results appear in Table 21.

Experiment I, using a very low nitrogen surface soil, shows no mineralization or nitrification of any consequence except in the case where the wet soil was thawed and kept in the laboratory for two months prior to analysis. Despite the low standard deviations of the means, the other results can not be rigidly interpreted because variances from 0 to 3 ppm could be due to chance error being high or low on both of the analyses.

Table 19. Results of Experiments I and II. Key to treatment codes: T, toluene administered in field; H, held 3 days at room temperature in closed poly-bag; D, dried 3 days at room temperature; F1, F3, F9, frozen for 1, 3, or 9 months before analysis; W, thawed, and stored in closed poly-bag at room temperature for 2 months. Nitrogen levels are averages of two analyses per treatment for Experiment I, and are averages of three analyses per treatment for Experiment II.

Experiment #	Sample # sample depth	Pre-treatment and storage (coded)	ppm NH_4^+-N* $\pm$ S.D. $\bar{X}^\dagger$	ppm NO_3^--N** $\pm$ S.D. $\bar{X}^\dagger$
I	A1 Surface	HDF3	1 ± 1	0 ± 0
I	A2 Surface	HDF1W	3 ± 1	0 ± 1
I	B1 Surface	THDF3	2 ± 1	1 ± 1
I	B2 Surface	THDF1W	3 ± 0	0 ± 0
I	C1 Surface	DF3	3 ± 1	0 ± 0
I	C2 Surface	DF1W	1 ± 0	1 ± 1
I	D1 Surface	F3	1 ± 0	2 ± 0
I	D2 Surface	F1W	0 ± 0	10 ± 0
II	A1 0-3 cm	HF9	3 ± 1	5 ± 2
II	A2 3-15 cm	HF9	1 ± 1	10 ± 0
II	B1 0-3 cm	THF9	7 ± 0	9 ± 1
II	B2 3-15 cm	THF9	1 ± 1	40 ± 1
II	C1 0-3 cm	DF9	4 ± 1	6 ± 0
II	C2 3-15 cm	DF9	2 ± 1	12 ± 1
II	D1 0-3 cm	F9	3 ± 1	6 ± 2
II	D2 3-15 cm	F9	1 ± 0	16 ± 1
II	E1 0-3 cm	TF9	4 ± 1	6 ± 1
II	E2 3-15 cm	TF9	2 ± 0	12 ± 0

* ± 1 ppm standard method error. for each determination.

** ± 2 ppm standard error for method for each determination.

† All results rounded to nearest ppm, including standard deviations of means.

Experiment II, using soil samples with some appreciable mineral nitrogen levels, yields more information. The value of toluene is ambiguous, since in the surface soil which was held 3 days at room temperature and then frozen, there seems to be a definite increase in both ammonium and nitrate nitrogen, while in the samples frozen immediately, there is no significant difference. In the 3-15 cm samples, those immediately frozen without toluene showed slightly more nitrate nitrogen present, while those held 3 days and having toluene present had nearly quadruple the nitrates of similar treatment without the toluene. According to Russell (1923), there are bacteria that use toluene as a food source. Perhaps toluene levels were not toxic in these samples, and stimulation of one group of bacteria was followed by a general increase in other microbiological activity as toluene levels were decreased and this bacterial group died back.

Comparing the practice of holding the sample at room temperature for three days with freezing immediately, nitrogen levels were not affected greatly by the three days of holding except in the case referred to above where toluene was present. The difference between samples A2 and D2 in the nitrate level may seem appreciable, except that field variations may well be to blame (see Appendix B) as they sometimes will meet or exceed this 6 ppm difference when sampling different parts of the source sample. This points out the importance of homogenizing a sample with some degree of thoroughness.

Comparing samples that were dried immediately with those frozen immediately and with those held 3 days and then frozen, reveals no significant changes due to pre-treatments.

From these two experiments the conclusion may be drawn that with the soil used in this investigation, pretreatment practices such as drying, freezing, or even holding field-moist at room temperature for a limited time, do not tend to alter the mineral nitrogen levels significantly except when toluene is present at a low level or when moist soils are held at room temperature longer than a few days.

Experiments III and IV investigated short and long term effects of drying on some April sampled specimens. The following small tables will illustrate the results.

Table 20. Results of Experiment III. April samples routinely analyzed after one month of deep-freeze storage, and reanalyzed after one, and two days drying. Standard error of determination for this group is ± 1 ppm, calculated using eight duplicate analyses.

Soil sample #	Field-moist		After drying one day		After drying two days	
	ppm NH_4^+-N	ppm NO_3^--N	ppm NH_4^+-N	ppm NO_3^--N	ppm NH_4^+-N	ppm NO_3^--N
IIK6	3	6	3	2	2	4
ID5	1	4	0	4	1	3
IIJ6	2	3	2	3	1	4
IC3	3	2	1	3	3	2
IG4	2	2	2	1	1	1
IIH4	1	1	1	1	1	1
IIG4	3	1	1	2	3	1
IIK5	1	3	1	3	3	1
IH3	3	0	2	0	1	2
IG6	1	6	2	3	2	5

Table 21. Results of Experiment IV. April samples routinely analyzed, dried 3 days, and stored at room temperature for three months in closed polyethylene bags before reanalyses.

Soil samples ranked by initial NH_4^+-N content	Field moist: 1st analysis		Air dry, 3 months storage: 2nd analysis	
	ppm NH_4^+-N	ppm NO_3^--N	ppm NH_4^+-N	ppm NO_3^--N
1	123	62	128	53
2	57	46	51	29
3	44	35	44	34
4	22	49	24	42
5	7	4	1	2
6	6	0	1	0
7	4	9	6	7
8	2	0	3	2
9	1	0	5	0

In Experiment III one way analysis of variance reveals no significant changes due to drying. Correlation analyses applied to the results of Experiment III show no particular direction for the changes in ammonium nitrogen with drying. With the nitrate changes in Experiment III, only the total changes between the first and the third analyses were significantly correlated, with the trend being in the direction of a slight nitrate loss. Such losses could be due in part to the nitrates having moved downward with the water and then having been deposited on the plastic under the soil as the water evaporated. But, as the analysis of variance suggests, the larger part of the changes were due to random error.

Correlation analysis applied to the results of Experiment IV yields an r^2 value of 0.96 for the ammonia nitrogen levels at both times, and an r^2 value of 0.99 for the nitrate levels at both times, which is highly significant at seven degrees of freedom. These high correlations show that what little variance there is, is to be attributed to chance errors, as is also suggested by the lack of significance found using two way analysis of variance. A two way analysis was necessary here to subtract out sample to sample variations from the total and thereby reduce the error variance for a more meaningful F test.

Conclusions

The conclusion to be drawn from these experiments is that for the soil used in this investigation, changes in the mineral nitrogen levels due to drying or freezing the samples as soon as they arrived at the laboratory and subsequent deep-freeze storage have been negligible.

Appendix B

The Magnesium Oxide-Devarda Alloy Procedure for Determining Ammonium and Nitrate Nitrogen in Potassium Chloride Soil Extracts: Principles, Limits of Detection, Accuracy and Precision

The magnesium oxide-Devarda alloy distillation procedure

Problems were encountered in using this procedure as outlined by Bremner (1965), which were mostly attributable to a lack of understanding of the reagents used and their reactions. Bremner (1965), for example, does not discuss the possible extent of ammonia adsorption by Devarda's alloy which caused a slight consternation in this author until it was better understood. Hillebrand and Lundell (1953), have warned that organic nitrogen compounds may be de-aminated in the procedure using Devarda's alloy. Bremner (1965), shows which organic nitrogen compounds and under what conditions, but does not discuss the implications of these facts to a soil mineral nitrogen determination.

Furthermore, Bremner (1965) indicates that the extracting solution need not be filtered before analysis, and that even suspended materials will not affect the results of an analysis by the Devarda alloy-MgO method. In the case of this study, filtration was found to be desirable, however, since unsettled material in the extraction solutions was found to have an effect on the analytical results.

Lastly, and perhaps most importantly, Bremner (1965), and others who describe the method such as Hesse (1971), and Jackson (1958), fail to give an indication of the analytical threshold range, or accuracy of the method

which can reasonably be expected under conditions of routine use. These problems will be discussed individually in the following sections.

Devarda's alloy as a reducing reagent

The MgO-Devarda's alloy distillation procedure for ammonium and nitrate nitrogen, as described by Bremner (1965), was used in this investigation. According to Hillebrand and Lundell (1953), the Devarda's alloy is a mixture of copper, aluminum, and zinc in the ratios 50 Cu: 45 Al: 5 Zn. The copper is inactive and is a desirable component only in that it provides a coherent yet brittle structure to the alloy. It has been observed in this laboratory that when using Devarda's alloy repeatedly in the same distillation flasks without cleaning, as the zinc and aluminum are solubilized, the copper will form a copper colored plating on the bottom of the flask, illustrating its inactivity in the reaction solution. Since the aluminum and the zinc metals are oxidized to ions by giving up electrons to the nitrate nitrogen atom, Devarda's alloy is not a catalyst, but a redox-reagent.

The reaction equations for nitrate reduction with aluminum and zinc follow:

$$3\ NO_3^- + 8\ Al + 5\ OH^- + 2\ H_2O \rightarrow 8\ AlO_2^- + 3\ NH_3 \qquad [1]$$

$$NO_3^- + 4\ Zn + 3\ OH^- + 2\ H_2O \rightarrow 4\ H\ ZnO_2^- + NH_3 \qquad [2]$$

In the KCl extraction, KNO_3 would be the reactant and the ionic products could be either potassium salts or hydroxides.

The above reaction schematics belie the complexity of the mechanisms involved. First, aluminum and zinc do not occur in their atomic states in a surface. Both undergo rapid oxidation when a nascent surface is exposed to air. According to Hannay (1967), at room temperature, the

thickness of this oxidized layer on aluminum is of the order of 20 Å, while at 100°C the thickness will be about 50 Å. The composition of this layer is mostly Al_2O_3 for the aluminum absorption, and mostly ZnO for the zinc absorption, with stoichiometric defects created by the presence of mixed oxides, especially where there are boundaries with charge dislocations between the metals in the alloy (Rees, 1954).

The rate limiting step in this process is thought to be the initial transfer of an Al atom into an interstitial position in the oxide layer as an Al^{+3} ion. A spontaneous donation of electrons by aluminum must occur to cause bonding in this initial layer. Thereafter, adsorption of new anionic oxygen layers will set up an electric field in this new surface layer which will draw Al^{+3} ions from beneath the first oxygen layer into interstices in this first oxygen layer with a consequent redistribution of electrons and a demand on the next lower layer of Al atoms to donate electrons. Repetitions of this process thicken the oxidized layer (Hannay, 1967).

Aluminum and zinc oxides are the active surface components of the Devarda's alloy, therefore. This alumina adsorbs water vapor from the air at room temperature. A rearrangement of one water molecule with one surface oxide ion results in a hydrated surface layer. A recombination of two of these surface hydroxyl groups to form water again temporarily leaves an exposed Al ion which, because of its electron deficient character, acts as a Lewis acid site. These sites are traditionally considered the active centers of alumina (Lippens and Steggarda, 1970).

A small extension of this information should result in the formation of a reasonable reaction mechanism for the alloy in solution. Generalization could lead to error, however, since Al^{+3} and Zn^{+2} seem to react by different pathways. Meyerstein and Mulac (1968) studied the reduction of

nitrite by zinc and found a large effect on the rate of reaction with variations in the ionic strength of the solution. Therefore, an outer-sphere complex formation mechanism was indicated for this reaction. Edwards (1964) confirms this diagnosis and explains that the formation of an outer sphere activated complex involves rapid electron transfer between the reacting species without an immediate change in their coordination spheres. In other words, if coordinated ligands are changed by either or both of the reactants, it is done after this rapid electron transfer has been initiated.

Benson (1968) also places Zn^{+2} reactions in this mechanistic classification, and indicates that the rate constant is greater than 10^7 moles per second. Aluminum, on the other hand, is classed as reacting by the formation of an inner sphere activated complex. The reaction rate for this type of formation seems to be the same as the rate at which the surface adsorbed water, in the inner sphere, is desorbed and replaced by a specimen from the outer sphere. This lends much weight to the characterization of an exposed aluminum atom by a desorbed water molecule as being the active site on an alumina surface. Any polarized specie in solution can become adsorbed by this process, including ammonia, about which more will be said below.

The formation of an inner sphere activated complex involves the attraction to the positive Al^{+3} ion of one of the ligands of the approaching specie. Such ligands as H_2O, OH^-, $O^=$, $O_2^=$, Cl^-, and carboxylate anions are acceptable bridging ligands, which serve to bind the two metal ions in an activated complex at the surface, partly inside the hydroxyl layer. In order to be effective, however, the electrons which pass through the ligand to the receiving ion must be slightly preceded by a ligand

replacement of one of the ions, otherwise electron flow will not result in any net gain or less of electrons in either specie of ion (Edwards, 1964).

In the case of nitrate being reduced by Devarda's alloy, the above discussion simply indicates that if the nitrate ion reacts with a zinc dominated active site, electron exchange will take place with extreme rapidity and will be followed by an immediate replacement of the $O^{=}$ ligands by the H^{+} ligands. If, on the other hand, an active site is approached dominated by aluminum, the nitrate ion will form an electron bridge with one of its oxide ligands, and as the oxide ligands are replaced by hydrogen ligands, electrons will flow along the bridging oxide ligand from the alumina complex to the nitrogen ion undergoing reduction.

The processes of oxidation, hydration, creation of an active site by dehydration, and the redox reaction of such an active site by adsorption from solution are all shown for an alumina surface in Figure 6.

Practical application was made of the above information in this investigation.

Since comparisons of blanks run with reagents only indicated that Devarda's alloy contributed a significant amount of ammonia to the determination, it became necessary to determine to what extent NH_3 would be adsorbed from air at room temperature, and whether this was a process at equilibrium with the ammonia concentration in the laboratory air, or whether this was an accumulative, specific, or preferential type adsorption as in some types of activated charcoal.

Lippens and Steggarda (1970) relate that NH_3 adsorption energies are somewhat lower than H_2O, and that molecular H_2O may be driven off active alumina by simply heating to 120°C for an hour. The same treatment, since NH_3 is held less tenaciously, will therefore also remove adsorbed ammonia.

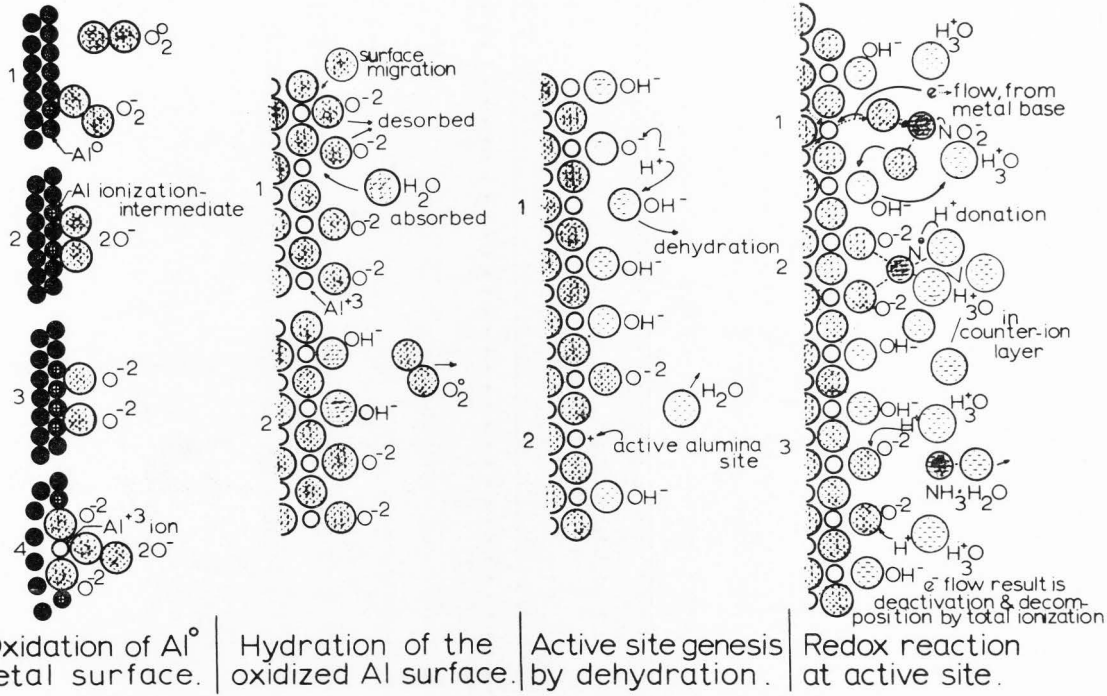

Figure 6. Reactions at an aluminum surface. Formation of an active site by oxidation, hydration, and dehydration, and a subsequent redox reaction at this active site involving a nitrite to ammonium reduction in solution are shown.

Since the ammonia and the water adsorptions are in proportion to their atmospheric partial pressures, and since the alloy being used was over a year old, it was deemed unnecessary to remove the ammonia from the Devarda alloy in the case of this investigation. If ammonia concentrations in the air did not change radically, it could be expected that the amount of NH_3 contributed by the Devarda alloy to each analysis would be uniform due to a well established equilibrium. Checks with blanks containing reagents only, throughout the period of this investigation, showed this assumption to be correct. On the other hand, ammonia removal would have been followed by a slow re-adsorption in time, which would have made the NH_3 contribution by the Devarda alloy non-uniform. This, in turn, would have complicated the calculation to the point where instead of standard subtraction values for reagent blanks being useful for calculations throughout the analytical effort, periodic changes would have had to be made to allow for increasing NH_3 contributions from the alloy and programs for calculations would have had to be revised accordingly. Naturally, the alloy's contribution was continually monitored through the running of reagent-only blanks, but the contributions were, indeed, found to be uniform throughout the 1-year period of the analyses.

The question of residual NH_3 contributions by the Devarda alloy which stayed from one analysis to the next was investigated in this laboratory.

Routinely obtained soil samples were run continuously, until four increments of 35 ml of distillate were collected from each analysis. In each of four such cases, the first 35 ml of distillate contained all of the ammonia, and the next 105 ml of distillate contained no more ammonia than control solutions without Devarda's alloy which were run simultaneously. These results confirm that the desorption of NH_3 from Devarda's

alloy is rapid at elevated (the boiling point, in this case) temperatures. The data appears in Table 24. Figures are given in the ml of standard H_2SO_4 required to titrate the distillates, and their magnitudes have no meaning other than showing on a comparative basis that there is no carryover of NH_3 from one analysis to the next in continuous use of the Kjeldahl flasks. Disuse of the apparatus over a few days, however, with some residual Devarda's alloy remaining in the flasks, will contribute some NH_3 when analyses are restarted due to readsorption from the air. It is therefore necessary to run blanks before each batch of analyses, which will reveal such a residual effect, or, if a measurement is not wanted, a good steaming out of the apparatus before analyses will also do.

The results in Table 22 also point up an interesting set of variations in the later distillate fractions. The first two samples with suspended materials, show similar values for the three later fractions in both the control and the Devarda's alloy treatments. In contrast, the clear, filtered samples show higher NH_3 content in the samples used for control, without Devarda's alloy, than in those where Devarda's alloy was used. A possible explanation for this phenomenon is that de-amination of organic amines was rapid in solutions with either Devarda's alloy or with suspended solids, and was slower in a solution without such suspended solids, so that ammonium was still detectable in each of the three later distillate fractions.

Experiments were designed and carried out to shed more light on such contributions from organic matter and the effects of suspended solids on the analysis. These experiments were intended to show mainly the Devarda alloy interactions with organic matter and suspended materials, and therefore will be presented in this section on the Devarda's alloy reaction.

Table 22. Lack of residual NH_3 contributions due to Devarda's alloy in successive distillations of the same sample in the same flasks. Four replications with four controls without Devarda's alloy, run simultaneously. All figures are in ml of standard acid used to titrate the distillates, and have no significance other than for comparisons between these distillation fractions.

Soil sample number and condition of solution	ml H_2SO_4 used in first 35 ml fraction of distillate	ml H_2SO_4 used in first 35 ml fraction of control distillate (no D.A.)*	ml H_2SO_4 used in next 3 35 ml distillate fractions of sample ($\bar{X}$) $\pm$ S.D. $\bar{X}$	ml H_2SO_4 used in next 3 35 ml distillate fractions of control ($\bar{X}$) $\pm$ S.D. $\bar{X}$
1. unfiltered solution: opaque	0.39	0.23	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01
2. unfiltered solution: opaque	0.51	0.30	0.18 $\pm$ 0.01	0.18 $\pm$ 0.02
3. filtered solution: clear	0.40	0.24	0.12 $\pm$ 0.00	0.17 $\pm$ 0.03
4. filtered solution: clear	0.43	0.21	0.13 $\pm$ 0.00	0.17 $\pm$ 0.00

*D.A. is Devarda's alloy.

Bremner (1965) presents results of analyses run on organic nitrogen materials by the Devarda alloy-magnesium oxide distillation to see how much deamination took place with resulting ammonia liberation. The compounds tested were 28 amino acids, 5 purines, 4 pyrimidines, 5 amides, 12 miscellaneous nitrogen compounds, and 2 amino sugars.

The amino sugars were the only compounds from which measurable ammonia was evolved. Interestingly, distillation with MgO only released one percent of the nitrogen present. If after this distillation Devarda's alloy was added, from two to four more percent of the nitrogen present could be liberated. These results becloud the issue somewhat, because in this type of analytical procedure MgO distillation proceeds first for ammonia determination, and then Devarda's alloy is added to the same solution for nitrate determination, confounding the effect of time with the effect of adding the alloy. The time of analysis was doubled, though, while nitrogen liberation was tripled or quintupled, giving support to the contention that the Devarda alloy plays a part in the deamination, even if it is only to provide a catalytic surface.

Perhaps the reason for the fact that the amino sugars are the only organic nitrogen bearing compounds which released nitrogen under the experimental conditions is due to the unique position of the amine group on the carbon α to the carbonyl carbon. This arrangement is able to form a carbanion on the α carbon with the subsequent release of an α carbon substituent. Usually this released substituent is the hydrogen of this α carbon, but it appears that in from zero to five percent of the cases, depending on the reaction conditions, the amine group will be released, hydrogenated, and leave the solution as ammonia.

As the amino sugars should be bound rather securely into the organic residues, insignificant amounts should be readily solubilized by the 2N KCl extraction procedure. Where a significant amount may become involved in the reaction, however, is in the unfiltered samples with suspended materials, the boiling of which may cause release of water soluble organic compounds including the amino sugars.

Before data is presented on experiments using such unfiltered solutions, another problem must receive attention which influences reactions of solutions with suspended materials.

Silicon dioxide, or the hydrated form of $Si(OH)_4$, is a polar molecule of some significant solubility. Huang (1966) reports solubilities of over 100 ppm in pH 7.5 solutions with silicate materials. Miller (1963) found silica solubility to be proportional to the water content of a soil, and found no solubility depression from the presence of common salts. The filtered solutions, therefore, probably contain a significant amount of silica, but the unfiltered solutions with suspended soil particles may be presumed to have, under heat and agitation, much more silica in solution. Any polar molecule or ion in solution, including water, ammonia, nitrate, nitrite, and silica, may adsorb temporarily at an active alumina site on the surface of the Devarda's alloy. The presence of much silica may, therefore, compete with and reduce the rate of nitrate and nitrite reduction reaction.

In addition to this rate reduction due to high silica concentrations, the inert particles suspended in the solution will physically interfere with the accessibility of the suspended alloy particles, so that no differentiation is possible between the two effects, and the

net effect of an unfiltered solution with suspended materials is to slow the reaction rate.

Figure 7 illustrates this effect as found in an experiment comparing filtered and unfiltered extracting solutions from the same large extraction. In this experiment, a 800 ml 2N KCl solution was used to extract 80 g of air dry soil, and 10 ml aliquots were used in each determination. Distillates were examined periodically, with the period (t = 0) beginning at the time boiling started in the flask. Periods were 1 1/2, 3, 4, and 5 minutes after boiling began. Error was largest at the smallest intervals, as is obvious from the figure.

It is immediately apparent from the results of this experiment that in the case of the soil used in this analysis, filtration is necessary because of the effect of suspended materials on the reaction. The best filter system for the soils in this investigation was found to be long stem funnels with fluted filter paper.

In a second experiment, two soil samples were extracted in the normal manner, using 100 ml 2N KCl per 10 g air dry soil. Sample number one was analyzed at four intervals of settling after shaking: directly after, 3 minutes after, 15 minutes after, and after 20 minutes of settling it was reshaken for a few minutes and then analyzed. A filtered aliquot was then also taken and analyzed. The second sample was shaken and analyzed immediately after removal from the shaker. Simultaneously, an aliquot was filtered and analyzed, and finally a third aliquot was analyzed after settling for 30 minutes. Results appear in Figure 8. All results are an average of two replications per treatment.

In analyzing Figure 8, it must be kept in mind that the accuracy of these analyses is ± 1 ppm under these conditions where the same

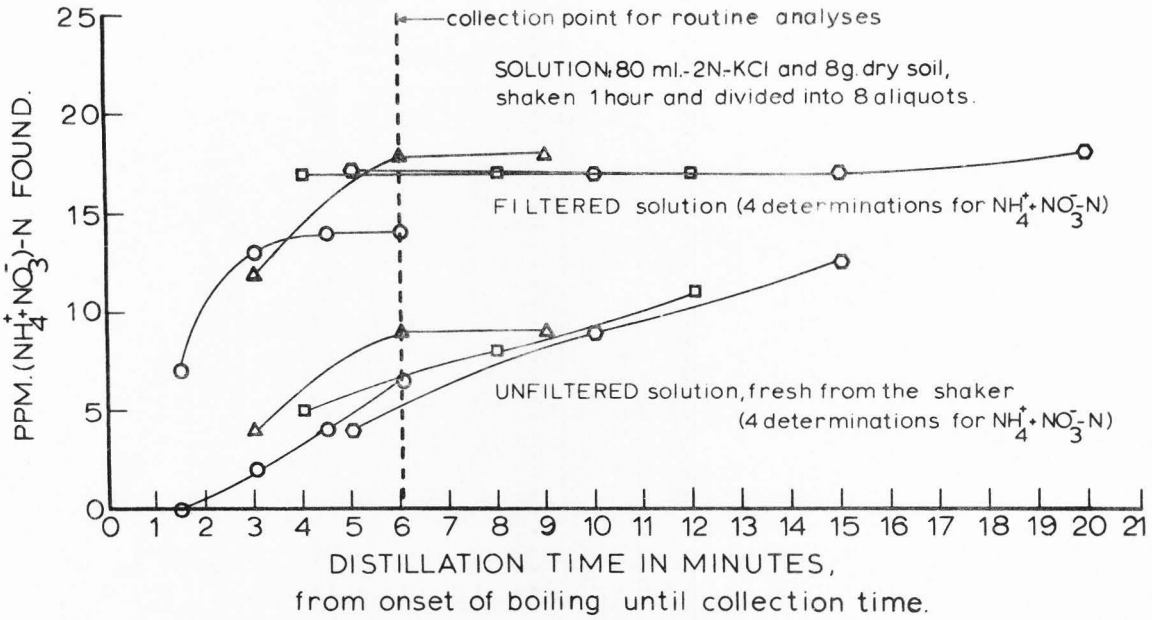

Figure 7. Effects due to distillation time and due to using filtered or unfiltered solutions with suspended material present. All eight analyses using aliquots from the same large soil extract.

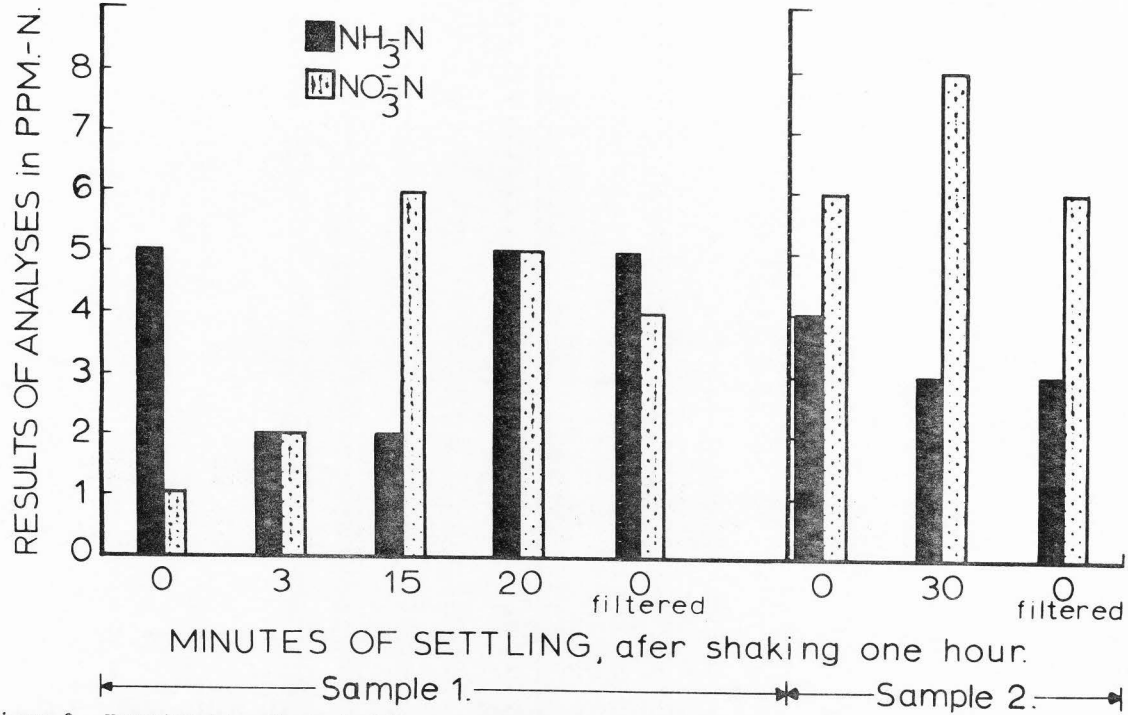

Figure 8. Unpredictable effect of different soil extract settling times on analytical results.

solution is used for every analysis. Therefore, there is no real difference between the treatments on sample 2, and Bremner's (1965) statement that 30 minutes of settling gave the same results as filtering holds for this case. In fact, even an unsettled sample gave similar results here.

Sample one, however, shows a rapid decline in ammonia nitrogen when settling has begun. This may be due to heavier particles, such as the organic and organo-clay complexes, settling out first taking with them some adsorbed ammonia despite the high KCl concentration. An actual organic matter contribution through de-amination is not supported, since the filtration of a freshly shaken aliquot has the same amount of available ammonia. Thus, freshness of the samples before filtration may be essential also, but the data are not sufficient to confirm such a need. Perhaps more importantly, the ammonia results for sample 1 suggest that the ammonia evolution reaction is not affected by any silica or suspended materials in solution. The reaction itself is aided by MgO, and may be outlined:

$$2NH_4\,Cl + Mg(OH)_2 \rightleftarrows 2NH_3 + Mg\,Cl_2 + 2H_2O \qquad [3]$$

The magnesium is very readily soluble as a chloride, and no interferences are to be expected since enough is added to accommodate about 14,000 µg N, or for our sample size, about 2,000 ppm NH_3-N, which is adequate considering the ammonia and nitrate content of the soils used in this investigation.

Sample 1, in Figure 8, reveals, as did Figure 7, that the longer the solution has settled, in other words: the less suspended solid

matter present, the more nitrate will be determined in a given time. The difference between the sample settled for 15 minutes and the filtered sample, as far as nitrates are concerned, could be due to a combination of experimental error and a contribution from slowly solubilized amino sugars which, if de-aminated in the presence of the Devarda alloy, would contribute to the nitrate nitrogen determination since this value is obtained without Devarda's alloy.

Threshold, accuracy, and precision of the procedure for nitrate and ammonium nitrogen

No indication of the range or accuracy of the method is given in any of the three texts that deal with the Devarda alloy-magnesium oxide method for nitrogen as applied to soils (Bremner, 1965; Hesse, 1971; Jackson, 1958).

A brief search of the literature was undertaken to determine what results others may have had using this method. Since this particular distillation is usually associated with a Kjeldahl procedure, and since very little literature could be found on the distillation alone, the Kjeldahl procedure was searched for in the literature. Indeed, it appears that some researchers have called this distillation procedure, when applied to inorganic nitrogen without a digestion, a Kjeldahl procedure (Scales and Harrison, 1920).

The overwhelming majority of workers using macro, semi-micro, or micro-Kjeldahl methods with digestion of organic nitrogen compounds find the procedures useful only in the milligram range of nitrogen content levels (Ebeling, 1967; Ebeling, 1968; Ma and Zuazaga, 1942; Munro and

Fleck, 1969; Fleck, 1969; Pregl and Grant, 1946; Steyermark, 1961; Acree, 1941). It is hard to determine from these articles whether the limiting step is the digestion, or the distillation and titration procedures.

A few researchers have refined their procedures to where less than 0.1 mg of nitrogen can be accurately measured. Allen (1931), for example analyzed 100 µg-N samples with an error of ± 3 µg-N. Stover and Sandin (1931) achieved ± 5 µg-N accuracy in urine samples of about 570 µg each.

Kirk (1950) hints at the existence of refinements to bring accuracy into the microgram range, but fails to describe any of them. And Hallett (1942) suggests using Conway microdiffusion units for the 0.3 to 10.0 µg range of nitrogen. This Conway micro-diffusion procedure was used on soils by Bremner and Shaw (1955). With 100 µg NO_3^--N additions to the soils, four recovery experiments with eight replications each, had standard deviations ranging from 0.2 to 1.4 µg of nitrogen. Standard solutions of 100 µg-nitrogen per 2 ml were run with a resultant error range of ± 0.6 µg of nitrogen.

Doyle and Omoto (1950), describe in detail the changes that need to be made in the distillation apparatus and procedure as used in this experiment (Bremner, 1965) to make it quantitative in the lower microgram range. The major changes include a silver tube condensor, and a millivolt range titrimeter with calomel and quinhydrone electrodes to eliminate titration errors as much as possible.

Since the procedure of Bremner (1965) was followed without such refinements, the literature was re-consulted to see if others using this procedure had reported accuracies of results in their publications.

Wagner (1940) examined titration of ammonia in the presence of boric acid and found that with apparatus much like that used in this study, the optimum range was 0.4 to 1.4 mg nitrogen. These findings were not for distillations titrated with 0.01 N H_2SO_4, but even so, a much greater range of usefulness should have been obtained. Using 0.005 N H_2SO_4, Scales and Harrison (1920) report soil analyses in the lower µg range with errors of less than 25 percent, meaning that a 28 µg-N sample could be determined with about a ± 6 µg-N accuracy.

More recently, in reporting analyses of soil samples, Allen and Grimshaw (1962) report an error of ± 8 percent, which means that a 25 µg-N sample was determinable with a standard error of ± 2 µg-N for the mean. A large variance could be indicated by such a standard deviation for the mean, but the authors did not elaborate on the number of trials so that estimating a variance was not possible.

Errors in the distillation procedure have been reported due to inserting the tip of the condensor into the boric acid solution (Yuen and Pollard, 1953; Bremner and Edwards, 1965) and it should be noted that the procedure of Bremner (1965) used in this study calls for the tip of the condenser to be above the boric acid, and not in it. In this study, no discernable difference was detected whether the condensor tip was under or above the boric acid surface, consequently care was not taken to keep the condenser tip out of the boric acid at each determination.

Limits of detection

To determine the threshold level of nitrogen, below which none could be detected by this apparatus and reagents used in this research, the

procedure of Eckslager (1969) was used. The smallest measurable value, $\underline{X}$, was computed as a function of the mean value of zero or blank experiments, $\overline{X}_0$, the standard deviation of these zero or blank experiments, S_0, and an appropriate constant from Gaussian tables, k, by the following relation which was first defined by Kaizer (1966):

$$\underline{X} = \overline{X}_0 + k\ S_0 \qquad [4]$$

Kaiser (1966) suggested the use of k = 3, because it represents a probability of 99.86 percent. Actually k = 3.18 represents such a probability, but the k = 3 was used for this data since the actual probability of 99.74 percent was quite acceptable. Throughout the year of analytical work, blanks and standards were run with every batch, and the calculation of the lowest determinable level was based upon these results for the blank trials. It was found that 1 ppm-NH_4^+-N and 2 ppm NO_3^--N were the limits of detection for this particular investigation. The determination of these limits is illustrated in Table 23.

Finding an upper limit for the method was not deemed necessary, since the 5 ml of 2 percent boric acid solution used to absorb the ammonia will effectively absorb about 5,000 µg-NH_3, and quantitative results have been obtained by many workers in the 500 µg-N range (Bremner and Edwards, 1965; Stover and Sandin, 1931). If larger amounts of nitrogen are present, however, it would be more convenient to use a stronger acid in the titrations. Using 0.01 $\underline{N}$ H_2SO_4, a workable range of 200 µg to 2,000 µg-N will result with the same apparatus and procedure as used in this investigation (Ma and Zuazaga, 1942; Munro and Fleck, 1969).

Table 23. Determination of limits of detection using mean and standard deviation of blanks run throughout the period of the analytical work. The limits of detection, $\underline{X}$, is equal to the mean value of the blank determinations, $\overline{X}_0$, plus three times their standard deviation S_0 (Eckslager, 1969).

Ammonia Nitrogen (magnesium oxide distillation only)		Nitrate Nitrogen (Devarda's alloy and magnesium oxide distillation with value of corresponding ammonia determination subtracted)	
Results given in cm^3 of 0.0052 $\underline{N}$ H_2SO_4 used to titrate distillates		Results given in cm^3 of 0.0052 $\underline{N}$ H_2SO_4 used to titrate distillates	
Number of blanks run	108	Number of blanks run	80
Mean, $\overline{X}_0$	0.1346 cc	Mean, $\overline{X}$	0.2395 cc
Standard deviation, S_0	0.0278 cc	Standard deviation, S_0	0.0405 cc
Lower limit of detection, $\underline{X}$	0.0880 cc	Lower limit of detection, $\underline{X}$	0.1310 cc
$\underline{X} = \overline{X}_0 + k\ S_0;\ k = 3$		$\underline{X} = \overline{X}_0 + k\ S_0;\ k = 3$	
Consequences		Consequences	
$\underline{X}$ converted to µg-N	6.41 µg-N	$\underline{X}$ converted to µg-N	9.54 µg-N
$\underline{X}$ range in terms of ppm-N (Soil samples ranged from five to nine grams)	0.7-1.3 ppm N	$\underline{X}$ range in terms of ppm-N (Soil samples ranged from five to nine grams)	1.1-1.9 ppm N
$\underline{X}$ in ppm-N for average sample of 6.5 grams	1.0 ppm N	$\underline{X}$ in ppm-N for average sample of 6.5 grams	1.5 ppm N
$\underline{X}$ used to evaluate data in this research	1 ppm N	$\underline{X}$ used to evaluate data in this research	2 ppm N

Accuracy and precision

Accuracy and precision for the procedure were determined by five separate and distinct types of tests, so that procedural error, soil heterogeneity error, sampling error, and the dependability of the experimental results could all be determined with confidence.

Procedural error

The first estimate of precision was based on the standard deviations of the blanks, S_0's, determined already in the above section on limits of the detection. This precision at the zero level of nitrogen was found to be ± 2.2 μg-NH_3-N and ± 2.9 μg-NO_3^--N for these two mineral nitrogen species. Confidence intervals for these values were computed using the $t/\sqrt{n}$ statistic at the 95 percent confidence level, but because of the high number of trials (high n), these intervals were unrealistically low, and the variation that can be expected from sample to sample is better described by the above values based on standard deviations. A hypothetical error of this magnitude on an average sized soil sample of 6.5 g dry soil would mean precision of ± 1.1 ppm NH_3-N or ± 1.5 ppm NO_3^--N. Precision of the method is therefore not much of a problem in field oriented work such as that of this study.

Accuracy of the method was determined by statistical analyses of standard solution results. Standard solutions were made up and run frequently in the course of this experiment. The total number of standards run, and their distribution by nitrogen levels are summarized in Table 24.

Table 24. Total number of standards run. Also ranges of their nitrogen contents expressed in the theoretical mls of 0.0052 $\underline{N}$ H_2SO_4 required to titrate their distillates, with the number of standards in each range.

Ammonia nitrogen standards		Ammonia + nitrate nitrogen standards	
Range in ml standard H_2SO_4 required	No. of samples	Range in ml H_2SO_4 required	No. of samples
0.03 - 5.76 (Total)*	162	0.03 - 5.76 (Total)*	213
0.03 - 0.20	46	0.03 - 0.14	35
0.26 - 0.68	35	0.15 - 0.29	31
0.72 - 1.03	30	0.34 - 0.52	42
0.38 - 1.92	37	0.55 - 1.14	35
2.50 - 5.76	14	1.37 - 2.58	29
		2.76 - 5.76	41

*Representing a range of 2.18 to 419.33 µg's of nitrogen, or, for an average size soil sample of 6.5 g dry soil with standard procedures, a range of from 1 to 215 ppm nitrogen, which covers the whole spectrum of nitrogen levels found in this study.

Statistical analyses of these standard solution runs consisted of correlating the theoretical titrant requirements for the standards with the amount of titrant actually used. The results of these analyses are reported in Table 25.

From Table 25, it may be seen that there is only 0.4 percent variation between theoretical and actual results, ascribable to random error. The ammonium nitrogen determinations seem to be lower than they should be by an average of about 0.04 ml of standard titrant, while the comparable figure for the (ammonia + nitrate)-nitrogen determinations is about 0.03 ml of titrant lower than theory. This error is rather consistent over the whole range of standards, indicating a systematic

Table 25. Correlations of amount of titrating acid theoretically required by the standard solutions, with the amounts actually used. Figures used are expressed in ml of 0.0052 N H_2SO_4. Mean deviation of experimental result from theoretical result, and the standard deviation of this mean, are also re-expressed in ppm-N for a hypothetical 6.5 g dry soil sample routinely processed, to give an indication of precision for the method as applied in this study.

Correlation analysis parameter	Ammonia nitrogen standards	Ammonium + nitrate nitrogen standards
	Value of parameter	Value of parameter
No. of analyses	162	213
Mean theoretically calculated acid requirement	0.8666	1.1357
Mean experimentally determined acid requirement	0.8149	1.1119
Coefficient of determination: R^2	0.9962	0.9964
Mean deviation of experimental results from theoretical results: b_0	-0.0375	-0.0308
Mean deviation, b_0, expressed in ppm N for a hypothetical soil sample with a ppm-N content of:	-1.40	-1.15
	30.42	41.51

type error. The standard subtractions due to dilution and reagent effects could have been readjusted empirically to decrease this systematic error, but since nitrate levels are determined by subtracting the corresponding ammonia determination result from the (ammonia + nitrate)-nitrogen result, the nitrate level results will be slightly high, by about 0.01 ml of titrant, in fact. As will be seen in the next

section, errors of this magnitude are negligible in the context of field sampling and soil heterogeneity errors.

Field and soil heterogeneity error

Soil heterogeneity error was estimated by frequent analyses of a standard soil sample. This standard soil sample consisted of a large surface soil sample which was air dried and homogenized, and then frozen as were all other samples. With each day's sample preparations, one or a few of the samples prepared were taken from this standard soil sample. Between November of 1972, and March of 1973, 51 such standard soil determinations were made. Thereafter, until the end of the analyses in September 1973, another batch of a similarly prepared standard soil sample was used for another 26 determinations.

Analysis of the data provided by these standard soil samples consisted of computing averages, standard deviations, deviations of means, ranges, and 95 percent confidence intervals for the two groups of data and for the two forms of mineral nitrogen determined. The 95 percent confidence intervals were constructed using the method and tables provided in the work of Eckschlager (1969). The formula used was:

$$L_{1,2} = \overline{X} \pm \overset{*}{K}_N \times \text{Range} \qquad [4]$$

The $\overset{*}{K}_N$ were found in tables, and were constructed by Eckschlager (1969) from the $t/\sqrt{n}$ statictic which is usually multiplied by the standard deviation for the creation of confidence intervals by the equally valid classical method.

Results appear in Table 26.

Table 26. Analysis of standard soil samples. Two experiments, one during the analysis of the September and October samplings, including 51 standard soil sample determinations, and the other during the analysis of the April sampling, including 26 such standard soil sample determinations. Results in ppm-NH_3-N or ppm-NO_3-N rounded to nearest whole ppm-N.

Batch number	Number of determinations	Average nitrogen content, ± 95% confidence interval values ppm-NH_3-N	ppm-NO_3-N
I	51	4 ± 1	4 ± 2
II	26	2 ± 1	5 ± 2

From this table it may be seen that a soil, if properly homogenized, will not display a heterogeneity error of discernible size if the nitrogen levels of the sample are low. This experiment was not valuable in terms of showing an error due to variations within the same sample, but it was of considerable value in providing workable confidence limits for low nitrogen sample results. An accuracy of ± 1 ppm for ammonia, and ± 2 ppm for nitrate determinations in low nitrogen level soil samples may be expected with a confidence level of 95 percent.

The last indication of error magnitudes in the procedure is given by the duplication of routine analyses. Differences between such duplicate sample results are a real measure of error within the sample, reflecting a combination of field variations with depth as well as variation due to failures in attempts at completely homogenizing each sample. An attempt was made in each duplication to take different appearing parts for the two parallel analyses so that profile heterogeneity would be emphasized over failure to homogenize.

The results of all these samples were used in the analyses in the main part of this thesis, and divergent results were averaged for use in the body of data upon which the conclusions of this study are based.

A breakdown of the duplicate analyses made appears in Table 27. The grouping of the data in Table 27 is misleading in that it suggests a larger mean deviation due to sampling date. A rearrangement of the individual data for each sampling by nitrogen level was made, according to the following scheme, as illustrated in Table 28, and analyzed against sampling date by one-way analysis of variance.

From Table 28, the conclusion is evident that sampling time was not an important variable, but nitrogen level is related to error between duplicate analyses of the same sample. This relationship was investigated further by combining the results of all the analyses for each of the groups defined in Table 28.

A comparison of the average deviation for each grouping, and for the standard deviation of the deviations in each grouping for each nitrogen specie analyzed appears in Table 29.

The trend is fairly obvious from these averages and standard deviations of the differences between duplicates, that higher differences in nitrogen levels within the sample may be expected with higher soil nitrogen levels. This relationship was tested by correlating the differences between each duplicated analysis with its mean nitrogen level for every duplication. For NH_3-N, with 176 duplicates, and therefore 174 degrees of freedom, the regression coefficient was 0.23, which was significant at the 1 percent level. Similarly, for NO_3-N, with 161 degrees of freedom, the regression coefficient was 0.205, which is

Table 27. Listing and characterization of duplicated analyses from the same soil samples. Number of duplicates run, for each nitrogen specie, and for each sampling appears together with mean values of deviations for each specie in each sampling.

Soil sampling Month, year	Number of NH_3-N duplicates run	Number of NO_3-N duplicates run	Average value of nitrogen levels deviations for each specie ppm NH_3-N	ppm NO_3^--N
September 1972	64	54	1	2
October 1972	29	26	5	3
April 1973	83	83	2	1

Table 28. Scheme for analysis of duplicated sample analyses results to show that variance within samples was due to nitrogen levels and not due to sampling dates. Samples grouped into a class by the highest value for either nitrogen specie in either analysis. One way analysis of variance, for each group, compared magnitude of differences in duplicate results with qualitative factors 1, 2, or 3, representing the September, October, and April samplings, respectively. F test results reported only as significant or not significant.

Group number and nitrogen level range in ppm-N	Sampling, and number from this sampling in this group for each specie		Average deviation between duplicates for each of the preceding numbers of duplicates for each specie		F test results for each specie N.S. = no significance, N.T. = no test possible	
	NH_3-N	NO_3-N	NH_3-N	NO_3^--N	NH_3-N	NO_3-N
			ppm	ppm		
	1. 39	24	1	1		
1. 0-5.00	2. 6	5	1	0	N.S.	N.S.
	3. 70	69	1	1		
	1. 12	22	3	2		
2. 5.01-10.00	2. 7	10	1	2	N.S.	N.S.
	3. 6	4	4	3		
	1. 13	7	2	5		
3. 10.01-20.00	2. 6	9	3	4	N.S.	N.S.
	3. 1	3	2	4		
	1. 2	1	3	1		
4. 20.01-50.00	2. 4	2	9	13	N.S.	N.S.
	3. 0	2	-	3		
	1. 0	0	-	-		
5. 50.00-100.00	2. 4	0	11	-	N.S.	N.T.
	3. 2	1	9	12		
	1. 0	0	-	-		
6. 100.01-200.00						
	2. 4	0	14	-	N.T.	N.T.
	3. 0	1	-	4		

Table 29. Median ppm-N level of each group, with the number of duplicated analyses in each group, listed with the averages and standard deviations of the differences between these duplicated analyses of the same sample. Means and standard deviations in ppm-N.

Median nitrogen level of group in ppm-N	Number of duplicates in group		Mean differences between duplicates		Standard deviations of differences between duplicates	
	NH_4-N	NO_3-N	NH_3-N ppm	NO_3^--N	NH_3-N ppm	NO_3^--N
2.5	114	100	1	1	1	1
7.5	25	37	2	2	3	2
15	20	19	2	4	2	3
35	6	5	7	6	5	11
75	6	1	10	12	13	-
150	4	1	14	4	16	-

significant at the 2 percent level, confirming the trend for larger in-sample deviations with larger nitrogen levels.

The actual error magnitudes here are not very meaningful, until the data are adjusted to cull out samples whose average nitrogen levels were found to be below the above determined limits of detection. Fully one-hundred of these duplicates were found to be below (or, the average was below) the detection threshhold for the method. Thus, analysis of in-sample error could be done on 239 duplicated samples. Using the values for 239 samples, with an average nitrogen content of 11 ppm NH_3 or NO_3^--N, the corresponding average error between duplicates was 4 ppm NH_3 or NO_3^--N. An average sample of 11 ppm NH_3 or NO_3^--N may thus be expected to have from 9 to 13 ppm NH_3 or NO_3^--N, which corresponds to a ± 2 ppm N uncertainty for this nitrogen level.

Comparison of the distillation procedure with a colorimetric procedure

One other experiment was conducted, comparing nitrate results of samples done with the procedure of this experiment with the results of these same samples analyzed by the colorimetric phenoldisulphonic acid procedure for nitrates as outlined by Bremner (1965). Fifteen samples were chosen to cover a range of from zero to fifty-three ppm nitrate nitrogen, as determined by the distillation procedure of this study. Regression analysis on the results show that the distillation procedure results, in ppm NO_3-N, were consistently lower than the results obtained by the colorimetric procedure. Since the coefficient of determination was high (R^2 = 0.98 with 13 degrees of freedom), a predictive equation was put together, and nitrate levels from the colorimetric procedure, with their standard deviations, were predicted from the nitrate levels given by the distillation technique. The results appear in Table 30.

Considering the results for duplicated analyses from the same samples as reported above, this comparison is not at all unfavorable to either method. Hesse (1971) estimates the accuracy of this colorimetric procedure at 5 percent; our standard deviation at the higher nitrate level reflects such an accuracy. In comparison, 14 of the duplicate analyses clustered around the 50 ppm-N level showed an average error of 14 percent difference between the duplicates, or ± 7 ppm-N at the 50 ppm-N level. Considering such a magnitude of error introduced by intra-sample variations, the trend of the regression shown in Table 33 may be accidental only and due to a coincidence of in-sample variations between the comparisons

Table 30. Result of comparison between nitrate levels as given by the distillation procedure and as given by the phenoldisulphonic acid method. Given the distillation procedure results, the corresponding value for the phenoldisulphonic acid method is predicted from the regression equation, also the computed standard deviation of this estimated value is given. All values in ppm NO_3^--N.

Nitrate level by distillation	Predicted nitrate level if done by colorimetric procedure	Standard deviation of this predicted nitrate level
0.00	0.19	± 3.04
1.00	1.33	± 3.04
5.00	5.89	± 3.00
10.00	11.60	± 3.03
25.00	28.71	± 3.05
50.00	57.24	± 3.42

having been in the same directions for the two larger nitrate levels. Such a coincidence of error at the larger end could influence a regression line's slope unduly. No conclusions as to the relative efficiencies or accuracies of these two analytical methods may therefore be drawn from the results of this experiment.

Summary of threshold, precision, and accuracy of findings

The results of the five experiments on threshold, accuracy and precision are summarized in Table 31.

Since the duplicate analyses were biased toward showing the magnitude of in-sample variations, and since NH_3 and NO_3^- nitrogen results were combined, the accuracy of the method for the individual species will be held to be that achieved with the standard soil samples and blanks, namely ± 1 ppm for NH_3-N and ± 2 ppm for NO_3^--N.

Table 31. Summary of results of experiments on threshold, precision, and accuracy of the method as applied to blanks, standard solutions, standard soil samples, and duplicated field sample analyses. Results reported as they would affect a hypothetical soil sample of 6.5 grams air dry weight, processed by the procedures of this investigation.

Experiment	Subject of investigation	Consequences for analysis of hypothetical soil sample with these nitrogen levels: ppm NH_3-N	ppm NO_3^--N	
Blanks	Lower limit of detection for the method	< 1	< 2	Indistinguishable from 0 ppm-N
	Precision of method	0	0	$\pm$ 1 ppm NH_3-N $\pm$ 2 ppm NO_3^--N
Standard solutions	Accuracy of method	30	42	- 1 ppm NH_3-N - 1 ppm NO_3^--N
Standard soil samples	Accuracy and precision of method using homogeneous soil samples	3	4	$\pm$ 1 ppm NH_3-N $\pm$ 2 ppm NO_3^--N
Duplicate analyses of field soil samples	Precision of field soils as affected by nitrogen levels in the samples	11 ppm NH_3-N or NO_3^--N		$\pm$ 2 ppm NH_3-N or NO_3^--N

Appendix C

Raw Data in Parts-per-million of Nitrate or Ammonium Nitrogen for Each Plot in Each Sampling

Table 32. September sampling--ppm NH_4^+-N and ppm NO_3^--N levels for each sampling depth increment for each plot. Format: ppm NH_4^+-N/ppm NO_3^--N.

PLOT:	I-A	I-B	I-C	I-D	I-E	I-F	I-G	I-H	I-I	I-J	I-K
Depth:											
0-3 cm	1/4	1/5	1/2	3/5	1/5	1/3	2/2	1/3	1/4	2/2	1/4
3-15 cm	5/9	2/9	11/10	2/14	1/10	2/12	1/12	5/9	4/18	2/15	5/10
15-45 cm	2/2	1/2	8/2	3/5	6/2	3/3	1/3	3/7	6/3	6/7	2/5
45-75 cm	4/2	1/2	4/11	4/2	3/2	2/2	4/2	4/8	5/5	3/4	4/4
75-120 cm	1/2	2/2	11/6	1/2	4/2	1/3	3/3	2/6	6/2	1/5	4/5
	II-A	II-B	II-C	II-D	II-E	II-F	II-G	II-H	II-I	II-J	II-K
0-3 cm	1/3	2/3	2/4	1/2	2/10	1/7	1/3	1/2	1/2	1/6	2/2
3-15 cm	2/10	4/12	1/10	3/10	1/6	1/7	4/9	2/8	1/7	1/8	1/6
15-45 cm	1/3	1/3	2/3	2/4	1/4	2/2	9/5	3/2	2/2	1/4	1/4
45-75 cm	2/2	1/3	2/1	1/4	4/2	2/2	1/4	1/3	3/2	9/14	2/4
75-120 cm	1/3	1/3	1/1	3/2	1/2	2/5	2/2	9/5	1/3	3/2	8/3
	III-A	III-B	III-C	III-D	III-E	III-F	III-g	III-H	III-I	III-J	III-K
0-3 cm	8/2	2/4	4/2	2/13	2/12	1/3	10/6	1/2	2/2	1/4	1/2
3-15 cm	2/10	2/12	1/12	1/12	1/9	7/18	10/9	10/13	2/11	1/12	4/11
15-45 cm	1/8	3/5	2/2	3/5	7/2	2/4	7/4	1/3	3/2	6/6	14/5
45-75 cm	2/2	2/2	6/6	2/4	1/6	4/2	5/2	2/7	1/3	2/2	12/9
75/120 cm	1/3	1/3	1/3	2/5	1/8	11/6	11/4	1/2	2/3	8/7	1/4
	IV-A	IV-B	IV-C	IV-D	IV-E	IV-F	IV-G	IV-H	IV-I	IV-J	IV-K
0-3 cm	2/3	5/12	4/3	1/4	1/3	4/2	5/4	4/7	1/4	2/4	2/6
3-15 cm	2/14	1/12	2/4	2/12	2/5	4/15	1/24	4/15	2/11	3/11	1/12
15-45 cm	1/5	2/4	1/5	7/2	4/8	6/5	5/5	4/2	2/4	2/7	1/3
45-75 cm	1/5	1/6	5/2	2/6	1/6	6/4	2/4	1/5	2/2	1/2	4/4
75-120 cm	1/6	2/6	2/5	5/3	6/3	1/2	5/5	5/3	3/2	2/4	1/2

Table 33. October sampling ppm NH_4^+-N and ppm NO_3^--N levels for each sampling depth increment for each plot. Format: ppm NH_4^+-N/ppm NO_3^--N.

PLOT:	I-A	I-B	I-C	I-D	I-E	I-F	I-G	I-H	I-I	I-J	I-K
Depth:											
0-3 cm	6/3	5/2	106/7	60/4	4/4	16/2	8/6	117/3	6/2	102/3	26/13
3-15 cm	6/7	21/27	6/22	7/16	5/19	6/8	3/19	12/5	3/2	13/2	23/2
15-30 cm	6/7	3/7	4/12	7/8	6/15	2/4	5/24	6/10	8/6	4/9	8/2
	II-A	II-B	II-C	II-D	II-E	II-F	II-G	II-H	II-I	II-J	II-K
0-3 cm	23/8	5/2	64/9	4/2	187/2	77/15	3/2	49/6	5/2	106/2	8/11
3-15 cm	11/14	8/5	13/8	4/12	7/9	4/31	3/6	11/13	5/22	9/7	7/9
15-30 cm	4/6	5/5	7/2	6/4	8/5	8/22	6/7	6/6	4/6	5/5	7/3
	III-A	III-B	III-C	III-D	III-E	III-F	III-G	III-H	III-I	III-J	III-K
0-3 cm	71/17	4/4	5/7	59/9	11/7	130/17	93/6	92/6	21/4	4/4	31/12
3/15 cm	83/10	6/31	10/6	9/26	4/29	6/21	6/4	9/15	20/20	6/6	15/4
15-30 cm	9/7	4/14	3/5	16/2	5/3	7/8	3/7	6/14	5/13	10/4	6/9
	IV-A	IV-B	IV-C	IV-D	IV-E	IV-F	IV-G	IV-H	IV-I	IV-J	IV-K
0-3 cm	65/11	53/14	10/2	12/3	5/2	9/7	4/5	23/2	3/3	133/11	186/4
3-15 cm	6/6	4/14	6/23	6/6	19/23	4/6	5/21	14/16	5/4	4/22	12/5
15-30 cm	5/10	5/10	5/17	1/2	6/7	4/8	2/9	4/6	4/6	9/3	6/2

Table 34. May sampling ppm NH_4^+-N and ppm NO_3^--N levels for each sampling depth increment for each plot. Format: ppm NH_4^+-N/ppm NO_3^--N.

PLOT:	I-A	I-B	I-C	I-D	I-E	I-F	I-G	I-H	I-I	I-J	I-K
Depth:											
0-3 cm	4/8	12/9	41/32	20/25	1/4	6/2	2/4	4/9	4/6	16/11	2/7
3-15 cm	2/2	7/21	1/2	2/6	1/2	2/3	3/2	3/2	5/3	3/2	9/2
15-45 cm	4/2	2/5	3/2	2/2	4/2	3/2	2/2	3/2	3/2	1/2	1/2
45-75 cm	3/2	3/2	2/2	3/2	2/2	2/2	2/2	1/2	1/2	1/2	1/2
75-120 cm	1/2	3/2	1/4	1/4	1/4	1/2	2/3	2/4	1/2	2/2	3/7
	II-A	II-B	II-C	II-D	II-E	II-F	II-G	II-H	II-I	II-J	II-K
0-3 cm	14/14	59/134	6/6	34/2	36/23	2/2	6/5	7/7	1/2	4/5	7/4
3-15 cm	4/3	2/3	5/14	4/2	3/4	4/2	6/2	3/2	5/2	1/5	1/2
15-45 cm	1/2	1/2	1/2	1/2	1/2	1/2	3/2	2/2	2/2	2/2	2/2
45-75 cm	2/2	1/2	1/2	1/2	1/2	2/2	3/2	1/2	2/2	2/2	1/3
75-120 cm	1/2	1/2	2/2	1/2	2/15	1/4	2/2	3/2	2/2	1/4	2/3
	III-A	III-B	III-C	III-D	III-E	III-F	III-G	III-H	III-I	III-J	III-K
0-3 cm	14/27	2/6	2/2	1/7	1/3	35/15	123/63	24/12	4/4	31/46	12/15
3-15 cm	1/4	4/2	1/3	1/2	3/3	1/3	1/2	9/2	1/4	10/2	1/3
15-45 cm	1/4	1/2	1/3	2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2
45-75 cm	1/2	1/2	2/2	2/2	1/3	2/2	1/3	1/2	2/2	1/2	3/2
75-120 cm	3/2	1/4	2/2	1/3	1/3	3/2	1/4	2/5	2/3	1/2	1/4
	IV-A	IV-B	IV-C	IV-D	IV-E	IV-F	IV-G	IV-H	IV-I	IV-J	IV-K
0-3 cm	57/46	9/12	2/6	86/94	1/2	1/6	1/7	4.9	3/2	19/13	41/30
3-15 cm	2/4	1/2	2/3	1/2	1/2	10/3	6/2	4/3	4/2	1/2	1/2
15-45 cm	1/2	1/2	1/2	1/2	1/2	1/2	2/2	1/2	2/2	1/2	2/2
45-75 cm	1/2	1/3	1/2	1/4	1/2	1/2	1/2	1/2	1/2	1/2	1/2
75-120 cm	2/2	4/2	1/2	5/2	3/2	1/2	1/4	1/2	2/2	1/4	1/2

Table 35. July sampling ppm NH_4^+-N and ppm NO_3^--N levels for each sampling depth increment for each plot. Format: ppm NH_4^+-N/ppm NO_3^--N.

PLOT:	II-C	II-E	II-G	II-J	III-A	III-C	III-D	III-E	III-J	IV-I
Depth:										
0-45 cm	1/4	1/3	2/2	1/2	3/2	1/3	1/3	1/3	2/2	2/2
45-75 cm	1/2	1/2	2/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
75-120 cm	1/2	3/2	2/2	2/2	1/2	1/2	1/2	4/2	1/2	1/2

VITA

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