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EFFECTS OF FOUR SMALL-GRAIN CEREALS ON NITRATE LEVELS IN PEAT-AMENDED SOILS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Andrew Allan Charlton Boyachek

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Plant Science

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read a Master's thesis entitled: ...EFFECTS OF FOUR SMALL-GRAIN CEREALS ON NITRATE LEVELS IN PEAT-AMENDED SOILS.....

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in partial fulfillment of the requirements for the degree of .....Master of Science.....

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EFFECTS OF FOUR SMALL-GRAIN CEREALS ON NITRATE LEVELS IN PEAT-AMENDED SOILS

BY

ANDREW ALLAN CHARLTON BOYACHEK

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

Boyachek, Andrew Allan Charlton. M.Sc., The University of Manitoba, May, 1983. Effects of Four Small-grain Cereals on Nitrate Levels in Peat-amended Soils. Major Professor; W. Woodbury.

The effects of wheat, barley, rye and triticale on soil nitrate levels were compared to nitrate levels in fallow soils with ammonium sulphate as a source of nitrogen.

Plant uptake and soil depletion of NO_3^--N occurred earlier in soil without additional nitrogen. The addition of ammonium sulphate prolonged the uptake of N by the plants. It appeared that barley destabilized soil NO_3^--N while wheat, rye and triticale stabilized soil NO_3^--N. The presence of crops had an apparent stimulating effect on the nitrification of the ammonium sulphate that had been added.

The addition of ammonium sulphate resulted in an increase of soil pH and acidification of the soil through nitrification. While differences in the pH between the inner and outer rhizosphere of both wheat and barley were not significant, changes in the rhizosphere pH varied with nitrate uptake. Soil nitrate levels were lower with barley in comparison to wheat. This was due to the different rates of uptake and/or different rates of immobilization as influenced by the roots of either of the two crops.

Washings of wheat stems and roots and barley stems and roots did not alter the nitrification of ammonium sulphate.

The use of vermiculite or perlite in soil mixes would eliminate interactions of organic matter and the soil microflora as observed in this study. The use of peat introduced large amounts of organic matter which influenced the microflora in various ways.

INTRODUCTION

Nitrification of ammonium salts into nitrite and nitrate has drawn a considerable amount of attention from both the soil scientist and the plant scientist. Absorption of nitrate ions by plant roots is the major source of nitrogen for the plant. The presence of nitrate ions in the soil and those factors which influence its presence and availability are, consequently, of the greatest importance.

Attention has been focused on the many variables which alter or in some way affect nitrification. While minimum, maximum and optimum levels have been established for many environmental variables such as season, soil pH, soil temperature, depth, aeration, organic matter, nutrient supply and inhibitors, the effect of plant roots on the nitrification process is still controversial.

The interest that has developed in the effect of plant roots via the exudates of the roots on the nitrifying bacteria and soil nitrate levels has resulted in a great deal of research and speculation. From this research two theories have evolved. Those in support of the first theory believe that plant roots and their exudates inhibit the production of nitrates or the nitrification process. This theory is supported by the fact that nitrogen found in the soil of cropped land was lower than in fallow soil. Even when allowances were made for the nitrogen taken up by the crop, this occurred. Contrary to this belief, others have postulated that nitrification is promoted or stimulated by plant

roots and their exudates. While proponents of these theories have aptly demonstrated the pros and cons of the effect of plant roots and their exudates on the nitrification process, no definite conclusion has been reached. Others have offered alternative explanations for the lower levels of nitrates under cropped soil in comparison to fallow soil. These alternate reasons include the immobilization and/or denitrification of soil nitrates.

The purpose of this thesis is to examine the effects of some cereal crops, via their roots and root exudates on the nitrification process in the soil. Thereby it is hoped that some information on the problem may be obtained.

LITERATURE REVIEW

Factors Affecting Nitrification

Environmental Influences on Nitrification

The ability of the nitrifying bacteria, Nitrosomonas and Nitrobacter, to function and proliferate in the soil is dependent upon various environmental influences. While the autotrophic bacteria, that consume ammonium to produce nitrite and nitrite to yield nitrate, respectively, function at adequate levels when environmental conditions are optimum, no soil system can provide the bacteria with optimum conditions. The sensitivity of the nitrifying bacteria to soil temperature, pH, soil moisture, and nutrient supply for the bacteria cannot be overlooked. Furthermore, soil aeration, organic matter, soil depth, particle size and plant roots and their excretions are all variables that interact to inevitably affect the efficiency of the oxidation pathway of ammonium to nitrate by the nitrifying bacteria.

The optimum temperature for nitrification is approximately 30° C. Above temperatures of 30° C the ability of the nitrifying bacteria to produce nitrite and nitrate declines rapidly. Below 30° C there is a progressive decline in the production of nitrite and nitrate. As the soil temperature approaches 0° C the activity of the nitrifying bacteria almost ceases. However, nitrification has been observed to occur at 0° C after the bacteria have become adjusted to the lower temperature, even though nitrite and nitrate production were not significant.

In spite of this high optimum temperature indigenous nitrifying bacteria are able to function adequately in soils that do not experience the higher temperatures of some temperate regions.

A soil pH that is near the neutral point favours the nitrification process. The Nitrosomonas species of nitrifying bacteria that converts ammonium to nitrite can effectively tolerate a slightly higher pH than the Nitrobacter species of nitrifying bacteria that converts nitrite to nitrate. The indigenous nitrifying bacteria in a soil seem to have an optimum soil pH value where the bacteria function adequately for that particular soil type. Extremes in soil pH can result in a significant decrease in the activity of these bacteria. Such extremes in soil pH have been caused by the addition of large quantities of ammonium fertilizer and over liming. If the variation in the soil pH due to either of these variables is not great, the indigenous nitrifying bacteria will eventually adjust to the change and will continue to function effectively.

Soil moisture and soil aeration are closely related in their effect upon the rate of oxidation of ammonium and nitrite. Poor soil aeration which in some instances can be attributed to high soil moisture content can result in a significant depression of nitrification. An oxygen content in the soil that closely resembles that of the air is the optimum level for the nitrifying bacteria to function. In soils saturated with water, denitrification is enhanced due to a lack of oxygen. The optimum soil moisture level lies in the area between one-half to two-thirds of the soil's moisture holding capacity.

Large quantities of organic matter present in a soil system can lower the nitrifying capacity of that soil. This is due to nitrogen

immobilization by other soil bacteria involved in the decomposition of the organic matter.

In nature it is almost impossible to achieve optimal environmental conditions for nitrification. With seasonal and temperature changes, variations in soil moisture and oxygen supply and nutrient supply, the nitrifying bacteria must continually adjust to the conditions. Conditions for nitrification to take place are most favourable in the surface layers of soil. At greater soil depths, temperatures are lower, soil moisture levels are higher while oxygen levels and energy substrates are lower. These are conditions that inevitably result in lower nitrification of ammonium. Consequently, it is in the surface layers of soil where nitrification readily occurs since the environmental variables are most favourable.

Besides the previously mentioned factors that influence the nitrification of ammonium and nitrite, there are other conditions which affect the nitrifying bacteria. These include the flushes of nitrification of ammonium and nitrite that occur with drying and wetting of the soil. Moreover, agriculture practices have altered the nitrifying capacities of the soil. Irrigation, application of various herbicides and pesticides, cultivation, liming and the use of various nitrogen fertilizers all change the soil's environment causing changes in the activity of the nitrifying bacteria.

The Effect of Plant Roots on Nitrification

The effect of plant roots and plant root excretions has received a considerable amount of attention from both the soil and plant scientist. While some have reported the stimulation of the oxidative process

in the presence of plant roots and plant root excretions, others have found no such stimulation of the nitrifying population. On the other hand, they have observed a suppression of nitrification under the influence of the plants' nutrient supply system.

Lyon et al. (1923) observed a decrease in nitrate production in soils producing crops compared to fallow soils. This occurred even after all allowances had been made for the plants' consumption of soil nitrogen. They suggest that the soil nitrate was consumed by other microorganisms which were influenced by the carbonaceous material produced by the plant roots. There was no inhibition of nitrification but rather a consumption of the nitrate produced. This was based on the fact that nitrate under maize and wheat could not be entirely accounted for in comparison to fallow soil. During the first 57 days of growth, maize resulted in a depression of nitrate production while wheat had an even more pronounced depressing effect. Addition of dried roots of oats, timothy, maize and clover also resulted in lower nitrate levels compared to soils where no plant roots were added. Consequently, they concluded the lower levels of nitrates could be attributed to consumption by other organisms favoured by the presence of carbonaceous material evolved by plant roots, rather than a suppression of nitrification. Furthermore, they believed that nitrate levels observed with different plants could be correlated to the quantity of carbonaceous matter deposited by their respective roots.

On the other hand, Theron (1951) provided a different explanation for the lower levels of nitrate observed under cropped soil compared to fallow soil. He suggested that assimilation of nitrate by other organisms would require that the plant liberate an excess of energy

substrate in the form of carbonaceous material which was theoretically impossible. He further substantiated this by the fact that nitrate began to accumulate in the soil after the millet produced on that soil had reached maturity. At this time the dead roots would presumably supply large quantities of energy substrate for the nitrate-consuming bacteria. Moreover, he believed that grasses would be nitrogen deficient if energy substrate was supplied for the consumption of nitrate utilizing bacteria. However, he found large quantities of ammonia under grasses. Therefore, Theron (1951) concluded that while ammonification was not affected by the presence of plant roots, nitrification was affected by the presence of plant roots. Substances exuded into the soil by the plant roots had a bacteriostatic effect on the nitrifying population, thus accounting for the lower nitrate levels in cropped soil. However, with annual plants an initial stimulation of nitrification occurred during early development followed by suppression as maturity progressed. Lyon et al. (1923) also noted an initial stimulation with maize.

The plant, Theron (1951) suggested, was a conserver of soil organic matter by limiting the turnover and eventual loss of nitrogen. With annual plants conservation would end shortly after maturity occurs; thereafter nitrification again commences. After the establishment of a perennial crop the conservation lasts until the crop is interrupted. Organic matter was consequently preserved until that point was reached.

In a study of eight different crops, Goring and Clark (1948) observed no significant differences in the population of Nitrosomonas and Nitrobacter between cropped and fallow. However, in the latter

stages of plant growth not all mineral nitrogen under crops could be accounted for compared to fallow soils. While others, Theron (1951) and Lyon et al. (1923), saw an initial stimulation of nitrification, Goring and Clark (1948) found no stimulation of nitrogen mineralized initially. Nitrogen mineralized under cropped soils was greater after 9 weeks of growth compared to 5 weeks of crop growth. Significantly less nitrogen was mineralized at 13 weeks in the cropped soil compared to fallow soil. Mineralization in fallow soil had a positive correlation with time.

Nitrogen mineralized had reached its peak in cropped soil when the crop had achieved its greatest growth. Thereafter, there was an unaccountable loss of mineral nitrogen from the soil. Correlation of a number of variables indicated that "the greater the amount of organic material lost by the roots and the lower the nitrogen content of that material, the less mineral nitrogen will be found in the soil," (Goring and Clark, 1948). They suggest that denitrification may be a possible explanation. The authors, however, believe that immobilization would account for the losses that occur under cropped soil. This was substantiated by a negative correlation between root weight and nitrogen mineralized and a positive correlation between percent nitrogen in the roots and nitrogen mineralized. During incubation studies with addition of root material from bromegrass, Power (1968) found the production of nitrate was dependent upon the percent nitrogen in the root material. Immobilization of nitrogen was noted with the addition of roots containing 0.84% N from unfertilized plants. Roots containing 1.44% N from fertilized plants resulted in mineralization. The amounts of nitrogen immobilized for roots low in nitrogen and the amounts mineralized for roots high in nitrogen were proportional to the amount of root

material added.

While Goring and Clark (1948) did not find any stimulation of Nitrosomonas nor Nitrobacter in potted soil under different crops, Molina and Rovira (1964) reported a stimulation in the population of both nitrifiers after 15 days growth under corn and alfalfa. After 46 days, though, no stimulation occurred. On the other hand, proliferation of Nitrosomonas was inhibited entirely by high concentrations of collected corn root exudate after it was added to a sterile media of Nitrosomonas. A lower concentration caused less inhibition. Nitrobacter was stimulated by corn root exudate. At high concentrations, alfalfa was inhibitory to Nitrosomonas while at low concentrations it was stimulatory. Alfalfa exudate did not affect Nitrobacter at any concentration. The authors speculated that the differences between the two entirely different environments under which their study and that of the former authors would account for the differences obtained. In the soil system, the exudates would be subjected to other on-going processes in the soil, i.e., absorption by clay particles and breakdown by heterotrophic bacteria. Exudates added to sterile cultures would not be affected in this way. Because of increased numbers of heterotrophic bacteria over the nitrifying population in their root system studies, Molina and Rovira (1964) suggest immobilization would account for lower nitrate levels rather than inhibition of the nitrifying bacteria.

In examining the populations of nitrifying bacteria in a cut-over and undisturbed forest system Smith et al. (1968) proposed three alternatives which could account for the larger numbers of Nitrosomonas and Nitrobacter bacteria found in the cut-over forest. In the first instance, removal of the water shed system would provide more ideal

environmental conditions. Secondly, the nitrifying bacteria would have a better access to ammonium. This was attributed to greater activity of heterotrophic bacteria and less competition from the vegetation. Finally, cutting of the forest removed any possibility of the vegetative growth exerting any influence.

Earlier, Rice et al. (1964) demonstrated that bacteriostatic factors to the nitrifying bacteria were present in the species of plants which developed in abandoned fields. They postulated that this was a characteristic in plants requiring only a small amount of nitrogen for survival. Fields went through four stages of regrowth. These were weed, annual grass, perennial bunch grass and true prairie. Using extracts of entire plant roots and plant tops applied to media supporting Nitrosomonas and Nitrobacter, the plant species found in the weed stage nearly all exhibited inhibitory activity at some point during their growth on the nitrifying bacteria.

In the annual grass stage Aristida oliganhta, a small grass, had the greatest inhibitory effect on the nitrifying bacteria. Andropogon scoparius, a predominant species in the third stage, was inhibitory to the nitrifying bacteria while in the latter stage this species as well as Erigeron strigosous had a pronounced inhibiting effect.

During later work, Rice (1965) in an attempt to identify the inhibitor, concluded that several of the inhibitors were polyphenols or gallotannins. These substances were extracted from plant leaves rather than from roots, though.

Another study (Rice et al. 1972), devoted to determining the effects of a three stage ecosystem development, indicated that nitrate levels were highest in the first stage. Thereafter, a progressive fall in

nitrate levels occurred in the second stage while in the third stage the lowest levels of nitrate were noted. An opposite trend occurred in the levels of ammonium. The results were significant. The authors, Rice et al. (1972), speculated that "inhibition of nitrification started during old field succession and increased in intensity as succession proceeds toward climax," Rice et al. (1972). No effect on the low nitrate levels could be attributed to the quantity of organic carbon present and nitrate uptake by plants. Consequently, only inhibitor substances produced by the plants would account for low levels of nitrification. They suggest that this feature would conserve not only nitrogen, but energy as well.

Somewhat similar results were obtained by Neal (1969). Root extracts of dominant and co-dominant grass species did not cause any inhibition of nitrite producing bacteria nor nitrite consuming bacteria. The situation was entirely different for increasing and invading grasses and forbs. While there was no inhibition in nitrite production except for a few isolated cases there was a marked inhibition in nitrite metabolism. In the instances where an inhibition of both oxidative steps occurred, Neal (1969) suggested that this was a mechanism whereby the species were able to compete more effectively for establishment.

Incubation studies carried out by Munro (1966b) indicated that Nitrobacter was sensitive to a heat liable substance found in grass roots of Hyparrhenia filipendula. This grass was common to a grassland climax in the Rhodesian Highveld. Results showed that the bacteriostatic substance was found in the outer tissues of the roots. Moreover, during the summer months it occurred in the stele. Counts of viable cells of the nitrite oxidizing bacteria demonstrated that the substances

were bactericidal. The substances were water soluble and heat labile. The author, Munro (1966b), concluded that the results from the in vitro study did not necessarily occur in vivo.

In addition, Odu and Akerele (1973) reasoned that the extract from plant tissues of toxic factors was not necessarily the characteristic of active roots to produce such substances with any consistency in the soil to affect micro-organism. Root and soil extracts did not result in any depression in the rates of nitrification. In spite of this fact, the possibility was not discredited that in natural conditions, plant roots are continuously producing exudates and coupled with a decreased capacity of absorption by the soil particles of these substances, toxic levels could be produced.

Contrary to the results of Munro (1966a and 1966b) and Neal (1969), Purchase (1974) working with washings of the same grass species as used by Munro (1966a) did not observe any inhibition of nitrification. While root extracts from the grasses studied inhibited nitrate production in the in vitro study, no inhibition was noted during in vivo experiments. Immobilization of mineral nitrogen accentuated by decaying grass roots would account for lower nitrification levels rather than an inhibiting effect, Purchase (1974) claimed. This was supported by the adaptability of plants to low nitrogen levels.

Earlier work conducted by Boughey et al. (1964) was supported by the studies of Munro (1966a; 1966b) and Neal (1969). Boughey et al. (1964) concluded that toxins are indeed produced by the Hyparrhenia species. This toxin, they speculated, may be destroyed by grass fires or removed by heavy rains. Their speculation was based on the observed flush of growth after the occurrence of either of these two factors.

In a study of the nitrifiers under the indigenous grass of the Hyparrhenia species Meiklejohn (1968) found low counts of the nitrifying bacteria. However, as Boughey et al. (1964) had observed, nitrifiers were more abundant at the start of the rainy season. Although the quantity of nitrifiers under the native grasses was low, the bacteria proliferated upon ploughing, fertilizing and cropping of that soil. Meiklejohn (1968) failed, though, to provide a plausible explanation as to why this occurred. Nevertheless, it would seem that the native soils were low in substrate which would support nitrifying bacteria.

Brar and Giddens (1968) proposed a different alternative for the low nitrate levels in a Balden grassland soil. They believed that low nitrate levels were caused by an absence of nitrifying bacteria. In an effort to discredit the toxic substance theory, no increase in nitrification rates were observed after an attempt was made to remove the substances with alcohol and hot and cold water extracts. Inoculation of the Balden soil sample resulted in increased activity by the nitrifiers. Moreover, liming of the soil did not bring about instantaneous action by the nitrifying bacteria.

Robinson (1963) provided evidence that nitrification under grassland soil was low due to the absence of nitrifying bacteria rather than toxic effects. The percolation of a grassland soil with an ammonium sulphate solution resulted in a lag phase of 50 days. Liming shortened the lag to some extent. Furthermore, inoculation of the limed soil with garden soil at the rate of 1 gram added to 35 grams resulted in rapid nitrification of the added nitrogen source. However, Robinson (1963) observed that the indigenous nitrifying bacteria could operate more efficiently than the populations in the inoculum. In addition,

low substrate levels in the grassland soils was a factor responsible for the low nitrification rates. This was supported by the fact that after an application of urea and lime to the grassland soil with a low pH, nitrification took place rapidly. In contrast the limed control exhibited a considerable lag in nitrification of any substrate present in the soil.

Poor competitive ability for substrate with heterotrophic microorganisms would account for the decreased and inactive nitrifying bacteria in the grassland soils. In contrast, arable soils have higher substrate levels and can, consequently, support higher numbers of nitrifiers.

Nakos (1975) also attributed low nitrifying capacities of soil to the absence of nitrifying bacteria. This idea was supported by the fact that no toxic substances were present in the forest soil under study. Furthermore, the liming of this acid soil did not increase the nitrification activity. In addition, inoculation of a nitrifying soil with a non-nitrifying soil failed to produce any suppression of nitrification. A somewhat positive correlation for nitrate production and negative correlation for ammonium consumption between the amount of inoculum added to a non-nitrifying soil was seen.

No evidence could be obtained by Soulides and Clark (1958) that toxic factors were deposited by grass roots during an incubation study with a soil that had supported grass plants. This occurred in soils that were not nitrogen amended. Soils amended with 466.6 ppm urea nitrogen produced different results, though. The content of ammonia in amended grassland soil was high while in the tilled soil nitrification was greater. Immobilization of nitrate could not account for the

poor nitrification rate in the grassland soil, consequently, the authors agreed that some toxic substances in the grassland soil exist to inhibit nitrification.

Ketcheson and Jakovljevic (1970) suggested that immobilization would account for nitrogen losses. Furthermore, gaseous transformation could also be an important consideration. Only 80% of added ammonium could be extracted after the shortest period between addition and extraction. However, this ammonium was nitrified eventually. A chemical fixation was suspected for the 20% loss that occurred. In addition, only 60% of the nitrogen added to the soil in the form of nitrate, supporting barley plants, could be recovered compared to a 90% recovery in fallow soil. The growth of roots, they hypothesized, leaves the soil in a favourable condition for the rapid disappearance of nitrate. Since the nitrate was formed rapidly in the soil, the nitrogen was even more susceptible to denitrification. A possible cause of this occurring was the lower oxygen content of the soil in the presence of plant roots and the H^+ donors in the rhizosphere.

In the absence of plant roots, added nitrogen was recovered to the extent of 90%. With plant roots present in the soil and after nitrogen present in the plant material had been evaluated, recovery of added nitrogen was only 60%.

Stefanson (1972) also indicated that lower oxygen levels due to actively growing roots and addition of exudates from those roots could result in lower nitrate levels caused by denitrification. Consequently, the soil system would be in a situation that promoted rapid denitrification.

In studying the numbers of Nitrobacter per area of root surface on wheat and nodulating and non-nodulating soybean roots, Rennie et al. (1977) concluded a number of relationships existed. Per area of root the numbers of Nitrobacter decreased due to increased root area as a function of time. Wheat had the highest concentration of nitrate producing bacteria per surface area of the plants' roots. Wheat and soybean roots induced no stimulation or inhibition of Nitrobacter populations when grown without any externally applied substrate. With the addition of soybean meal to soil containing wheat and soybean plants a proliferation of Nitrobacter was noted. The effect was greater for soybeans than for wheat. Addition of ammonium did not result in any proliferation.

Vigorously growing plants were observed by Katznelson (1946) to support larger populations of soil micro-organisms. The population of the soil rhizosphere was dependent on the treatment applied to the soil. Soil with no fertilizer supported fewer micro-organisms than a soil which had received barn yard manure. While the numbers of nitrifying bacteria surrounding the roots of mangels were low, it was suggested that "the intense activity of other organisms in the rhizosphere of vigorously growing plants may suppress these bacteria," Katznelson (1946). In comparison to the low populations of nitrifying bacteria, large numbers of ammonifying and denitrifying bacteria were noted. Activity of these two different micro-organisms occurred when the plants were growing profusely.

Similar trends occurred when Slavnina (1971) observed ammonification in the rhizosphere of winter rye, wheat and oats. Exchangeable ammonia was from one and one-half to three times higher in the root zone

as opposed to the area outside the root zone. The tests conducted with soil removed from field plots were significant. Fluctuations during the season of the year in the inner rhizosphere corresponded to changes in the ammonia level of the outer rhizosphere. It was suggested that "the intensity of ammonification will vary with the hydrothermal conditions, crop grown, soil group and even subgroup," Slavnina (1971).

Nitrate levels were not necessarily always higher in the inner rhizosphere compared to the outer rhizosphere. Plant utilization was thought to be responsible for this occurrence. Nitrate content of the soil declined as the growing season came to an end. Generally, oats were able to mobilize greater quantities of exchangeable ammonia and nitrate than was rye. During the growth of the plants, nitrate content of the soil was higher in the tillering and earing stages. Slavnina cites this fact from an earlier study, Slavnina et al. (1958).

Regulation of the release of nitrate nitrogen was, according to Gupta and Reuszer (1967), a characteristic of any particular soil. Inoculation of soils which had supported alfalfa, bromegrass and corn over a 9 week period produced varying results. The alfalfa plot produced about 70% more nitrate than the other plots. However, there was no difference between any of the plots in the percent of total available nitrogen nitrified. Khan and Moore (1968) suggested two different mechanisms may occur in the soil whereby nitrate levels are found to be lower. Either plants remove nitrate rapidly thereby preventing loss by denitrification or they provide more favourable conditions for denitrification.

Rye grass seedlings grown for 28 days in 350 grams of soil by Cornish and Raison (1977) mineralized significantly more nitrogen with the

addition of 60 ppm phosphorous. As well, there was a significant increase in root weight. The addition of nitrogen to the soil supporting the plants did not increase mineralization. Incubation of three different soils with 0, 30 or 60 ppm phosphorous produced no differences in the amount of nitrogen mineralized. Growth of plants in the three soils with 0 or 60 ppm phosphorous and no additional nitrogen demonstrated that the nitrogen mineralized was proportional to plant growth. Mineralization was not related to a phosphorous deficiency. The increase in root growth due to the added phosphorous produced a greater rhizosphere effect. Nitrogen mineralizing bacteria, ammonifying and nitrifying organisms, are higher in the rhizosphere, consequently they can deposit their products at a site easily accessible to by the plant roots. Immobilization would, therefore, be less likely to occur.

Rouatt et al. (1960) found with spring wheat that ammonifying, denitrifying and other micro-organisms were significantly higher in the rhizosphere. This also occurred with barley and soybean plants. Moreover, there was a significant increase in the numbers of amino acid requiring bacteria in the rhizosphere of all three plant species. It was suggested that due to the presence of root exudates forming a source of substrate, soil micro-organisms would be preferentially stimulated. Though no attention is directed to the nitrifying bacteria, denitrifying bacteria proliferated in the rhizosphere. Consequently, it would be assumed that since denitrifying bacteria are stimulated whereas the nitrifying organisms are not stimulated. This would account for the lower nitrate levels under plant roots.

Poor grass growth in a Hyparrhina grassland, Purchase (1974) suggested, was caused by a nutrient deficiency which also restricted

nitrifying bacteria. Addition of ammonium sulphate to a Hyparrhina grassland increased the proliferation of nitrite producing and consuming bacteria as well as an increase in plant yield. Large populations of nitrifiers under high yielding grasses would not support the theory that these bacteria are suppressed by toxic root substances, as found by Theron (1951).

While Cornish and Raison (1977) have shown that nitrogen mineralized was dependent upon root growth which was influenced by phosphorous levels, Purchase (1974) believed that a phosphorous deficiency in savanna grassland soils restricted the activity of the nitrifiers as well. Inoculation of medium showed that nitrite oxidizers were more sensitive to low phosphorous than were ammonia oxidizers. However, no evidence was supplied to demonstrate that under plant growth a phosphorous deficiency actually existed to restrict nitrification.

Introduction of rape, ryegrass and lettuce root washings into a soil column with a steady state of nitrification by Moore and Waid (1971) reduced nitrate levels from 400 mg N per Kg soil per day to 100 mg N per Kg soil per day. Additional leachings of the soil column caused the disappearance of ammonium to occur at the same rate of nitrate appearance. Cessation of the leaching resulted in increased nitrification. Ryegrass, however, had a longer lag phase in the commencement of nitrification as compared to rape and lettuce. Reintroduction of the leachings into the soil columns caused a slight decrease in nitrification with lettuce and rape leachates. With ryegrass the effect was more severe for a longer period of time. Wheat and onion were also found to decrease nitrate production in a steady state of nitrification in a soil column.

Moore and Waid (1971) assumed that denitrification or immobilization occurred in the soil columns used. They suspected the nitrification process was interrupted. Since no nitrite was evident, the oxidation of ammonium was believed to be the point at which the mechanism was inhibited. Several reasons were advanced for the occurrence of this interruption. Active substances in the root washings could prevent the nitrifying bacteria from functioning. Through chemical or biological reactions taking place in the soil the toxic factor may be activated or increased. Another alternative, they suggested, was the blocking of the sites of nitrification in the soil by organic substances found in the root leachates. Removal of this blockage by the soil micro-organisms would eventually leave the site available for nitrification again.

During studies of wheat in field plots Carpenter et al. (1952) observed an accumulation of nitrates during tillering of the plants. This occurred 21 days after the wheat plants emerged. The 12 to 24 inch soil depth had the largest concentration of nitrates. Furthermore, soils which had been fallow the previous year were higher in nitrates than were stubble soils. While nitrate levels were low at heading of the wheat plants, an increase took place in their levels prior to maturity. It was concluded that soils with high concentrations of nitrogen produced greater quantities of nitrates and furthermore, the production continued into the latter growth stages of wheat.

In spite of the fact that a vast amount of information has accumulated on the effect of roots and root exudates on the nitrifying bacteria, no general consensus has been reached on the actual process that occurs. A number of theories have been consistently upheld. The possibility that plant roots secrete toxic substances that inhibit the

nitrifying bacteria has been shown to exist. On the other hand, the deposition of root debris, root excretions and dying roots, all material highly carbonaceous, has been observed to promote immobilization of inorganic nitrogen. Furthermore, the conditions present in the rhizosphere have been found to provide an environment conducive to denitrification.

The approaches used to study the problem have been different and varied. Studies in the field have been employed. Incubation of soil samples in the laboratory have been carried out. Extracting and leaching of root substances have been studied. Perhaps the variation in the methods used to study the activity of the nitrifying bacteria have provided artificial environments which do not reflect the conditions that occur in actuality. Nonetheless, the results cannot be disputed. Since the variations existing in soil characteristics and soil-plant relationships are so different, no judgement can be made which would embody all of them.

MATERIALS AND METHODS

The ability of the nitrifying bacteria to nitrify indigenous ammonium and ammonium added in nitrogen fertilizer may vary in different soil-plant systems and soil systems alone under varying conditions. A number of experiments were conducted in the laboratory to evaluate the processes which affect nitrate levels in the soil plant system.

Experiment 1. Incubation of soil at four temperatures and two nitrogen sources at six rates of application.

The soil used during this study was the type employed by the Plant Science Department at the University of Manitoba for the growth of plants in the greenhouse. The soil was blended to produce a mixture containing two parts soil, one part peat and one part sand. The soil used was a dark medium textured soil. Prior to blending of the soil, the components were sieved to pass through a number 2 mm screen. This eliminated any large clods of soil, large pieces of peat and any large particles of sand or stones. Mixing of the aggregates took place until a homogeneous mixture was obtained. The soil produced after mixing had a pH of 7.8 and a conductivity of 0.47 mmhos cm^{-1}. The bulk density of the soil was 1.2 g/ml and had a field capacity of 57% moisture. Throughout the incubation period the moisture level of the soil was maintained at 50% of field capacity.

Urea (H_2NCONH_2) and ammonium sulphate ($(NH_4)_2SO_4$) were added in granular form on a weight-to-weight bases to individual samples from

the bulked soil to give 0, 50, 100, 200, 500 and 1000 ppm of added N. To plastic containers with aeration provided by means of holes in the covers, 180 grams of soil on an oven-dried bases was added. Each level of added nitrogen was produced in duplicate. Distilled deionized water was added to bring the moisture to the required level. The containers with the soil were placed with air temperatures of 1° C, 4° C, 19° C and 20° C. Samples of about 4 grams of soil were removed weekly over a 12 week period. Nitrate was measured initially and at the weekly intervals by using the Brucine method for nitrate determination as described by Chapman and Pratt (1961). Standard curves were run together with the samples at each analysis in order to minimize possible effects of day-to-day variations in the chemical procedures. The quantity of nitrate produced was considered the rate at which nitrification occurred.

Experiment 2. Soil nitrate content as influenced by the presence of plant roots.

In order to assess nitrate levels in the soil under the influence of growing roots, pots containing various species of cereal grains were evaluated. The experiment was a split-split plot design. Two levels of applied nitrogen were used. Sampling took place on four different dates of five different crops.

The soil used to grow the plants consisted of the same aggregates blended together in the same proportions as in the previous experiment. From the bulked soil sample, two subsamples were prepared. To one subsample of soil, 200 ppm N of granular ammonium sulphate $(NH_4)_2SO_4$ was added on a weight-to-weight bases. The other subsample received no additional nitrogen. Both subsamples were thoroughly mixed individu-

ally until the soil was homogeneous. Ammonium sulphate was chosen as a nitrogen source since it was a familiar source of nitrogen.

The soil from each subsample was placed at the rate of 1000 grams on an oven-dried basis in 15 cm diameter pots lined with plastic bags. The purpose of the plastic bags was two-fold. In the first instance it facilitated the removal of the entire plant and subsequent separation of root material from the surrounding soil. Secondly, the lining prevented the loss of any water applied. Consequently, this would prevent the loss of any soil nitrate through leaching.

Three replicates of five different crops were used. The crops consisted of no crop which acted as the control; wheat, Triticum aestivum var. Naypo; barley, Hordeum vulgare var. Bonanza; rye, Secale cereale var. Prolific; and tricicale, X Triticosecale var. Welsh. Five seeds were placed in each pot. Upon emergence each pot was thinned to two plants and subsequently to one plant per pot. Plants were grown under greenhouse conditions receiving 16 hours of daylight and 8 hours of darkness.

Nitrate content of the soil was determined initially. Thereafter, soil and plant material were removed at 3, 6, 9 and 13 weeks. During sampling, top growth was removed at the surface of the soil. A soil sample of about 10 grams of soil was removed from the rhizosphere before the roots were entirely separated from the soil. The roots were shaken free of any remaining loose soil and then washed to remove any other soil. The remaining soil was then sieved to remove any root parts that remained.

The tops and root material and soil were oven dried prior to analysis. The nitrogen present in the tops was determined by the macro

Kjeldahl method while that in the roots was determined by the micro Kjeldahl method after being ground to a fine powder. Soil nitrate was determined by the Brucine method as described by Chapman and Pratt (1961).

The level of nitrate found in the soil sample was converted from ppm NO_3^- to mg N found per 1000 grams of soil. The amount of nitrogen found in the soil was combined with the amount of nitrogen found in the shoots and roots. This value was taken as the amount of nitrogen present in the system that had been in the form of nitrate.

Experiment 3. Measurement of soil pH at two levels of added nitrogen.

In order to measure the effect of changes in the soil pH caused by nitrification of ammonium sulphate, $(NH_4)_2SO_4$, incubation of soil samples containing no added nitrogen and 200 ppm NH_4^+ - N was carried out. Plastic containers containing 50 grams of oven-dried soil were prepared. The soil used was the same as that employed in the previous experiments. At weekly intervals over a 10 week period three replicates of each nitrogen level were removed. The NH_4^+ - N was added in an aqueous solution of ammonium sulphate. Nitrate in the soil samples was determined by the Brucine method of nitrate determination as described by Chapman and Pratt (1961). The soil pH was determined by a 1:2 soil water paste with a pH electrode.

Experiment 4. The influence of added nitrogen on the rhizosphere pH and nitrate levels in fallow and cropped soil.

The purpose of this study was to determine the effect that barley roots and wheat roots had on the nitrate levels and rhizosphere pH due

to nitrification of ammonium sulphate. The treatments consisted of a control where no crops were grown and wheat and barley plants were grown.

Pots lined with plastic bags were filled with 600 grams of soil on an oven-dried bases. The soil was a blend of those aggregates as described in Experiment 1. The soil was amended with ammonium sulphate $(NH_4)_2SO_4$ in granular form to give 200 ppm nitrogen. The plastic bags made removal of the soil and roots easier as well as preventing the leaching of nitrate from the soil. In those treatments where a crop was grown two seeds of wheat, var. Naypo, and of barley, var. Bonanza were placed in the pots. After plant emergence, the plants were thinned to one plant per pot. Sufficient pots were prepared to allow for the removal of three representatives for four sampling intervals of 3, 6, 9 and 13 weeks. Thirteen weeks was chosen as the final date for sampling because at this time the wheat and barley plants had fully matured.

The pots were placed in a growth chamber with 16 hours of light and 8 hours of darkness at temperatures of 20° C and 15° C, respectively. Moisture content was maintained at two-thirds of field capacity for the 13 weeks. Distilled deionized water was added as required to bring the moisture content of the soil up to the required level.

Soil for the analysis of pH and nitrate in uncropped soil consisted of the removal of 100 grams of fresh soil. In the cropped soil about 100 grams of fresh soil was removed from the outer area of the roots. This soil was designated the outer rhizosphere soil. It consisted of the soil surrounding the roots that was removed by gentle shaking of the roots by hand. The inner rhizosphere soil consisted of about 100 grams of fresh soil. This was removed from the inner root mass that had

formed by a much harsher shaking than was used previously. Any root material present in these soil samples was removed.

The soil pH was determined by a 1:2 soil water paste with a pH electrode. The soil nitrate was determined colorimetrically by the Brucine method as described by Chapman and Pratt (1961). Plant nitrogen was determined by the macro Kjeldahl method.

These experiments were designed to employ conditions which were well away from the optimum for the nitrification process and for growth of the nitrifying bacteria, i.e., very high moisture, high temperature and high levels of ammonium. On the grounds that by working under optimum conditions one might expect to see only inhibitory effects of plant roots whereas under conditions where environmental factors are limiting, both positive and negative effects might be seen.

Experiment 5. The effect of root and stem leachate on nitrate production during incubation of a soil.

Pots containing three wheat plants (var. Naypo) per pot and pots containing three barley plants (var. Bonanza) per pot were grown under greenhouse conditions in sufficient numbers to facilitate removal of three pots of wheat and three pots of barley over a 6 week period.

The soil used in this incubation study was the same type as used in Experiment 1. Soil in the amount of 50 grams on an oven-dried bases was placed in plastic containers. Enough samples were prepared that would allow the removal of three replicates of a control (no added leachate), wheat root leachate, barley root leachate, wheat stem leachate and barley stem leachate over a 6 week period. An aqueous solution of ammonium sulphate, $(NH_4)_2SO_4$, was applied to individual soil samples to

give 200 ppm nitrogen. This was done four days after the wheat and barley plants had emerged in the pots. Seven days after plant emergence the wheat and barley plants were removed. The roots and stems obtained from the three pots of wheat and the three pots of barley were bulked individually and leached. The leaching apparatus used in this study is described by Lacroix and Staniforth (1964). At weekly intervals thereafter plant roots and stems were removed and leached.

The roots and stems were subjected to leaching for 4 hours in distilled deionized water. The leachates were then concentrated to 150 ml by means of a Rotorevaporator. Individual soil samples received 1/50 of the leachate of the wheat stem, the wheat root, the barley stem and the barley root. Additional distilled deionized water was added to bring the soil moisture up to two-thirds of field capacity. The control soil sample received only distilled deionized water. At weekly intervals thereafter the remaining soil samples received 1/50 of their respective leachates from the developing plants' stems and roots.

Soil samples were kept in a growth chamber for 16 hours at 18° C and 8 hours at 15° C over a 6 week period. Samples were allowed to incubate for weekly periods after the addition of the leachates. The entire 50 gram samples of each replicate were removed at weekly intervals and analyzed for nitrate by the Brucine method of nitrate determination as described by Chapman and Pratt (1961).

RESULTS

Introduction

These experiments were carried out over a 3 year period. Thus variation in the soil, with respect to year, origin and time in storage was to be expected. The original data and appropriate statistical data are tabulated in the Appendix.

Soil mixes similar to those used here are almost universally used in greenhouse and growthroom studies even on agronomic crops in this Department. Their use is usually justified on the basis of increased water retention and better porosity as a result of incorporation of peat. Morita and Montgomery (1980) reported that Canadian peats harvested in Quebec contained between 2 and 10% of dry weight of readily hydrolyzable carbohydrates. The amount and sugar composition of the polysaccharides varied with location of the deposit and depth within the profile. If this material is available to the soil microbes, the increased C/N ratio would result in an increased immobilization of added inorganic N. As well, soil fungi have the ability to degrade lignin-like materials (Garrett, 1963).

Christianson *et al.* (1979) demonstrated accumulation of NO_2^- in prairie soils incubated with high levels of N as ammonium sulphate or urea. Accumulation of nitrite may depend upon combined effects of elevated pH, osmotic potential, ammonia and nitrite, all of which seem to be more inhibitory to *Nitrobacter* than to *Nitrosomonas* (Nakos and

Wolcott, 1972; Wetselaar et al., 1972). Accumulated nitrite could be lost to chemodenitrification through reaction with phenolic materials to form nitrophenols which react further with NO_2 to form N_2O and N_2 gases (Christianson et al., 1979). Rapid microbial degradation of available carbon in the peat could have resulted in anaerobic conditions and a redox potential favoring conversion of NO_3^- to NO_2^- by microbes which can utilize NO_3^- as a terminal electron acceptor in the absence of oxygen (Focht, 1978). Focht (1978) points out that disappearance of nitrate is an adequate criterion for denitrification only if the system is carbon limiting. Addition of organic matter can affect immobilization or denitrification of nitrate. A high C/N ratio and high oxygen would favor the former while low C/N ratio and low oxygen would favor denitrification.

Probably the soil mixes were not carbon limiting. Clearly, variation in the soil and peat used in the soil mixes in these experiments would be expected to have important effects on the form of soil N during the experiments. The results then do not relate to cereal crops grown in the usual prairie soil which is low in organic matter, however, there is considerable research activity within the Department of Soil Science in respect to cereal production on peat and muck soils in Manitoba.

An additional complication is that the reagent used (Brucine) was probably determining both NO_2^- and NO_3^- in these experiments.

Experiment 1. Incubation of soil at four temperatures and two nitrogen sources at six rates of application.

Data on nitrate production at the different temperatures and at the different levels of applied N are given in the Appendix (Tables 1

and 2). Since the differences between 19° C and 21° C and between 1° C and 4° C were very small, the averaged values for the higher temperatures are presented in Figures 1 and 2.

Nitrate production was very low at 1 and 4° C. This was expected since other work (Alexander, 1965) showed the optimum temperature for nitrification to be about 30° C and minimal activity near 0° C. The low temperature curves appeared to be sigmoidal.

At the higher temperatures, at all levels of applied ammonium sulphate and urea, the curves show a well defined lag period of three weeks followed by a rapid rise in nitrification over the next two weeks. The lag and the sigmoid nature of the increase phase probably indicate that low populations of nitrifying bacteria were present in the soil mix used. It is somewhat surprising that the duration of the lag was not affected by the level of applied N since these microbes are autotrophic and gain their energy through oxidation of NH_3^+ and NO_2^-. Increased levels of organic or ammonia nitrogen have been shown to shorten the lag period in nursery soil mixes (Baker, 1957). Some factor other than availability of substrate must have been limiting.

During the period from 3 to 5 weeks, with the exception of the 1000 ppm urea treatment, the slope of the early portion of the curves increased with the level of applied N, as expected from the autocatalytic process.

At the lower levels of applied N, the soil NO_3^- level reached a plateau at about 5 to 6 weeks. Thereafter, the nitrate level fluctuated somewhat, with marked minima being evident at 8 and 11 weeks.

At the higher levels of applied N, particularly at 1000 ppm, accumulation of soil NO_3^- showed two distinct phases. These were separ-

Figure 1. Changes in soil nitrate following the addition of N as $(NH_4)_2SO_4$ at six rates over 12 weeks at a mean temperature of 20° C.

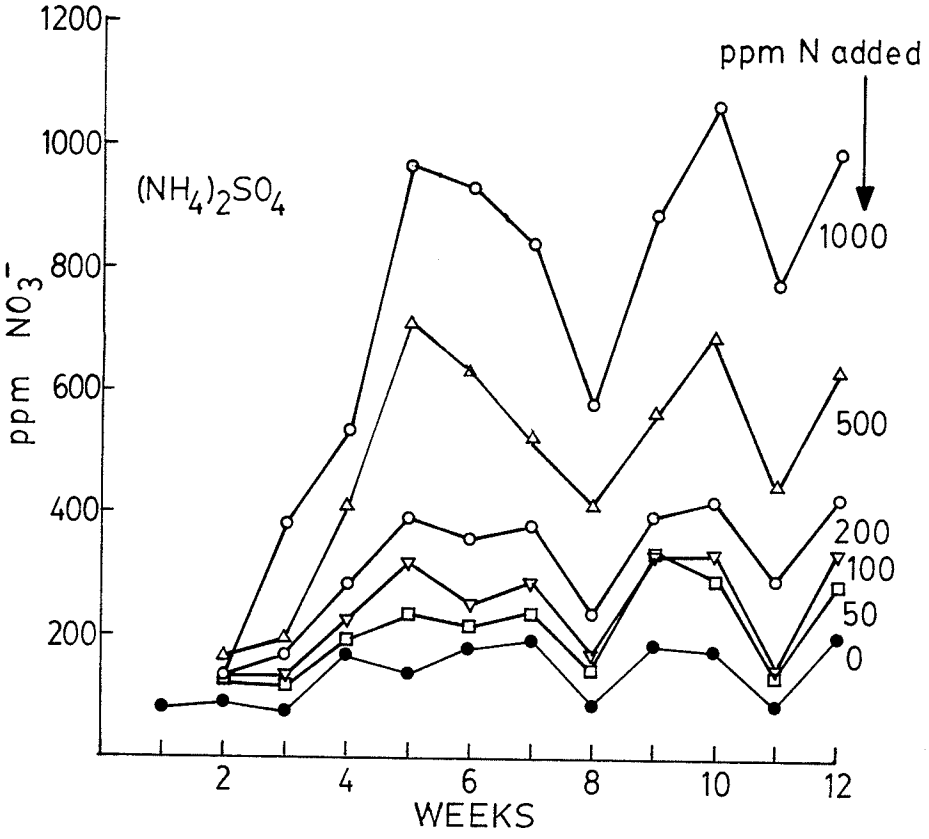
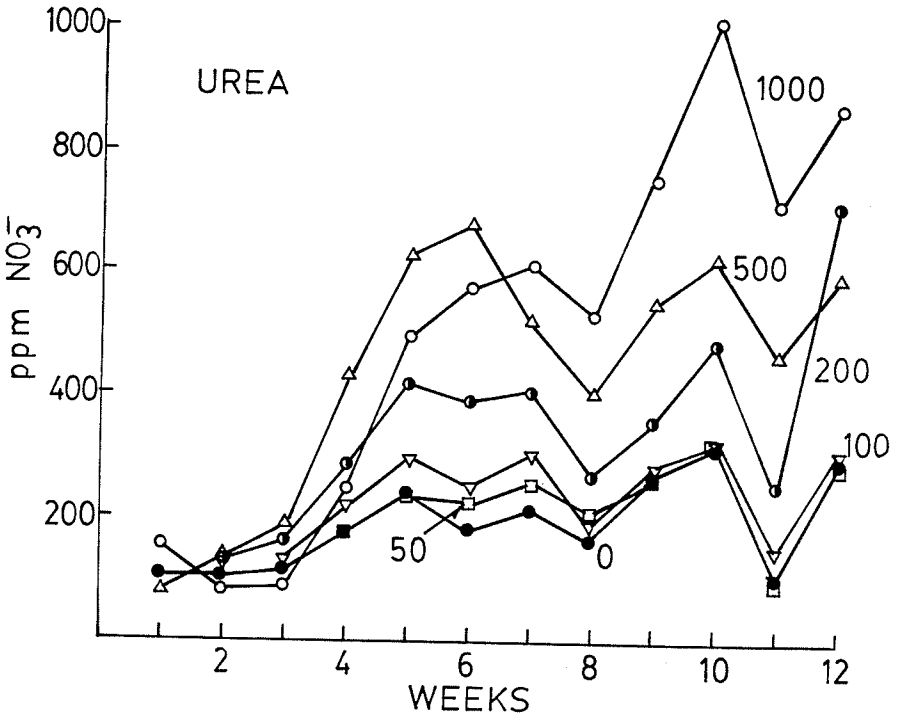

1200
1000
800
600
400
200
ppm NO_3^-
$(NH_4)_2SO_4$
ppm N added
1000
500
200
100
50
0
2
4
6
8
10
12
WEEKS

Figure 2. Changes in soil nitrate following the addition of N as urea at six rates over 12 weeks at a mean temperature of 20° C.


UREA
1000
800
600
400
200
ppm NO_3^-
1000
500
200
100
50
0
2
4
6
8
10
12
WEEKS

ated at about 8 weeks (where a minimum also occurs at low N) but there was a gradual decline in NO_3^- between weeks 5 and 8 followed by a sharp secondary flush of NO_3^- accumulation. The fact that the decrease and increase around the 8 week minimum both occurred over a period of several weeks at high levels of applied N suggests that the minimum was the result of events within the pots and not due to analytical errors which might have explained the minimum in the low N treatments.

Unfortunately, the second minimum in soil NO_3^- at 11 weeks does not receive similar internal support but it did occur in all treatments.

The 8 and 11 week minima are transient declines amounting to 30 to 40% of the NO_3^- present earlier. This occurred in all cases except with 1000 ppm N as urea. Remarkably, their time of appearance like the duration of their lag period was not influenced by the N treatment except that at high N, the decline began at 5 to 6 weeks.

These transient declines in nitrate and/or nitrite could involve denitrification by chemical of microbial reactions which would require low oxygen conditions which would simultaneously limit the rate of nitrification. Denitrification may be made less probable by the fact that following the decline, the levels rose to values as high or higher than existed before. However, this could represent nitrification of endogenous ammonia and organic N in the soil which were not measured in the present experiments. Jones and Hedlin (1971) reported organic N levels of about 0.3% in the Ap horizon of three Manitoba soils (3000 ppm) at a C/N ratio of 4 to 5. Assuming similar values of organic N in our soil samples, mineralization of this material could account for much of the recovery of nitrate levels following the transient declines. As well, the declines could be due to assimilation of nitrate and

ammonia by micro-organisms using whatever portion of carbohydrate in the added peat that was available to them as an energy source. Morita and Montgomery (1980) reported that sphagnum peat deposits in Quebec contained between 2 and 10% of easily hydrolyzable carbohydrate. It is possible that soil fungi were able to mobilize some of the phenolic constituents of the peat (Garrett, 1963). What effect modification of the peat might have on its participation in chemodenitrification through reaction with nitrite as suggested by Christianson et al. (1979) remains to be determined.

The curves for 500 and 1000 ppm added N as urea (Figure 2) appear to be rather different than the other treatments. In particular, the first NO_3^- peak was progressively delayed. Up to 200 ppm added N, NO_3^- curves for ammonium sulphate and urea were very similar. Jones and Hedlin (1972) showed that the rate of urea hydrolysis in Wellwood soil was nearly proportional to added urea levels up to 800 ppm while in Lakeland soil the rate was low and did not increase beyond about 150 ppm urea. In Holland soil, hydrolysis rate was very low and was not influenced by the concentration of urea. Differences in the level of urease activity in the three soils are perhaps associated with differences in microbial activity. But the differing response of Wellwood and Lakeland soils would indicate differences in the nature of the enzyme itself. This was due, possibly, to differences in pH and buffering capacity of the soil or location of the enzyme (i.e., within bacterial cells vs. bound to soil colloids) (Campbell et al., 1976). Bereo and Thien (1979) examined phosphatase activity in the rhizosphere of corn roots. They suggested that the Michaelis constant of the enzyme decreased with increasing organic matter content of the soil because enzyme-substrate

binding was enhanced in the presence of organic colloids. Thus, the delayed appearance of the first NO_3^- peak at high levels of urea could be explained by the fact that enzymatic activity was saturated at urea levels above about 200 ppm N.

This cannot explain the fact that early nitrate production was considerably slower at 1000 ppm than at 500 ppm added N. The overall process must have been inhibited by high urea. High pH resulting from hydrolysis of urea may have been a factor. As well, high pH might have resulted in losses of volatile NH_3^+. Possibly biuret or ammonium cyanate (Baker, 1957; Beaton et al., 1976) which are known toxic contaminants of many urea preparations could be involved.

Under field conditions, assuming uniform distribution through the surface 15 cm, 224 kg/ha of applied N would give a concentration of 100 ppm. Thus, the higher levels of N used in these experiments are unrealistic assuming uniform distribution. However, with banding of the fertilizer and relatively slow movement of mineral N away from the band, much higher local concentrations can occur (Pang et al., 1973; Passioura and Wetselaar, 1972). Levels of soil N above 200 ppm also occur in many horticultural soils (Baker, 1957). High concentrations of ammonium sulphate and urea are known to modify nitrification and denitrification through effects of osmotic potential, high pH, ammonia and nitrite concentrations (Focht, 1978; Pang et al., 1973; Passioura and Wetselaar, 1972). These soil parameters would also be expected to influence activity of other microbes in a selective way and could also influence growth and function of roots directly or indirectly.

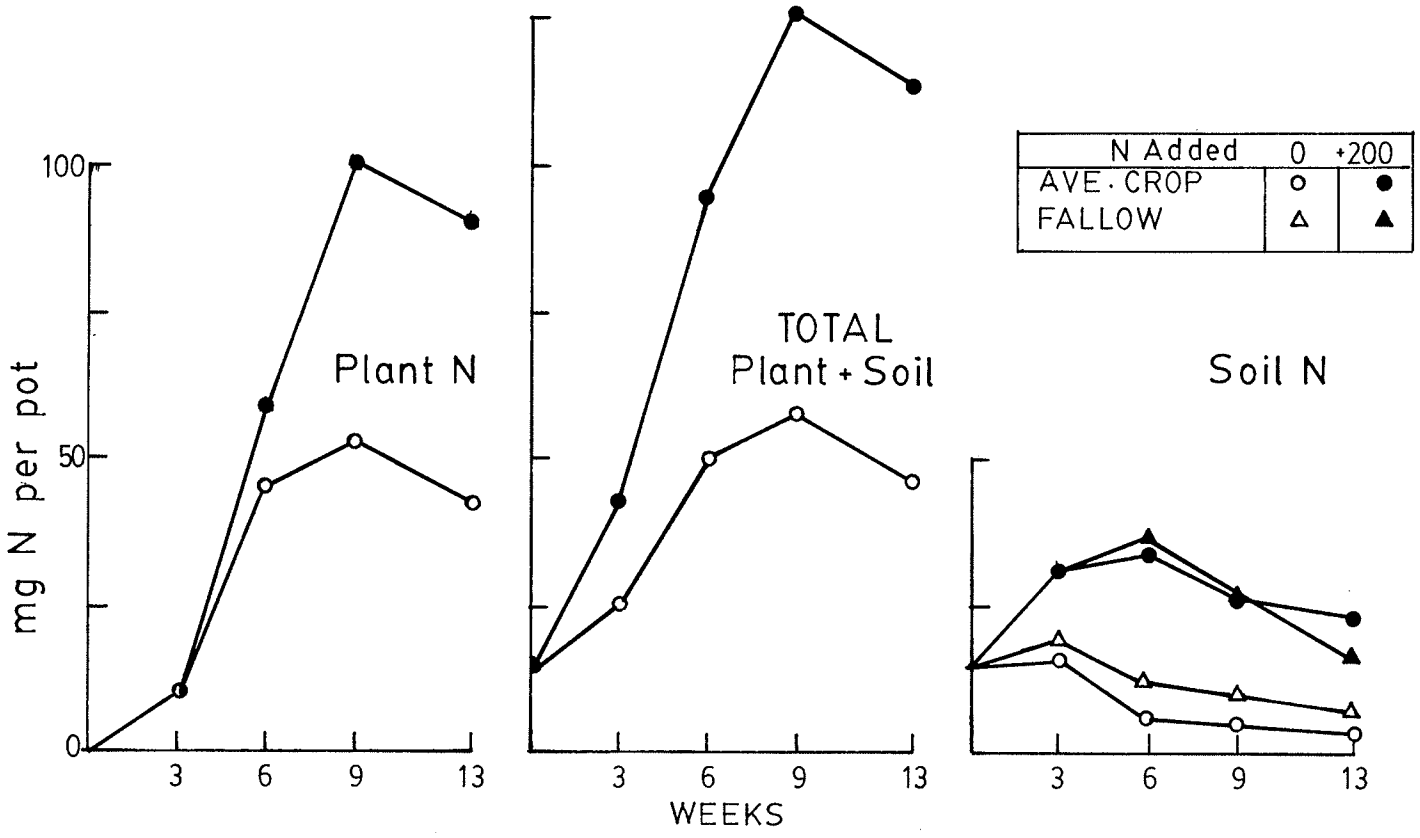
Experiment 2. Soil nitrate content as influenced by the presence of plant roots.

In this experiment wheat, barley, rye and triticale were grown in a soil mix with 0 and 200 ppm of added N as ammonium sulphate. In discussing the results it will be assumed that plant N originated as soil NO_3^-. Usually NO_3^- is the form of soil N most available to plants; NH_4^+ is of low availability since it is bound to soil colloids. It is recognized that plants can assimilate NH_3^+ even in a soil system (Reisenauer, 1976). Unfortunately, given the complex pattern of chemical and biological "demands" which center around NH_4^+ and NO_3^- in a soil, definite experiments even using isotopic N are very difficult to carry out.

The results of this experiment suggest that under the conditions used, the crops may have had differential effects on nitrification. A summary of the data when soil, plant and total N are averaged for the four crops and compared to the "fallow" system is presented in Figure 3.

Without added N, the crops depleted soil NO_3^- - N. This occurred after 6 weeks. On the other hand, soil NO_3^- - N in the cropped soil with additional N supplied was maintained above that of the "fallow" soil except at 13 weeks. In cropped systems, the major increase in plant N was between 3 and 6 weeks. This occurred for both the fertilized and unfertilized soil, however, the plants in the N amended soil continued to accumulate N. Plant uptake and soil depletion of NO_3^- - N followed the same trends for the unamended and N amended soils. In the unamended soil this trend occurred earlier than in the amended soil. Plant N uptake in the unamended soil was nearly complete at 6 weeks. Soil NO_3^- - N levels were at their highest at 3 weeks and thereafter fell as plant N

Figure 3. Changes in levels of Plant N, Total N (Plant N + Soil N) and Soil N averaged for four crops and fallow soil with 0 and 200 ppm N as $(NH_4)_2SO_4$.



uptake had been completed. In the N amended soil, the soil NO_3^- - N was highest after 6 weeks followed by a drop in soil NO_3^- - N after plant N uptake had been completed at 9 weeks. It appears the addition of ammonium sulphate prolonged the N uptake period of the plants studied. The loss of soil NO_3^- - N in the unamended soil after 3 weeks and the amended soil after 6 weeks could be attributed to immobilization or denitrification.

This decline in the soil NO_3^- - N beyond 3 and 6 weeks in the unamended and N amended soil must have been a function of events in the soil mix itself since it also occurred in the cropped as well as the "fallow" soil systems. This took place in spite of the fact that plant N accumulation had occurred after 6 and 9 weeks in their respective N treatments.

The decline in the average plant N (Figure 3) beyond 9 weeks may be of interest. It occurred in all crops at both levels of N (Figures 4 and 5). It was most pronounced in barley at high N. Triticale, on the other hand, did not experience this loss at high N. A loss of plant N has been reported previously. Wieland and Stutte (1979) reviewed this situation. They used the technique of pyroluminescence to show that soybeans lost various nitrogenous gases to the atmosphere, indicating denitrification by the foliage. Other workers have suggested that important losses of NH_3^+ may occur at senescence especially from high N foliage. When the data for individual crop treatments are considered (Figures 4 and 5; Appendix Tables 5 and 9) fairly large differences in plant N are seen.

The increase in the accumulation for plant plus soil N (Figure 4; Appendix Tables 4 and 8) compared to the fallow soil system for the two

Figure 4. Changes in levels of Plant N, Total N (Plant N + Soil N) and Soil N with 0 ppm added N. Soil N has an expanded "y" axis.

● - Fallow

W - Wheat

B - Barley

R - Rye

T - Triticale

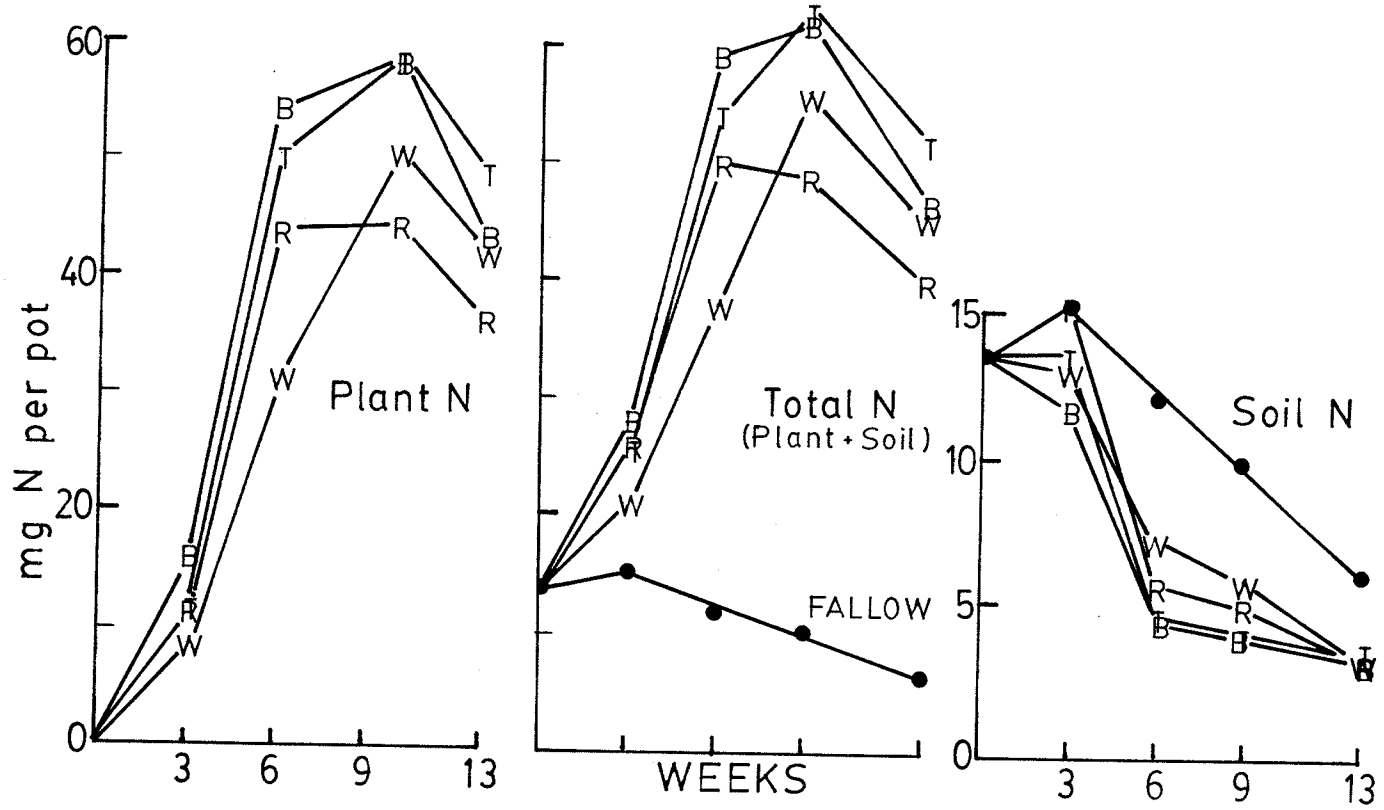

mg N per pot
60
40
20
0
3
6
9
13
Plant N
Total N
(Plant + Soil)
FALLOW
WEEKS
15
10
5
0
Soil N

Figure 5. Changes in levels of Plant N, Total N (Plant N + Soil N) and Soil N with 200 ppm N as $(NH_4)_2SO_4$. Soil N has an expanded "y" axis.

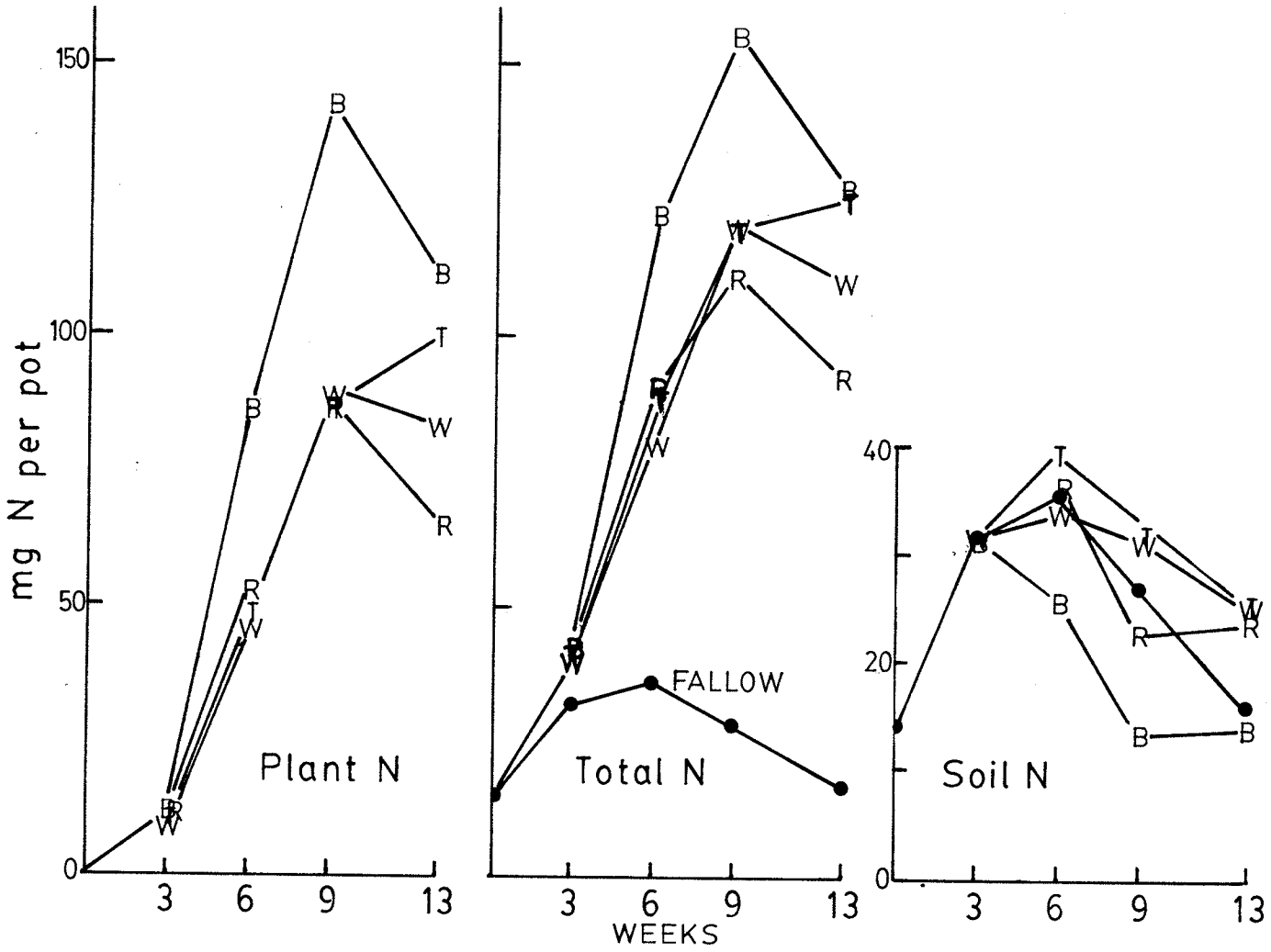
● - Fallow

W - Wheat

B - Barley

R - Rye

T - Triticale


mg N per pot
150
100
50
0
Plant N
Total N
Soil N
FALLOW
3
6
9
13
WEEKS
40
20
B
T
W
R

N treatments suggests that the plants promoted the nitrification of soil NH_4^+. Upon nitrification of the soil NH_4^+, plants either took up soil NO_3^- - N or it was immobilized or lost through denitrification immediately. Possibly a combination of all three events takes place.

Accumulation of plant N by barley and triticale was earlier and higher than by wheat or rye in unamended soil (Figure 4). The same trend was seen with barley in the amended soil (Figure 5). At both levels of added N, the wheat and rye curves for plant N crossed over after 6 weeks. At this time the rate of N uptake by rye leveled off. This would indicate that rye completed N uptake earlier than did the other crops.

The data for soil NO_3^- - N with added N (Figure 5; Appendix Tables 3 and 7) seem to indicate that the crops were influencing nitrification. The effects were particularly evident beyond 3 weeks. Under barley soil NO_3^- - N declined sharply after 3 weeks. This could be attributed to a single factor or a combination of interactions. The amount of N taken up by barley had increased substantially at this time. Moreover, at this point the weight of barley roots had increased almost twice as much as that of the other three crops (Appendix Table 6). Consequently, the mass of roots acting on the microflora was greater for barley than the other crops. Assuming that plant N uptake was as NO_3^-, it would seem that barley destabilized soil NO_3^- - N whereas the other crops stabilized NO_3^- - N levels compared with the fallow soil system.

Soil NO_3^- - N levels in the amended soil under wheat, rye and triticale were essentially identical to each other and similar to those observed for the fallow soil. Possibly the differences between barley and the other three crops could be due to root exudation and shifts in

the rhizosphere pH both of which could influence the microflora within the rhizosphere. Since wheat and rye are similar in their genetic makeup and triticale being a hybrid between wheat and rye, similarities in soil NO_3^- - N under these crops might be expected.

In the unamended soil, soil NO_3^- levels (Figure 4) for barley were again lower, while the rye, wheat and triticale soil NO_3^- - N were again similar, however, they were all lower than the fallow soil. This indicates the addition of ammonium sulphate resulted in higher soil NO_3^- - N levels despite the additional uptake by the crops. Uptake of NO_3^- - N by the plant from the soil would remove a potential source of N for denitrifying bacteria (Firestone, 1982). Consequently, it would seem that the crops stimulated nitrification of added N. The data for total NO_3^- - N accumulation for the soil-plant systems in unamended and amended soil (Figures 4 and 5) would also indicate that this had occurred.

Measurement of soil respiration might have supplied useful information in this study. Monteith et al. (1964) showed that soil respiration under barley was much higher than in fallow soil and under several other crops. This is discussed later in relation to the rhizosphere of wheat and barley.

The changes induced by plant roots must be operating against a background of pH changes caused by fertilizer N and by the nitrification process (Jones and Hedlin, 1970; Wetselaar et al., 1972). Smiley (1979) observed pH differences as large as 2.2 units in the rhizosphere and 1.2 units in the bulk soil when ammonia and nitrate were the source of N. It is known that on the uptake of NH_4^+, plant roots lower the pH of the rooting medium. On the other hand, uptake of nitrate causes a pH increase (Mengel and Kirkby, 1979). These effects were investigated in

Experiments 3 and 4.

Experiment 3. Measurement of soil pH at two levels of added nitrogen

This experiment was designed to establish the effects of fertilizer addition and nitrification on soil pH and NO_3^- levels as background material for the next study. In the next study the effects of roots on the soil pH and the rate of nitrification were evaluated.

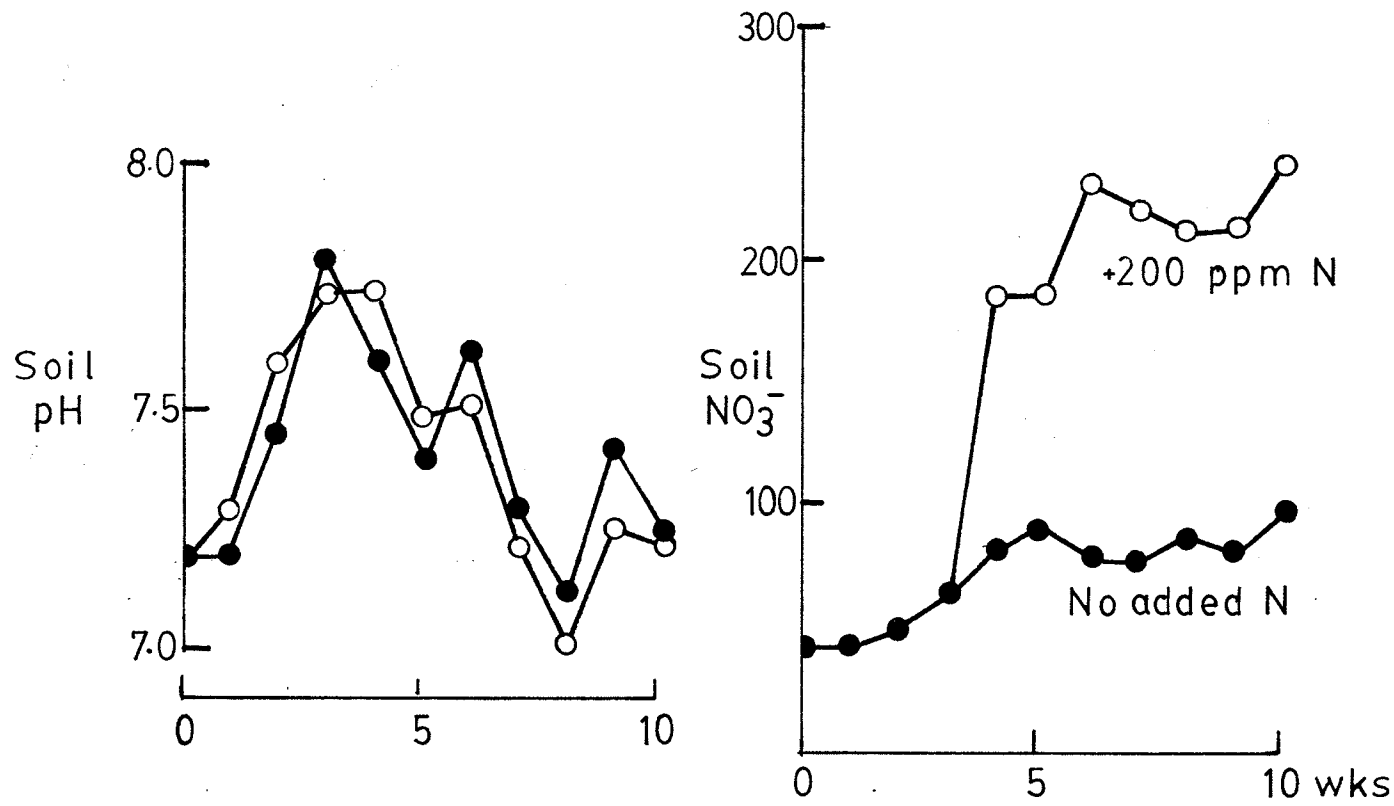
The accumulation of NO_3^- in the soil amended with 200 ppm N as ammonium sulphate was significantly higher than in the soil without added N (significant differences for Treatment, Date and T x D interactions at both 1 and 5% l. of s.; Appendix Table 13). During the first three sampling periods, nitrate levels remained low, after which nitrate levels increased significantly (Figure 6; Appendix Table 11). Nitrate levels during the latter part of the lag period in unamended and N amended soils were significantly different. However, significant differences in nitrate levels became more apparent at the later sampling dates.

At the beginning of the experiment, the pH of the unamended soil was 7.2 while the amended soil had a pH of 7.3. After 1 week an increase in the pH of both soils was observed up to the third sampling date (Figure 6; Appendix Table 11). Thereafter there was a gradual decline in the pH up to the 7th week. There was, however, a transient increase at week 5. A second pH rise occurred in both soils at week 8. In general, the pH of the N amended soil remained higher than in the unamended soil through week 4 while thereafter its pH was lower. Statistically, these differences were not significant (Appendix Table 12).

Figure 6. Changes in the pH and NO_3^- content of soil incubated with no added N and 200 ppm N added as $(NH_4)_2SO_4$.

● - No Added N

o - 200 ppm N


Soil pH
8·0
7·5
7·0
0
5
10
Soil NO_3^-
300
200
100
+200 ppm N
No added N
0
5
10 wks

However, they are consistent with the effects of added NH_4^+ in raising pH and acidification of the soil through nitrification (Jones and Hedlin, 1970; Justice et al., 1962; Mengel and Kirkby, 1979). The small pH difference between treatments probably indicated a high buffering capacity of the soil. In Wellwood soil (pH 7.2) Jones and Hedlin (1970) observed that the pH rose to 7.3 over the first week only to decline to pH 5.5 at 4 weeks. A decline in the soil pH beginning at week 5 coincided with increases in nitrate levels.

As was observed in Experiment 1, added N did not affect the duration of the initial lag in nitrification. Furthermore, it had little effect on the time at which other changes in nitrate levels and pH occurred.

Experiment 4. The influence of added nitrogen on the rhizosphere pH and nitrate levels in fallow and cropped soil.

In this experiment soil pH and nitrate levels were measured in the inner and outer rhizosphere of wheat and barley and in fallow soil. The soil had been amended with 200 ppm N as ammonium sulphate. It should be noted that NO_3^- levels increased more gradually than in other experiments and that rapid transients in NO_3^- and pH seen in other experiments were not observed because of the long sampling intervals.

As expected, addition of 200 ppm N resulted in a significant increase in nitrification in fallow soil (Figure 7; Appendix Tables 14 and 16). As well, pH of the amended soil was lower than in the control soil reflecting acidification due to nitrification (Figure 8; Appendix Tables 15 and 17). The large increase and decrease in pH in early sampling in Experiment 3 were not observed in this experiment, because

Figure 7. Changes in levels of NO_3^--N in the inner and outer rhizospheres of wheat, barley and fallow soil with 200 ppm N as $(NH_4)_2SO_4$ and fallow soil with no added N.

O - Fallow 200 mg N

● - Fallow 0 mg N

W - Wheat

B - Barley

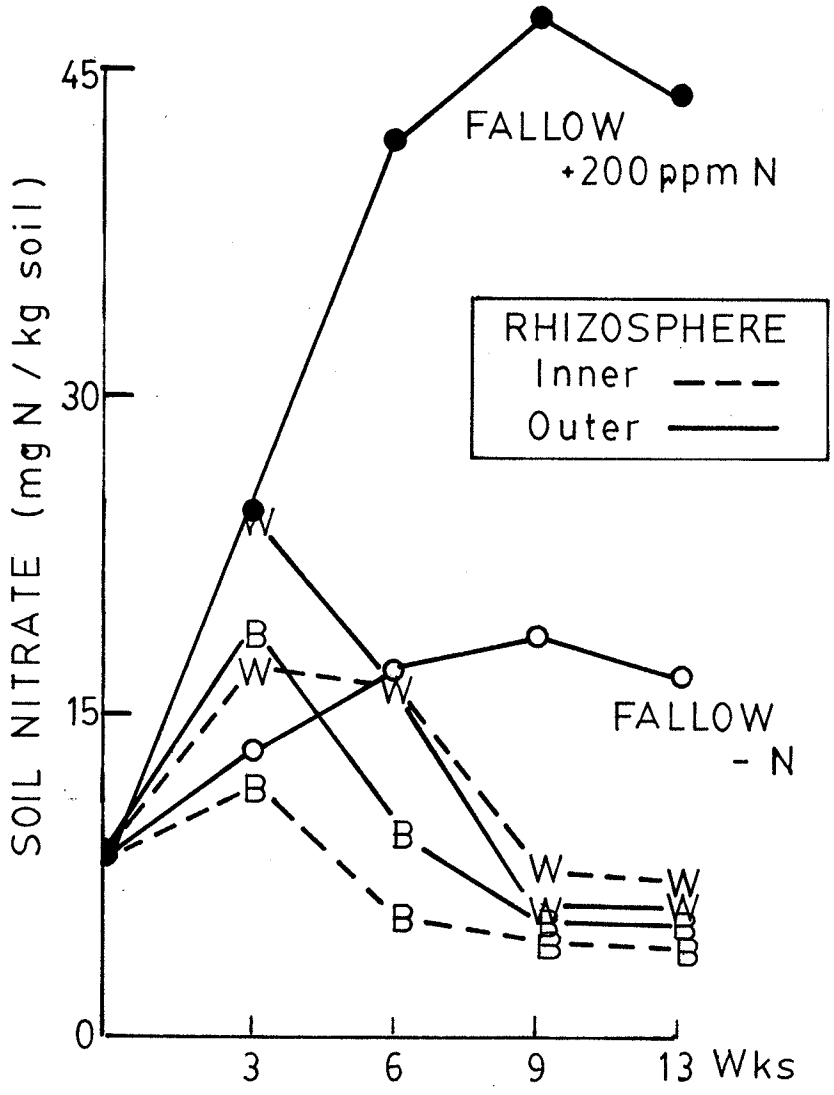
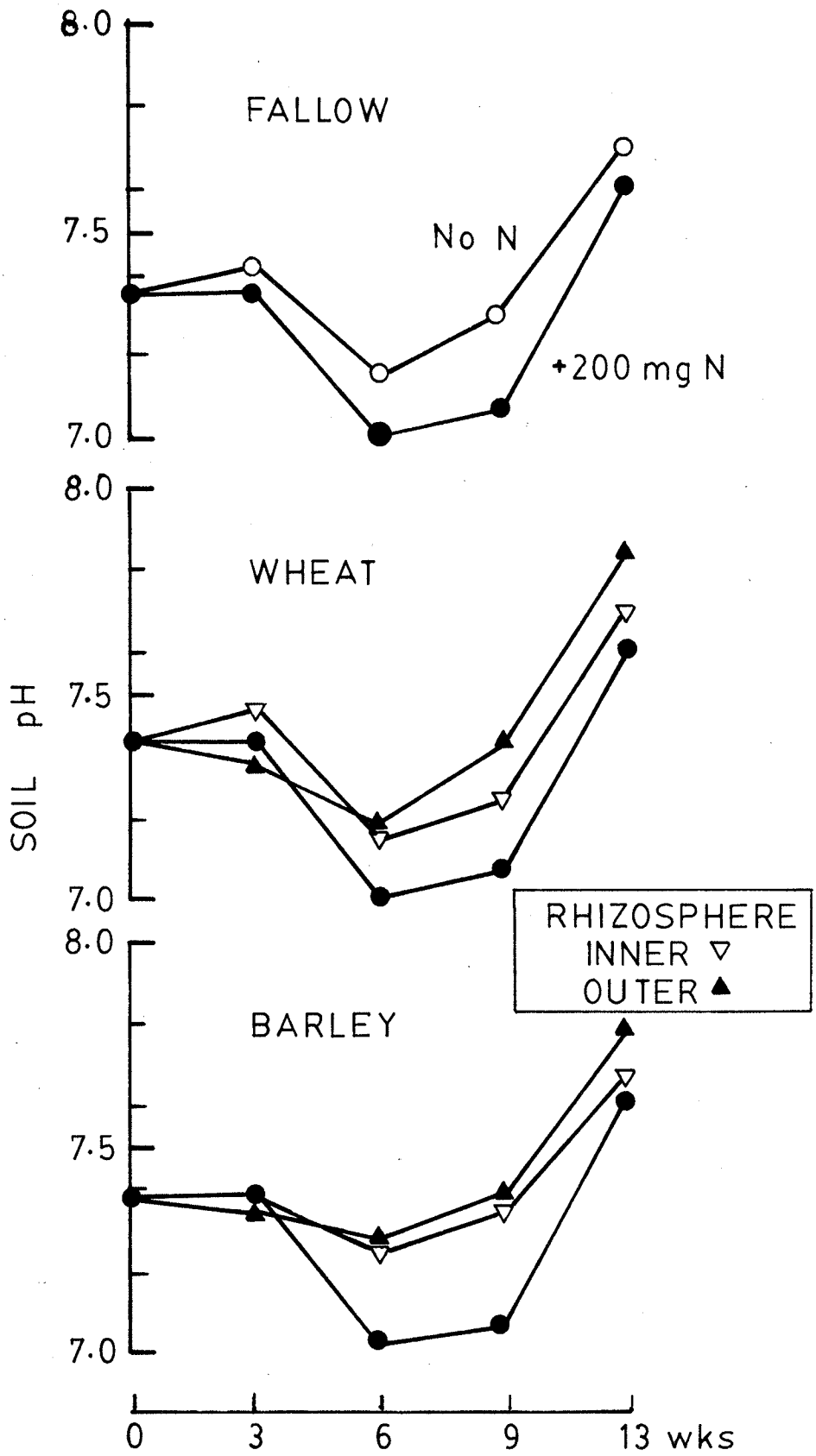

45
30
15
0
SOIL NITRATE (mg N / kg soil)
FALLOW
+200 ppm N
RHIZOSPHERE
Inner
Outer
FALLOW
- N
W
B
3
6
9
13 Wks

Figure 8. Changes in the pH of the inner and outer rhizospheres of wheat and barley roots and fallow soil with 200 ppm N as $(NH_4)_2SO_4$ and fallow soil without added N.


8·0
7·5
7·0
FALLOW
No N
+200 mg N
WHEAT
BARLEY
SOIL pH
RHIZOSPHERE
INNER ▽
OUTER ▲
0
3
6
9
13 wks

of the long sampling interval. The lowest pH was 7.0 at 6 to 9 weeks as observed in Experiment 3, but the initial pH and final pH of about 7.4 and 7.7, respectively, in this experiment were higher than the values of about 7.2 in Experiment 3. The wider pH change probably indicates greater microbial activity in this soil mix or lower buffering capacity.

At 6 and 9 weeks, rhizosphere pH was 0.1 to 0.4 units higher than the pH in fallow soil (Figure 8). The pH rise probably reflects assimilation of NO_3^- by plant roots (Mengel and Kirkby, 1979; Smiley, 1979). The steep rise in pH after 9 weeks in all treatments, since it occurred after nitrification and plant uptake of N were completed (Figures 3 and 9), must reflect increased microbial activity. A similar sharp rise in pH was observed in Experiment 3 (Figure 6) at 8 weeks.

Differences in the pH between the inner and outer rhizospheres (Figure 8) were small and usually did show statistical significance. However, pH is the negative log of the hydrogen ion concentration. A difference of 1.0 represents a ten-fold change in concentration; a difference of 0.1 units would be approximately a 25% difference. Probably the H ion concentration, rather than pH, would better relate to each of the many competing variables centering on nitrate.

Changes in rhizosphere pH (Figure 8) appear to reflect variation in nitrate uptake (Figure 7). Thus up to week 3, the pH of the inner rhizosphere of both wheat and barley was higher and nitrate depletion was greater close to the root. Beyond 6 weeks after NO_3^- depletion was complete or nearly complete, the inner rhizosphere showed a lower pH probably as the result of processes other than nutrient uptake.

The curves for barley rhizosphere pH are flatter than for wheat. In particular the decline at 6 weeks was less pronounced in barley. Earlier, more rapid uptake of NO_3^- by barley (Figures 4, 5 and 9) would produce a greater rise in pH which would offset the decline in pH associated with rapid nitrification which occurred at this time.

Data for wheat and barley grown at 200 ppm N in Experiments 2 and 4 are assembled in Figure 9. In the second experiment with added N, soil NO_3^- - N under barley declined more rapidly than under wheat. These results were similar to those found in this experiment (Figures 7 and 9). However, with 200 ppm of added N, NO_3^- - N under wheat (Figure 5) and under the averaged crops (Figure 3) was the same as in the fallow treatment, whereas with barley the NO_3^- declined rapidly from week 3. Those results suggest that barley may have inhibited nitrification whereas wheat, rye and triticale maintained nitrification rates. The present results may, in part, be consistent with that interpretation under wheat, nitrate levels were higher than under barley. However, these results can be explained on the basis of different rates of nitrate uptake by the two crops or by the differing rates of N immobilization by the microflora under the influence of the two crops.

The two experiments differ in another respect. In Experiment 2, NO_3^- - N in the fallow treatment declined continuously from 6 weeks whereas in the present study, it continued to increase through 9 weeks and declined only slightly thereafter. Fallow soil NO_3^- levels appeared unstable in Experiment 2, but stable in Experiment 4. N uptake by wheat was little affected by different soil NO_3^- responses in the two experiments, but uptake by barley differed considerably.

Figure 9. Changes in Fallow N, Cropped Soil N and Plant N for wheat and barley with 200 ppm N as $(NH_4)_2SO_4$ in Experiments 2 and 4.

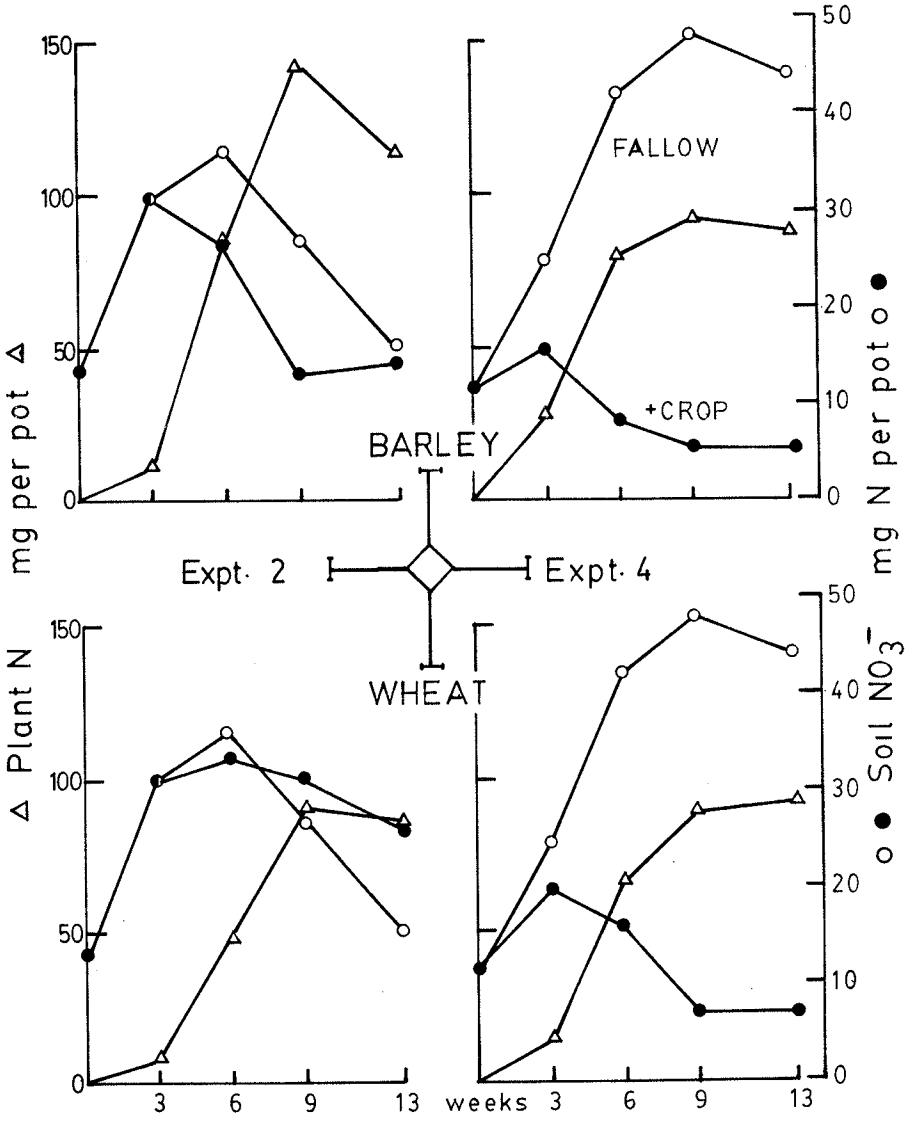
Left scale - Plant N per pot

Right scale - Fallow N and Cropped Soil N per pot

○ - Fallow Soil N

● - Cropped Soil N

△ - Plant N


BARLEY
WHEAT
Expt. 2
Expt. 4
FALLOW
+CROP
mg per pot Δ
mg N per pot o ●
Δ Plant N
o ● Soil NO_3^-
weeks
150
100
50
0
40
30
20
10
3
6
9
13

Monteith et al. (1964) measured CO_2 fluxes on a Rothamsted clay loam. Bare soil released 4 g/m^2/day. Beans, oats and shortgrass increased the rate by 2 to 3 g/m^2/day, but barley increased the rate by as much as 10 g/m^2/day in excess of the bare soil. Since the average photosynthesis rates were 17 g/m^2/day, it seems unlikely that barley roots would waste more than 1/2 of daily photosynthesis on respiration. Considerable stimulation of the microflora in the presence of barley roots would seem to be indicated. Martin (1977) found that 20% of recent photosynthate in wheat was lost rapidly to the soil as the result of senescence and death of the root cortex.

Hallem (1981) found that the cortex of wheat roots died out beginning within a few centimeters of the root tips at 16° C, but that at lower soil temperatures (8° and 12° C) the cortex remained white and turgid. Volkmar (1981) looked at the effects of soil temperature (12°, 16° and 20° C) on the growth of barley roots in the presence and absence of mycorrhizae (VAM). At all three temperatures, VAM resulted in a 20% increase in shoot weight (dry). There was, however, a three-fold decrease in root weight. In spite of the decreased mass of roots, the total length was increased indicating proliferation of the high order lateral roots. Up to 90% of the finest roots were VAM infested indicating that VAM promoted proliferation and infected these slow growing and slow senescing (Milthorpe and Moorby, 1974; Deacon and Lewis, 1982) lateral roots. Chemical analysis showed that mycorrhizal barley roots retained higher concentrations of soluble carbohydrate and other nutrients than did non-mycorrhizal roots. These and other results indicate that a variety of factors, physical, chemical, genetic and/or microbial would regulate senescence of the root cortex and release of

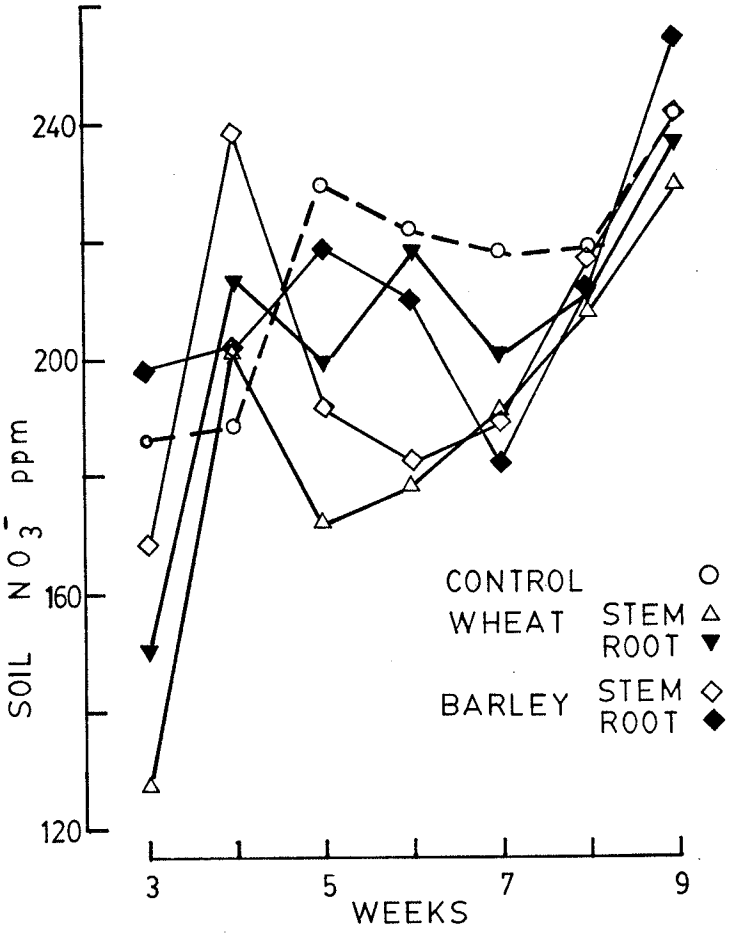
organic matter to the rhizosphere. If this is so, then the effects of barley on soil NO_3^- would involve earlier and more rapid uptake of NO_3^- compared to wheat plus increased immobilization of NH_4^+ and NO_3^--N by the rhizosphere microflora. Possibly in Experiment 4, soil conditions produced a microflora which was responsive to wheat root exudates with the same results.

Experiment 5. The effect of root and stem leachate on nitrate production during incubation of a soil

The effect of added leachate on the production of nitrate in soil incubated with 200 ppm ammonium sulphate is shown in Figure 10 (Appendix Tables 18 and 19). Production of nitrate in this experiment again followed a sigmoid curve. A lag phase was evident up to the end of the second week of incubation. From the second to the third week nitrate production was rapid. The lag phase was not dependent on the type of leachate added to the incubated soil samples. From week 3 to the end of week 8 a plateau was formed. At the end of week 9 nitrate levels in all classes of leachate had increased considerably over those levels observed in the plateau. It would have been of interest to have continued the experiment to determine what trend the curves would have followed. However, the amount of material used in the leaching apparatus had become impossible to handle. It would seem that the type of leachate used had no significant effect in general on soil NO_3^- at any particular time.

Molina et al. (1964) provided results that are similar to some extent. Alfalfa root exudate did not inhibit or stimulate Nitrobacter at neither "high" nor "low" concentrations. However, Nitrosomonas was inhibited by "high" concentrations while stimulated at "low" concentra-

Figure 10. Changes in soil NO_3^- levels for soil samples amended with 200 ppm N as $(NH_4)_2SO_4$ and incubated with wheat stem and root leachate, barley stem and root leachate and soil without any leachate. Weeks 0, 1 and 2 have been omitted so that the "y" axis could be expanded.


240
200
160
120
SOIL NO_3^- ppm
3
5
7
9
WEEKS
CONTROL
WHEAT STEM
ROOT
BARLEY STEM
ROOT

tions of alfalfa root exudates. Stimulation of Nitrobacter occurred with corn root exudates while Nitrosomonas was inhibited by both concentrations used. These effects were observed on Nitrobacter and Nitrosomonas supporting media which had been amended with either corn or alfalfa root exudate. In spite of the results observed, they suggest that exudates in the rhizosphere would be altered in some way. Either absorption of the exudates by clay particles and/or interaction of the exudates with the heterotrophic population could change the effect of the exudates on the nitrifying population in the soil compared to a media supporting the nitrifying bacteria.

During the present study, the leachate collected from the wheat roots and barley roots was applied directly to the soil. Consequently, the leachate in the soil would encounter environmental influences in the soil similar to those found in soil supporting plant roots. There was one exception, though. This was the presence of the root. The presence of the plant root would possibly influence the heterotrophic bacteria present in the rhizosphere and, consequently, alter the exudates in some way. Therefore, the exudates encountered in the soil by the nitrifying bacteria when roots are present could be entirely different from those encountered when no roots are present. Moreover, the concentration of leachate applied during the present study can in no way duplicate the quantities of exudate produced in the soil by actively growing roots.

While Purchase (1974a) observed that grass eluate increased the lag period in nitrate production, subsequent nitrate production was not affected. Inhibition of the nitrifying bacteria did occur in liquid cultures with eluates from living and decaying roots. Munro (1966b)

experienced similar results. A heat-liable substance in the roots of Hyparrhenia filipendula was found to be toxic to nitrifying bacteria. Eluates were collected from the root segment or chopped up roots, a process that was not imitated in the soil and, consequently, raises some doubt to the validity of the results. Similar extraction procedures have been employed by Odu and Akerele (1973), Neal (1966) and Rice (1964) yielding the same results.

Nonetheless, washings of living roots of ryegrass, wheat, salad rape, lettuce and onion were found to reduce the rate of nitrification in a "steady state" system by Moore and Waid (1971). Odu and Akerele (1973) and Moore and Waid (1971) question the validity of making inferences from data collected in the laboratory to situations that actually exist in the soil with living roots. Martin (1977) found that 20% of recent photosynthate was released from wheat roots into the soil probably as a result of senescence and death of cortical cells of the root. Losses of this magnitude through the season would sustain the rhizosphere microflora on a continuous basis. In the present experiment the exudates or leachings were at weekly periods and may have been degraded by the microflora during the lag period.

Previous studies on the effect of plant leachate have been confined to root extracts. As stated before, these types of studies do not simulate field conditions. Under field conditions rain, heavy dew and guttation could return material from the above ground parts to the soil (Tukey, 1970). Though the amount of material returned from the above ground plant parts is small in comparison to the quantity of material released by the roots, it should be taken into consideration. While most extracts lowered the mean NO_3^- level slightly, the wheat-stem and

barley-stem extracts produced a lower level of NO_3^- in the incubated soil than their respective root extracts. In combination, the root and stem extracts may have provided interesting results. However, it would be impossible to determine the proper concentrations of both extracts that could be found under field conditions. In the present study the stems of the plants underwent a continuous leaching for an extended period. The leachate was then applied at weekly intervals, consequently, it was possible that it was broken down by the microflora.

GENERAL DISCUSSION

Since the experiments were carried out over a period of three years, differences in both the soil and the peat were probably involved. It is doubtful that any one of the experiments could be repeated at will. Probably the same variation would occur in greenhouse and nursery soil mixes (Baker, 1957). Use of vermiculite or perlite in such mixes would at least eliminate the complication of organic matter--microflora interactions arising from the use of peat. Since these interactions would influence the availability of other elements such as Mn, Fe, Cu, P and S, and since the microflora can profoundly influence plant growth in ways not directly related to nutrient availability, it would seem that agronomists and physiologists concerned with detailing crop performance through greenhouse and growth room studies could reduce variability in their studies by abandoning peat as a soil amendment. The use of nutrient and sand culture systems would produce rhizospheres which are totally unrelated to those in the field.

In Experiment 1, with both ammonium sulphate and urea at all levels of added N, the initial lag period and marked transient disappearances of soil NO_3^- at 8 and 11 weeks occurred. It is surprising that these features were not influenced by the level of N added except perhaps at the highest amended rates. In Experiments 3 and 5, less pronounced transient declines in NO_3^- occurred at 7 to 8 weeks. It is noted that in Experiment 3, pH changes reflected changes in nitrate levels as well

as microbial activity. Because of the longer sampling interval in Experiments 2 and 4, these transients were not seen. If they were present and happen to coincide at all levels of added N, peak levels of NO_3^- were about 150 ppm greater than expected from nitrification of the added N. This would indicate considerable nitrification of endogenous N whether it was NH_4^+ or organic. This "excess NO_3^-" was much less apparent in other experiments.

Without simultaneous measurement of NO_2^-, NO_3^-, pH and redox potentials, it is not possible to decide whether the disappearance of NO_3^- is due to NO_2^- formation, denitrification or immobilization. Perhaps one can argue against denitrification since the NO_3^- returned to levels as high or higher than before. Measurement of soluble Mn^{+2} might be useful since redox potentials for $NO_3^- - NO_2^-$ and $Mn^{ox} - Mn^{+2}$ are nearly identical. Focht (1979) indicated that high levels of NO_3^- and soluble Mn^{+2} were never found in the same soil extract.

In Experiment 2, the influence of barley on soil NO_3^- was clearly different from that of other crops and in Experiment 4, NO_3^- disappearance from the rhizosphere of barley was earlier and greater than with wheat. The abrupt decline in NO_3^- after 3 weeks under barley was probably not the result of plant uptake since triticale took up as much or more N than did barley. However, the kinetics of uptake by triticale were different especially during early growth. This assumes that all plant N originated as soil NO_3^- which may not be true (Reisenauer, 1978). Monteith et al. (1964) showed that soil respiration under barley was much greater than under other crops which would indicate that barley roots stimulate the microflora to a larger extent than the other crops. Russel (1973) indicates that there are a few other comparative studies

on the effects of crops on soil respiration. Such data would have been very useful in the present experiments. To some extent changes in levels of NO_3^- and pH in the rhizospheres of wheat and barley in Experiment 4 support the suggestion that microbes in barley were more effective at immobilizing nitrogen. Wheat, rye and triticale appear to have enhanced the levels of soil NO_3^- especially in the later stages of the growth cycle. Based on the different effects of wheat and rye, it is suggested that these crops favor elevated NO_3^- levels at different times and that the root factors involved in these crops are retained in triticale, a wheat - rye hybrid. It would be interesting to examine this suggestion using the recently developed wheat - barley hybrid.

In a recent thesis Reid (1982) investigated the nutrient content of wheat and barley plants growing in an organic soil at four temperatures between 10 and 25° C. Sampling was done at the early boot and the 3 to 4 leaf stage (which was at about 1/2 the days to the boot at all temperatures). Analysis of his data indicates that at low temperatures, barley tended to accumulate the largest amounts of most elements during the second half of growth whereas wheat tended to accumulate more of the total amount before the 3 to 4 leaf stage. The difference between wheat and barley was especially pronounced in the case of Mn, Fe, Cu, Zn and P. Availability of these elements is known to be influenced by pH, redox potential and microbial activity (Mengel and Kirkby, 1979; Russel, 1973). These findings could be explained if barley stimulated the microflora more than did wheat. This is consistent with the more rapid disappearance of NO_3^- under barley compared to wheat or the other crops. In this respect the report by Focht (1978) that high levels of Mn^{+2} NO_3^- are never found in the same soil extract is particularly

interesting although his concern was for denitrification in anaerobic soils.

In Experiment 5, the effect of materials leached from roots and shoots of wheat and barley on nitrate levels were examined. In retrospect this was probably a useless experiment in that the dominant effect of peat was not appreciated at the time. Most studies of the effect of plant exudates on nitrification have been concerned with materials originating in the roots although Tukey (1970) showed that large amounts of materials can be transferred to the soil from the above ground parts.

CONCLUSION

The use of soil mixes containing peat was clearly a mistake in the sense that it introduced relatively large amounts of organic matter, the composition of which probably varied from one experiment to another. This seems to have affected the microflora in different ways. In view of the uncertain effects of such a varying microflora on availability of nitrate and of other elements inferred from results of Reid (1982) and other effects of the microflora on plant growth, it seems that peat should not be used in soil mixes intended for studies on the performance of crop plants. Another disadvantage of peat is that the root system is difficult to examine, fine roots grow through individual leaflets and cannot be removed.

Probably the most important finding is that barley results in early and rapid disappearance of nitrate from the soil. In part this effect seems to involve stimulation of the microflora within the rhizosphere. The fate of the N is not known. Of equal interest is the observation that other crops seem to have stabilized and/or elevated soil nitrate levels particularly in the later stages of growth. The differing effects of wheat, rye and triticale seem to indicate distinct effects on the microflora (Deacon and Lewis, 1982; Graham, 1978). Furthermore, triticale may retain factors from both of its parents. If this is true then triticale and wheat - barley hybrids may provide unique and interesting material for investigation of the biology of the rhizosphere. It

will be interesting to know what extent these crop effects are dependent upon the addition of organic matter and N fertilizer and which components are involved.

While the use of soil mixes containing peat may have been a mistake in one sense, it may have provided conditions which allowed us to "see" the result of changes in the rhizosphere which would not have been observed had soil alone been used. In addition, these experiments in combination with Reid's (1982) results may provide an insight into the behaviour of these crops in organic soils.

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APPENDIX

TABLE 1. Mean NO_3^- - N for soil amended with ammonium sulphate and urea at 19 and 21° C over 12 weeks.

	ppm N as Ammonium Sulphate						ppm N as Urea				
Week	0	50	100	200	500	1000	50	100	200	500	1000
1	81	92	89	98	93	83	102	102	102	85	154
2	87	122	130	138	156	134	101	127	142	144	85
3	76	110	131	160	179	382	107	124	159	195	89
4	157	186	220	275	403	532	173	225	285	442	248
5	142	231	308	378	708	964	244	281	416	630	496
6	174	209	241	355	643	927	229	247	385	682	570
7	192	227	272	372	520	818	256	299	400	524	611
8	85	141	154	229	409	575	157	186	214	402	529
9	181	329	327	389	566	887	260	284	354	554	752
10	173	293	325	408	679	1060	312	313	478	624	1007
11	83	122	132	285	444	772	99	143	250	465	714
12	198	276	324	412	625	978	283	304	716	597	870

TABLE 2. Mean NO_3^- - N for soil amended with ammonium sulphate and urea at 1 and 4° C over 12 weeks.

	ppm N as Ammonium Sulphate						ppm N as Urea				
Week	0	50	100	200	500	1000	50	100	200	500	1000
1	69	62	66	70	72	66	68	61	85	94	76
2	52	69	68	66	69	68	59	62	65	61	47
3	49	53	53	53	54	51	53	47	48	45	46
4	87	97	91	100	83	100	106	109	104	102	83
5	75	101	79	79	74	63	71	65	61	59	60
6	85	82	90	88	90	82	83	91	84	77	70
7	77	96	82	88	83	64	88	84	87	72	58
8	58	83	85	91	86	71	76	82	76	61	49
9	89	133	130	134	120	93	129	123	127	102	74
10	116	166	169	174	187	140	162	165	162	144	84
11	69	130	153	159	166	117	134	143	140	121	77
12	90	146	176	206	211	160	133	155	187	180	84

TABLE 3. Mean N in 1000 g of soil for 5 different crops in soil amended with 0 and 200 ppm N over 13 weeks.

Weeks	Fallow	Wheat	Barley	Rye	Triticale
		(mg N per 1000 g of soil)			
		0 ppm N			
3	15.2	13.1	11.7	15.0	13.4
6	12.2	7.2	4.4	5.8	4.6
9	9.9	6.0	4.1	4.9	4.2
13	6.2	3.2	3.1	3.1	3.7
		200 ppm N			
3	31.6	31.4	31.2	30.1	32.1
6	36.3	33.7	26.3	35.9	39.5
9	26.9	31.6	13.2	25.2	31.8
13	16.0	26.2	14.4	27.2	25.5

[a] The mean N in the soil for all crops at week 0 was 13.6 mg per 1000 g of soil.

LSD 0.05 = 5.2; 0.01 = 7.0

TABLE 4. Mean N found as soil NO_3^- plus plant N for 5 different crops over 13 weeks in soil amended with 0 and 200 ppm N.

Weeks	Fallow	Wheat	Barley	Rye	Triticale
		(mg N per 1000 g of soil)			
		0 ppm N			
3	15.2	21.1	27.7	26.4	25.3
6	12.2	37.7	58.6	49.9	54.1
9	9.9	56.2	61.9	48.4	63.0
13	6.2	45.0	46.2	40.0	52.3
		200 ppm N			
3	31.6	40.6	42.6	43.0	42.8
6	36.3	79.3	122.2	89.9	86.9
9	26.9	119.7	155.1	110.9	119.0
13	16.0	109.8	125.8	92.3	124.8

[a] The mean N in the soil for all crops at week 0 was 13.6 mg per 1000 g of soil.

LSD 0.05 = 14.9; 0.01 = 19.9

TABLE 5. Mean N per plant for 4 crops over 13 weeks in soil amended with 0 and 200 ppm N.

Weeks	Wheat	Barley	Rye	Triticale
		(mg N per plant)		
		0 ppm N		
3	8.0	16.0	11.4	11.9
6	30.5	54.3	44.1	49.5
9	50.2	57.8	43.5	58.8
13	41.8	43.1	36.9	48.6
		200 ppm N		
3	9.2	11.4	12.9	10.7
6	45.7	85.9	53.9	47.4
9	88.0	141.9	85.7	87.2
13	83.7	111.3	65.1	99.2

LSD 0.05 = 15.3; 0.01 = 20.5

TABLE 6. Mean root weight of 4 crops at 4 sampling dates and at 2 levels of added nitrogen.

Weeks	Wheat	Barley	Rye	Triticale
		0 ppm N		
3	2.0	3.2	2.0	2.5
6	5.9	12.9	7.6	6.1
9	6.3	9.4	7.0	6.2
13	3.7	3.8	6.0	4.9
		200 ppm N		
3	2.3	2.7	2.8	2.3
6	7.9	13.8	7.1	4.9
9	9.2	11.6	9.3	6.5
13	3.1	7.8	6.2	5.1

TABLE 7. Analysis of variance for soil nitrogen.

Source	df	SS	MS	
Reps	2	27.9787	13.9894	0.41
Nitrogen	1	12931.4041	12931.4041	371.88**
Error a	2	67.9323	33.9662	
Date of harvest	3	1737.1929	579.0643	63.09**
Nitrogen X Date	3	477.5609	159.1870	17.34**
Error b	12	110.1410	9.1784	
Treatment	4	591.0245	147.7561	14.31**
Treatment X Nitrogen	4	468.3675	117.0919	11.34**
Treatment X Date	12	308.9175	25.7431	2.49**
T X N X H	12	319.6357	26.6363	2.58**
Error c	64	660.6750	10.3230	

TABLE 8. Analysis of variance for plant N and soil N.

Source	df	SS	MS	
Reps	2	114.7902	57.3901	0.33
Nitrogen	1	53966.7254	53966.7254	311.35**
Error a	2	346.6601	173.3301	
Date of harvest	3	33953.5977	11317.8659	75.93**
Nitrogen X Date	3	8230.5939	274.5313	18.41**
Error b	12	1788.7014	149.0585	
Treatment	4	51500.9537	12875.2384	153.84**
Treatment X Nitrogen	4	6032.4979	1508.1245	18.02**
Treatment X Date	12	14564.2822	1213.7735	14.50**
T X N X H	12	3745.4622	312.1239	3.73**
Error c	64	5356.4450	83.6935	

TABLE 9. Analysis of variance for plant N.

Source	df	SS	MS	
Reps	2	67.8500	33.9250	0.21
Nitrogen	1	17566.2704	17566.2704	109.20**
Error a	2	321.7340	160.8670	
Date of harvest	3	58898.2625	19632.7542	150.95**
Nitrogen X Date	3	10829.7580	3609.9193	27.76
Error b	12	1560.6921	130.0577	
Treatment	3	6923.0721	2307.6907	26.80**
Treatment X Nitrogen	3	2618.9208	872.9736	10.14**
Treatment X Date	9	3489.8321	387.7591	4.50**
T X N X H	9	2297.3059	255.2562	2.96**
Error c	45	3875.3020	86.1178	

TABLE 10. Mean soil pH at 2 levels of added N over 10 weeks.

Week	0 ppm (added N) pH	200 ppm
1	7.2	7.3
2	7.4	7.6
3	7.8	7.7
4	7.6	7.7
5	7.4	7.5
6	7.6	7.5
7	7.3	7.2
8	7.1	7.0
9	7.4	7.3
10	7.2	7.2

[a] The N amended and N unamended soils had a mean pH at week 0 of 7.2.

TABLE 11. Mean soil NO_3^- at 2 levels of added N over 10 weeks.

Week	0 ppm	(added N) NO_3 ppm	200 ppm
1	39		39
2	55		58
3	63		66
4	83		186
5	85		188
6	78		229
7	79		222
8	83		218
9	81		218
10	97		242

[a] The N amended and N unamended soils had a mean NO_3^- level of 38 ppm at week 0.

LSD 0.05 = 22.0; 0.01 = 29.0

TABLE 12. Analysis of variance for soil pH.

Source	df	SS	MS	
Reps	2	0.2230	0.0115	1.02
Date of harvest	9	2.6735	0.2971	26.29**
Treatment	1	0.0001	0.0001	0.01ns
Treatment X Date	9	0.1615	0.0179	1.58ns
Error	38	0.4304	0.0113	

TABLE 13. Analysis of variance for soil NO_3^-.

Source	df	SS	MS	
Reps	2	603.2333	301.6167	1.74
Date of harvest	9	121959.7500	13551.0833	78.20**
Treatment	1	128528.8166	128528.8166	741.73**
Treatment X Date	9	55592.0167	6176.8907	35.65**
Error	38	6584.7667	173.2833	

TABLE 14. Mean N in 1000 g of soil with a control receiving 0 ppm N, a control with 200 ppm and the soil from the inner and outer rhizosphere of wheat and barley amended with 200 ppm N.

Weeks	Control 1	Control 2	Wheat		Barley	
			inner	outer	inner	outer
			(mg N per 1000 g soil)			
3	13.3	24.7	16.9	23.1	11.8	18.3
6	16.7	41.9	15.9	16.3	5.9	9.6
9	18.6	47.6	8.2	6.0	5.2	5.8
13	17.2	43.8	7.0	6.7	4.4	5.9

[a] In this experiment 600 g of soil was used, however, the values listed here have been converted to the amount of N found in 1000 g of soil.

[b] The unamended and amended soils had a mean N level of 11.5 mg/1000 g of soil.

LSD 0.05 = 3.3; 0.01 = 4.3

TABLE 15. Mean soil pH of unamended and N amended fallow soil and the inner and outer rhizosphere of wheat and barley. Soil amended with N.

Crop	pH (weeks) 3	6	9	13
Fallow unamended	7.4	7.2	7.3	7.7
Fallow amended	7.4	7.0	7.0	7.6
Wheat inner	7.4	7.1	7.2	7.7
Wheat outer	7.4	7.2	7.4	7.6
Barley inner	7.4	7.3	7.3	7.7
Barley outer	7.3	7.3	7.4	7.8

[a] The mean soil pH at week 0 was 7.4.

LSD 0.05 = 0.07; 0.01 = 0.10

TABLE 16. Analysis of variance for soil N.

Source	df	SS	MS	
Reps	2	10.9336	5.4668	1.21
Treatment	5	8378.7044	1675.7409	371.58**
Error a	10	45.0981	4.5098	
Date of harvest	3	190.3467	63.4489	16.35**
Treatment X Date	15	2032.0473	135.4698	34.91**
Error b	36	139.7016	3.8806	

TABLE 17. Analysis of variance for soil pH.

Source	df	SS	MS	
Reps	2	0.0119	0.0060	0.0058
Treatment	5	0.2782	0.0556	0.54ns
Error a	10	1.0351	0.1035	
Date of harvest	3	2.8502	0.9501	558.88**
Treatment X Date	15	0.1989	0.0133	7.82**
Error b	36	0.0017	0.0017	

TABLE 18. Mean NO_3^- levels in soil amended with 200 ppm N to which wheat and barley root and stem leachate had been applied over a period of 9 weeks.

Week	Control	Treatment			
		Wheat stem	Wheat root	Barley stem	Barley root
		(ppm NO_3)			
1	58	51	59	54	58
2	69	70	72	65	67
3	186	126	150	167	197
4	188	202	213	238	200
5	229	172	199	191	218
6	222	178	217	182	209
7	218	192	200	189	182
8	218	207	210	218	212
9	242	228	236	242	254

[a] The mean NO_3^- level for all soil at week 0 was 39 ppm.

LSD 0.05 = 32

TABLE 19. Analysis of variance for soil NO_3^-.

Source	df	SS	MS	
Reps	2	370.2373	185.1187	0.48
Treatment	4	7981.2299	1995.3075	5.15**
Date	8	514608.5040	64326.0630	166.17**
Treatment X Date	32	20887.5701	652.7366	1.69*
Error	88	34065.7627	387.1109	