THE EFFECT OF SUBSOIL MINERAL NITROGEN ON GRAIN PROTEIN CONCENTRATION OF WHEAT

by

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SUMMARY

In the ley farming system traditionally used in the cereal zone of southern Australia, nitrogen (N) input for cereal production was largely derived from that added by legume-based pastures. In recent years cropping has increased, the importance of pastures has diminished in many areas and pastures have been replaced with grain legumes and oilseed crops. As a consequence input of biologically fixed N into the farming system has declined and the use of N fertiliser increased. Nitrogen deficiency is a major factor limiting productivity and quality (grain protein concentration) of wheat in some areas.

Subsoil nitrate (NO_3-N) may be a useful N reserve for field crops when N in the surface layer is unavailable or not accessible because the soil is dry. Leaching of N mineralised in, or applied as fertiliser to, the surface layer of soil appears to be the main avenue of NO_3-N build-up in the subsoil. There is some evidence to suggest that, under the farming conditions of southern Australia, NO_3-N leached deeply into the soil profile, but still in the active root zone, may be taken up by wheat crops late in the growing season and thus may constitute a potential source of N for enhancement of grain protein concentration (GPC). Preliminary measurements in the field at the Waite Institute showed a loss of mineral N from the surface horizon of the soil during the growing season, part of which was attributed to leaching to the deeper layer.

This project examined the uptake of mineral N from the subsoil after anthesis and its effect on the GPC of wheat. The overall objective of the studies was to examine the importance of subsoil mineral N and investigate the ability of wheat to take up N from the subsoil late in the season under different conditions of N supply and soil water availability. Greenhouse experiments were designed to investigate the importance of subsoil mineral N availability on GPC of wheat and the factors that contribute to the effective utilisation of N. The recovery of N from subsoil, the effect of split N application on GPC and short term N uptake by the wheat at different rooting densities were studied.

The first experiment examined the ability of wheat plants to take up subsoil mineral N and the subsequent effect on GPC. Wheat was grown in pots 105 cm deep using a soil

that was sandy and very low in N. Samples of wheat were taken during the season to measure shoot and root weight, root length, concentrations of NO_3-N, total N, grain yield and GPC. Two weeks after anthesis, 150 mg N (equivalent to about 150 kg N ha^{-1}) as KNO_3 was added in solution to the treatment pots at a depth of 60 cm and its fate was examined. Application of N after anthesis significantly increased root growth at the site of N placement in subsoil. Grain yields, N uptake per unit root length and GPC were increased. The apparent recovery of applied N in plant tops was high (about 72%). The amount of N remobilised after anthesis from the roots and shoots in the control plants was high, equivalent to 81% of the N in the grain, while only 27% was remobilised in the N-treated plants. The results from this experiment support the hypothesis that subsoil mineral N may be taken up late in the season and contribute to GPC.

The second pot experiment considered the effects of depth, time and amount of N application, as well as soil water regime on GPC. A single post-anthesis application of N was compared with treatments where the N was placed in the topsoil or subsoil during the early part of the season under different water treatments. The experiment was designed to simulate possible conditions in the field *i.e.* subsoil N derived from decomposition of organic material or from fertiliser applied early in the season and leached into the subsoil. There were five N treatments applied as KNO_3: (N0) no N; (N1) 150 mg to topsoil at sowing; (N2) 75 mg to topsoil and 75 mg to subsoil at sowing; (N3) 150 mg to subsoil at sowing; (N4) 75 mg to topsoil at sowing and 75 mg to subsoil one week after anthesis. When the wheat reached anthesis, water treatments were introduced to provide (1) well-watered control ; (2) dry topsoil but ample water supply in the subsoil. The plant and soil measurements were the same as the first experiment. An additional series of pots in which 150 mg N was applied at sowing (N1), was established and watered as for the well-watered treatment to follow the leaching of N from the topsoil. When the fate of the N was followed down the profile in these pots during the pre-anthesis period, it was found that one month after N application some NO_3-N was leached in to the subsoil.

Application of N significantly increased the yield and GPC irrespective of the depth or the time of application and the recovery of N was similar in all treatments. This

shows that placement of N had no effect on N uptake by plants. The plant yield and GPC responses to the different N treatments were not affected by restricting the supply of water during the post-anthesis period. The GPC of plants treated with 150 mg in the topsoil at sowing (N1) was comparable with N2, N3 and N4 treatments suggesting that as long as the N is not leached beyond the root zone, it has an equivalent effect as an application to the subsoil. Root growth increased at the site of N placement in the subsoil. The ability of wheat to recover N from subsoil was high (about 70%). Nitrogen fertiliser increased the water use and water use efficiency compared with the control.

A further experiment was conducted to test the hypothesis that a split application of N has an equivalent effect on GPC as a subsoil N reserve which is used after anthesis. Wheat was grown in a sandy loam soil in pots 105 cm deep. There were four N treatments applied as KNO_3: (N0) no N; (N1) 150 mg to topsoil at sowing; (N2) 75 mg to topsoil at tillering and 75 mg to topsoil at booting; (N3) 150 mg to subsoil after anthesis. Water treatments were applied as in the previous experiment. Drying of the topsoil decreased grain yield but increased the GPC of wheat. Placement of 75 mg N in the topsoil at tillering and 75 mg N topsoil at booting or 150 mg N at 60 cm depth after anthesis produced higher GPC than the control. The effect of the split N application (N2) on GPC was comparable to the subsoil N application after anthesis (N3), due to the leaching of the N into the subsoil where it was taken up after anthesis. Root growth increased at the site of N placement in the subsoil. N fertiliser increased the efficiency of water use compared with the control.

Root length density declines with depth and in some cases root growth in the subsoil is poor. It may not be sufficiently large to utilise N effectively, or it may limit the ability of the plant to take advantage of transient increases of available N from mineralisation or exploit subsoil N reserves. Therefore it is important to examine the effect of different root length densities on N uptake from the soil. Short term (48 hour) N uptake by wheat at different rooting densities with different levels of plant demand (imposed by the use of shading treatments) was studied in 2 experiments. This experiment examined the importance of root density and plant growth rate on the rate uptake of N by wheat.

Wheat was grown in 2.4 kg sandy loam soil in pots 20 cm deep. The two rooting densities were produced by planting 3 or 9 seeds per pot. There were four N treatments applied as KNO_3: (a) no N; (b) 50 mg per pot; (c) 100 mg per pot; (d) 150 mg per pot to topsoil four weeks after sowing. After applying N, shade treatments were introduced to provide (1) non-shade or (2) shade. A second experiment also included a treatment which reduced effective leaf area (LA) by covering the basal half of each leaf with foil at the time that shade treatment were imposed. Two sets of plants were harvested, before and after the N and shade treatments were imposed.

Shading had no significant effect on root dry weight or root length, but average shoot dry weight of shaded plants was lower than that of non shaded plants after the 48 hour period. The rate of N uptake increased when 50 mg N was applied but was not increased further by higher rates of application. This response to N was independent of root length density. Restricting the growth of the plants by shading or decreasing the area of leaf exposed to light had no effect on the response of the plants to 50 mg N, but it decreased the ability of the plant to respond to higher levels of N. These results show that the ability of plants to exploit transient increases N in soil depended more on the shoot growth rather than rooting density. The rate of uptake was not affected by the density of roots but was sensitive to shoot growth. Therefore a transient increase in subsoil mineral N due, for example, to mineralisation and leaching, may improve the N uptake by plant, but the ability of the plant to respond to this N will depend on its growth rate.

The findings from this work showed that the subsoil mineral N is potentially a good N reserve for the wheat late in the season when there is no water available in the topsoil for the plant. The ability of plant roots to recover N from the subsoil is high and N uptake from subsoil after anthesis can increase the GPC of wheat. The results also showed that as long as the N is not leached from the root zone topsoil N application at sowing or split N application at tillering and booting has an equivalent effect as application to the subsoil. Results provided a sound basis for field work to investigate the effect of subsoil mineral N on GPC of wheat.

DECLARATION

I hereby declare that the thesis presented here contains no work which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Mohammad Lotfollahi

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Lotfollahi M, Alston A M and McDonald, G K (1997). Effect of nitrogen fertiliser placement on protein concentration of wheat under different water regimes. Australian Journal of Agricultural Research 48, 1-10.

CHAPTER 1

GENERAL INTRODUCTION

Nitrogen (N) is one of the most important elements for plant growth and the adequate supply of N has been recognized as a major limitation of agriculture (Olson and Kurtz 1982, Freney and Simpson 1983, Vallis 1990). Nitrogen is also very dynamic in agricultural ecosystems because it enters and leaves the soil and plant system by more pathways than any other plant nutrient. The amount of inorganic N (NO_3-N + NH_4-N) available in the soil and which is taken up during the growing season affects the yield and grain protein concentration (GPC) of wheat.

There has been some concern in southern Australia about a decline in the average GPC of wheat (Palmer 1990, Dyson and Fewings 1990). The amount of N in the grain comes from two sources, N remobilised from leaves, roots and stem during grain filling and N taken up from the soil post-anthesis. If the soil water content falls, or if the availability of N in the soil is low, the N in the grain, and hence GPC, depends largely on the mobilisation of N and its translocation to the grain. Although there is debate about the cause of declining GPC (*i.e.* whether it is a symptom of declining fertility caused by more intensive cropping or a result of higher yields achieved by better management), it is apparent that the supply of N to the grain during grain filling period is often inadequate to produce grain with satisfactory GPC.

Russell (1963) suggested that GPC < 11.7 % (< 2.3 % grain N) were indicative of soil N deficiency, and on this basis nitrogen deficiency has been reported to be a widespread factor limiting cereal productivity and quality of wheat in southern Australia (McDonald 1989, Reuter 1989, Xu *et al.* 1991 and 1992, Xu and Elliott 1993).

In the wheat-growing areas of southern Australia the topsoil which contains a large amount of labile plant nutrients becomes progressively drier during anthesis and grain filling and the N uptake by plants may be low late in the season (Alston 1976).

Subsoil mineral N may be a useful N reserve for a field crop when N in the surface layer is unavailable or not accessible because the topsoil is dry.

In southern Australia, leaching of NO_3-N can occur during winter because of the combined effects of a winter-dominant rainfall and relatively low demand for N by the crop. Observation from the field at the Waite Institute illustrate this. Soil water content increased with increasing depth (until 80 cm) late in the season and there was also a loss of mineral N from the surface horizon of the soil, part of which may be due to leaching to the subsoil. Consequently in southern Australia there is often an accumulation of NO_3-N in the subsoil which often is not exploited by the crop until after anthesis (Storrier 1965, Greenland 1971). Increasing the amount of N in the soil by the use of N-fertiliser or more frequent inclusion of legumes in the rotation may increase the amount of NO_3-N that is leached from the surface soil, and increase the pool of subsoil N (Kissel *et al.* 1974, Ladd 1990, Weier and Macrae 1993).

Ladd (1990) argued that late season uptake of N from the subsoil can play an important role in determining GPC of wheat. There is considerable evidence that post-anthesis uptake of N from the subsoil can increase GPC and, in some cases, grain yield (Smika and Greb 1973, Strong and Cooper 1980, Strong 1982, 1986). However the effectiveness of uptake, its efficiency in increasing GPC and the importance of factors such as available soil water and root length density on N uptake require further study to develop a better understanding of the factors that may improve the utilisation of subsoil N.

The work reported in this thesis was undertaken to investigate the importance of subsoil mineral N availability on GPC of wheat. Firstly the recovery of N from the subsoil was examined in a series of greenhouse experiments. In these experiments different rates of N was applied to the topsoil and subsoil at different times under different water regimes. The influence of N fertiliser on shoot and root growth, nitrogen status of plant, soil and water use was measured in these experiments. Secondly, the effect of split N application on GPC was investigated using pre and post anthesis N applications. Thirdly, short term N uptake by the root at different rooting densities was

studied in the greenhouse. The overall objectives of the studies was to examine the importance of subsoil mineral N and investigate about the ability of wheat to takeup N from the subsoil late in the season.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Nitrogen (N) is a vitally important plant nutrient that is required in large quantities for crop production. Legume-based pastures continue to be the main source of N for cereal crops in large areas of southern Australia, although in higher rainfall areas their importance has declined and there is greater use of N fertiliser. There has also been a trend to lower grain protein concentration (GPC) in many parts of the cereal belt that has occurred co-incidentally with these changes. This suggests that the supply of N during grain filling is inadequate to maintain GPC. The amount of inorganic N (NO_3-N + NH_4-N) available in the soil over the growing season affects greatly the yield and GPC of wheat. Nitrate (NO_3-N) and ammonium (NH_4-N) production in the soil profile are related to mineralisation, microbial activity, leaching, soil organic matter, climate and agronomic practice. An understanding of the N cycle and interaction between soil N and N status of wheat are important in efficiently managing N in cereal crops. In southern Australia, subsoil accumulation of NO_3-N is common, but it usually remains within the root zone of crops (Storrier 1965, Greenland 1971) because rainfall is generally insufficient to cause leaching beyond the roots. The NO_3-N can be taken up by the wheat late in the season and Ladd (1990) stated that late season uptake of N from the subsoil can play an important role in determining GPC of wheat.

This chapter provides a review of literature on the N in cereal-legumes rotation in southern Australia. It will also consider the effect of environmental factors and soil management on the soil N. Nitrogen requirements of a wheat crop, with special attention to the N uptake by plants and NO_3-N concentrations in the plant tissue is another part of this chapter. This review integrates the results of studies (up to 1993) on the effect of N on the GPC. The GPC in the southern Australia and the effect of subsoil mineral N on GPC of wheat to provide a focus on the possible uptake of the subsoil N

by wheat in the late season. This review will discuss the factors affecting soil mineral N more generally with particular reference to subsoil mineral N, depth of mineral N.

2.2 Nitrogen in cereal-legume rotations in southern Australia.

Cereal-legumes rotation and climate, particularly rainfall, are the predominant factors affecting cereal responses in grain yield and GPC to application of N fertiliser.

2.2.1 Cereal-legume rotations

In environments where water is not a limiting factor for plant growth, N should be supplied in amounts appropriate for the maximum economic expected yields and GPC. In these conditions, the supply of N from the soil is usually insufficient for optimum economic plant growth and the main decision is on the level of application. In a Mediterranean environment, the problem of N nutrition of crops is more complex due to the unpredictable occurrence of seasons when water is limited. The problem is even more complicated in countries like Australia where the supply of N for cereal crops is dependent partly on input by legumes. The question is "Do legumes supply enough N for maximum production of the subsequent cereal crop"? In the Australian literature numerous references to this question are given, examining both the total soil N increase and the wheat yields after leguminous pastures. As far as total soil N accumulation is concerned the studies are reasonably consistent. Ford (1968) reviewed inputs from pasture legumes and found an average 70 kg ha^{-1} y^{-1} total N. Similar figures have been given by Greenland (1971), Kohn *et al.* (1977) and Dahmane (1978).

The amount of 70 kg ha^{-1} total N input is that needed for wheat yields of 2-3 t ha^{-1} in a semi-arid environment (French 1978b). However, Ladd *et al.* (1981a) found that from amounts of 40 to 60 kg ha^{-1} of organic N returned to the soil by medic, only 40 percent was available the next season and the remaining 60% had a half-life of 6 years. Additional mineral N can be released from the soil organic matter, under favourable conditions for mineralisation.

2.2.2 Available mineral N in the soil after legumes.

During the growing season, the N fixed by legumes is the organic N in shoots and roots, plus the amount released to the soil from dead roots and leaves. For grain yield of subsequent crops we are interested not only in the increase of soil fertility as total soil N after legumes, but also its availability, which is determined by the fate of the residuals.

The most common uses of pasture or grain legumes are grazing, herbage conservation (hay, silage), seed harvesting or incorporation in the soil. All these treatments remove part of the N except for herbage incorporation into the soil as green manure, which is not a common practice in Australia. The management practices which remove most N are seed harvesting of grain legumes and herbage conservation of pastures. Any N returned to the soil through animals or in herbage remaining in the field is subject to further losses, mainly as gas to the atmosphere. Watson and Lapins (1972) and Brown (1977) found total losses N of up to 82% from residues left on the surface. During the summer period losses of dry matter between 50 to 80% have been recorded (Cameron 1966, Parrott and Donald 1970, Brown 1977). Finally, from all the organic N which is returned to the soil and is not lost, only part will be available to the next crop.

In the field, the mineral N after legumes appears to be higher than after cereal crops. Strong and Clarke (1977) measured 72, 71 and 48 kg ha^{-1} mineral N in March after lathyrus, field peas and lupin respectively, compared with 13 and 20 kg ha^{-1} after oats and wheat. The soil mineralisable N after leguminous pastures was higher than under grassy pastures (Rixon 1969, Dahmane 1978). French (1978 a) found that high NO_3-N in soil after fallow was usually associated with leguminous ley history. Dann (1969) measured higher soil NO_3-N under sub-clover than under naturally occurring pastures.

The higher N availability after legumes compared with non-legumes crops is associated with the high N concentration (low C:N ratio) in legume residues. During decomposition of organic residues with low C:N ratio mineral N is readily released for

subsequent crops from the decomposing tissue (Barrow 1960). However the increased amount of mineral N after legumes does not mean that there is always sufficient for maximum production of ensuing crops in years of ample rainfall.

2.2.3 Legumes in rotations and wheat yield.

The main reason for improved wheat yields in southern Australia in the late 1940s was the input of N by leguminous pastures (Donald 1963, 1964, French *et al.* 1968). The positive residual effects of leguminous pastures on subsequent wheat yield have been demonstrated by many field experiments in Australia (Watson 1963, Meagher and Rooney 1966, Tuohey 1973, Rixon 1969, 1972).

The results of long-term rotation experiments have shown that wheat grown in sequences with leguminous pastures have done better than in sequences without legumes, *e.g.* fallow-wheat. (Elliott and Jardine 1972, Clarke and Russell 1977). Legume pastures also contribute to the subsequent crop by improving soil structure (Andrew 1965). Stoneman (1973) showed that the percentage of water stable aggregates increased with the number of years of subterranean clover leys, but soil structure declined very rapidly after cropping. Consequently, the increased yield of subsequent cereal crops is not only a result of the mineral N contribution of legumes in preceding years, but also as a result of improved soil structure. The mineral N contribution by legumes to the subsequent crop needs to be isolated from other factors by soil analysis or plant analysis.

Department of Agriculture of South Australia (1990) reported that from the mid-1970s cereal farmers in southern Australia have sown more grain legumes, paid less attention to their pastures, and applied more N fertiliser. Rotations that allow easier control of diseases and weeds in the cereal phase have become popular because they give high farm returns in the short term. These short-term decision are paving the way for long-term disaster. Already in some areas they have jeopardised the sustainability of agriculture. Closer rotations require more tillage and more rapidly degrade the soil. More N and other nutrients will be removed from the soil when there are more crops.

Furthermore, because there is less pasture or pastures have been replaced with grain legumes, less N and organic matter are returned to the soil. Where pastures are not productive and have little medic or clover there is an even greater decline in fertility. The falling GPC of southern Australia wheat crops is a result of this kind of farming system.

2.2.4 Nitrogen and water

Wheat in southern Australia is grown as a rainfed crop. Water deficits are usually encounted late in the growing season and this may interact strongly with N to determine grain yield and GPC. Both yield increases and decreases due to N application are known, depending on water supply. For example, Barley and Naidiu (1964) showed that applications of N to wheat increased the rate of soil water depletion and consequently increased water stress during the grain filling stage. There are many studies that show that responses to N depend on the availability of water during the growing season and the incidence of environmental stress (Blum and Pnuel 1990, Van Oosterom *et al.* 1993). Therefore it is important to understand the limitations of the climate when examining N responsiveness.

2.2.5 Major features of the Mediterranean environment

The major features of the Mediterranean environment climate are mild, wet winters and hot, dry summers. Annual rainfall varies considerably and ranges between 275 and 900 mm. More than 65% of the annual rain falls in winter (Smith and Harris 1981, Hamblin *et al.* 1987, Buddenhagen 1990). Dry areas frequently receive their annual precipitation in sporadic but heavy falls with consequent rapid runoff and erosion. The length of the growing season is defined by the availability of water for plant growth. It depends on the locality and season, but ranges from 5 to 7 months (Prescott and Thomas 1949). General descriptions of the climate of southern Australia are given by Leeper (1970), Gentilli (1971) and Nix (1975). Total annual rainfall in the cereal belt of southern Australia varies from 300 to 600 mm. Most rain falls during the

winter, and the April-October rainfall consist of 80% of the annual total. The growing season is determined by the beginning of effective rains, and its length varies from 4 to 6 months. Water deficit is a major limitation to productivity, and a delay in its development through cropping pattern adjustments or water conservation is the key to increased productivity (Arnon 1979, Van Oosterom *et al.* 1993).

2.2.6 Water stress in Mediterranean environments

In Mediterranean environments the amount of rainfall and its distribution are important constraints to winter cereal production (Ceccareli *et al.* 1991). The total water content of the soil profile increases during the winter period, reaching a maximum in late winter-early spring. During spring, when rainfall declines and evaporative demand increases the soil profile dries, through crop extraction of water or direct evaporation from the soil surface or both (Cooper 1983, Gregory *et al.* 1984). The depth of wetting during winter varies from site to site and season to season, depending on the total rainfall and its distribution within the growing season as well as type of soil. Cropping strategies are influenced by the probable timing of stress periods. The rate of development of stress depends on the amount and pattern of rainfall during the season and on soil type, but it is the development of water stress which greatly influences grain yield and the responsiveness of crops to N.

2.2.7 Interaction between water and nitrogen

Water and N are frequently the two most critical factors in limiting crop production in the environment of southern Australia. A strong interaction between the two is known to exist (Young *et al.* 1967, Singh and Prihar 1978, Eck 1988, Engel 1991) and one may improve the use efficiency of the other to a certain extent (Singh *et al.* 1975, Kanemasu *et al.* 1983, Benbi 1990). This interaction is greatly modified by soil type, root distribution pattern and other factors that influence water availability and use, *e.g.* root diseases. An optimum combination of water and N is essential for enhancing the water use efficiency and maximising crop yields. There are many ways

by which water use efficiency can influence N efficiency in cereals. Firstly, added water may increase root growth and thus increase absorption of N; secondly, soil water affects mineralisation of N from the soil organic matter; thirdly, N is absorbed by plants largely through mass flow which requires water; fourthly water movement into the soil is required to move N fertiliser into the root zone to make it available but excess water also removes soil N by denitrification and leaching (Sander *et al.* 1987). As water use efficiency increases, the response to N fertilisers will be improved and will be reflected in higher crop yields. Nitrogen is a mobile element in the soil and moves from colloids to roots mostly by mass flow (Russell 1980). Where water in the soil is low, the response to N fertiliser may be reduced with reduced flow of N to roots. Low soil water content reduces growth and total dry matter yield, thereby decreasing the demand for N by the crop. Under dryland conditions a high proportion of the fertiliser remains in the soil and will probably be available for subsequent crops. High amounts of applied N fertiliser may however decrease yields due to lodging and haying off. Haying off is attributed to high rates of available soil N stimulating early vegetative growth and water use, thereby depleting soil water reserves and inducing water stress in the crop. This decreases harvest indices because of water stress during the latter part of the growing season (Storrier 1975). In semi-arid regions, variability in rainfall and the associated variability in the degree of water stress may account for up to 85% of the variability in the yield of wheat (French and Schultz 1984).

2.3 Nitrogen supply: soil

The N nutrition of cereal crops is a complex and dynamic process. It depends on the acceration of organic N in the soil, its availability and uptake by the crop and its partitioning with in the plant. Each of these processes is influenced by environmental and edaphic factors and can be managed by addition of fertiliser.

2.3.1 Soil nitrogen

Knowledge of soil type, climate and the behaviour of N in the soil is important for management of N. Nitrogen is required by plants in greater amounts than other nutrients (Viets 1965) and the amount of inorganic N affects the yield and GPC of wheat. Viets also comments that N is the most unpredictable of the nutrient elements in the soil and emphasised the dependency of N availability on weather conditions and mobility of N in the soil. Nitrogen should be applied in amounts appropriate for the maximum expected yields in environments where water is not a limiting factor for plant growth. Under these conditions the main decision is on the amount of N application because the supply of N from the soil is usually insufficient for maximum yield. The problem of N nutrition of crops is more complex in a Mediterranean environment due to the unpredictable occurrence of seasons when water is limited. Where the supply of N for cereal crops is dependent mainly on input by legumes, as occur in southern Australia, the problem is even more complicated because the amount of N available to cereals grown after legumes is not usually enough to sustain maximum growth of wheat (Papastylianou 1980).

The amount of inorganic N in the soil is affected by a variety of biochemical and chemical processes. Natural gains in total soil N occur through symbiotic and non-symbiotic fixation of molecular N_2 by microorganisms and from the return of NH_4-N and NO_3-N in rain water. Nitrogen losses occur through volatilization, crop removal and leaching. The change of molecular N_2 to combined forms through biological N_2 fixation is of particular interest both practically and theoretically. Nitrogen may be lost from a soil-plant ecosystem by various processes, principally through involvement of soil inorganic N. Loss mechanisms include erosion (which depletes both soil inorganic N and organic N pools), ammonia volatilisation, denitrification of NO_3-N and leaching of NO_3-N in soil to depths below the zone of uptake by roots.

2.3.2 Factors affecting the mineral nitrogen availability

Several factors will affect the availability of mineral N. The most important are ammonia volatilisation, denitrification, mineralisation, immobilisation and leaching. Leaching of N mineralised in or applied as fertiliser to the surface layer of soil appears to be the main avenue of NO_3-N build-up in the subsoil.

2.3.2.1 Ammonia volatilisation

Ammonia volatilisation represents a major loss of nitrogen from agricultural system involving animals (Jarvis and Pain 1990) but its importance is generally much less in dryland grain crops. It has also been found that some ammonia can be lost from the foliage of arable plants. This may be serious in crops that are heavily over-fertilised with N, or are badly diseased (Goulding *et al.* 1993), but in normal crops the quantities involved are probably less than 10 kg N ha^{-1}, although there is some evidence to the contrary (Schorring *et al.* 1989). The form of N fertiliser may affect the amount of N volatilised. Under certain conditions, for example, in dry calcareous soils, large quantities of N may be lost as ammonia if urea fertiliser is applied to the surface of soil (Fenn and Hossner 1985).

2.3.2.2 Denitrification

The other main path way of NO_3-N loss, is the conversion of nitrate to a mixture of nitrous oxide (N_2O) and nitrogen (N_2) which is evolved to the atmosphere from soil that is wet and sufficiently warm for microbial activity (Fillery 1983, Sahrawat and Kenney 1986). Denitrification has long been recognized as loss of a valuable plant nutrient but, more recently, environmental problems associated with N_2O have been recognized. It is important to ensure that future changes in agricultural practice, including those designed to decrease NO_3-N leaching, do not lead to an increase in N_2O production because they increase the residence time of NO_3-N in soil. Nitric oxide (NO) and nitrogen dioxide (NO_2) also influence atmospheric chemistry and can be evolved from and absorbed by soils and crops. As yet information on the factors

involved is rather limited (Jenkinson, 1990a). Denitrification can be considered desirable when it occurs below the rooting zone because it reduces the NO_3-N content of ground water. Denitrifying microorganisms are known to be present at considerable depths in soil, and it is possible that some of the NO_3-N leached into the subsoil may be denitrified before reaching the water table. Meek *et al.* (1969) concluded that much of the NO_3-N leached into the subsoil in irrigation waters was lost through denitrification. Stewart *et al.* (1967) found that NO_3-N concentration in soil under feedlots decreased sharply with depth and concluded that the decrease was due to denitrification. In semi-arid winter rainfed environments with annual vegetation, denitrification is generally regarded as a minor mechanism of N loss (Noy -Meir and Harpaz 1977)

2.3.2.3 Mineralisation

Mineralisation is one of the most important processes of NO_3-N and NH_4-N production in soils. The mineralisation capacity of soils is of considerable importance in regard to optimising N fertilizer recommendations. Nitrogen mineralisation rates are influenced by microbial and faunal activities in the soil as well as by the C:N ratios of decomposing organic materials. The proportion of soil organic N mineralised annually generally ranges from about 2-4% (Scarsbrook 1965) but higher values have been reported. For example, Ladd and Amato (1986) reported net gains of NO_3-N in a soil profile equivalent to 4.9% of the organic N of the topsoil (0-10 cm). Cassman and Munns (1980) showed that more than one half of the N mineralised in soil from a profile to 108 cm deep and incubated *in vitro* was due to decomposition of organic N in the subsoil (below a depth of 18 cm). Schultz (1972) found 152 kg ha^{-1} NO_3-N in the 60 cm zone after grassland in which all herbage was burned in autumn before sowing. This high amount of mineral N was attributed to mineralisation of total soil N after favourable summer rains.

Mineralisation of N in soils is influenced by many factors. The nature of the soil, the accessibility of decomposable material and microbial activity are important. For example, the amount of mineral N in a fallow soil is higher than that in a cropped soil

because the populations of microorganism in the cropped soil are high compared with the fallow soil and there is considerable competition between microorganisms and plants for mineral N in the soil. Mineralisation of N was studied by Robertson *et al.* (1993) who reported that decomposition and net mineralisation of N were stimulated by soil cultivation and a 6 month fallow resulted in accumulation of N in the organic soil pool. Perhaps the cultivation stimulated the microbial activity. The addition of decomposable plant residues to soil initiates an increase in the rate of microbial activity, which can either mineralize or immobilize N. The principal factor determining which of these two processes occurs is the C:N ratio of the material being decomposed.

The water content and temperature of the soil are major factors determining biological activity. Van Veen and Frissel (1981) showed that by increasing the temperature to 30° C biological activity increased and by increasing the water stress (from -10 to -2000 kPa) activity decreased. The effect of soil temperature and water on mineralisation was examined by Cassman and Munns (1980). They used four incubation temperatures (15, 20, 25 and 30° C) in a factorial combination with six soil water potentials (-10, -30, -70, -240 and -1000 kPa). There was a significant water x temperature interaction. Nitrogen mineralisation increased in the 30° C treatment above that expected from the additive effects at 30° C. These results indicate that both quantity and distribution of soil water affect *in vitro* estimates of N mineralisation. Stanford *et al.* (1973) showed that rates of N mineralisation for each 10° C rise in incubation temperate over the range 4-35° C doubled. The overall effect of soil water on N mineralisation was similar to the effect on nitrification except at very high or low potentials (Miller and Johnston 1964). Total soil N was decreased by successive cycles of submergence and drying. Nitrogen was nitrified during the aerated portion of the cycle and lost when the soil was submerged (Patrick *et al.* 1964). Predicting the amount of mineral N released to the crop under a specified climate condition requires understanding of the quantitative relationship between soil water and temperature and mineralisation (Stanford and Epstein 1974). At water levels between the permanent wilting and field capacity percentages ammonification and nitrification take place as

expected. Nitrification rates of both fertiliser and soil N increased by addition of lime to fertiliser acidified soils (Clay *et al.* 1993); this is possibly because lime will change the soil pH and the microbial activity will be different in various soil pH.

The mineralisation of organic N during the growing season might be expected to contribute significantly to the N requirement of the crop during the phase of active N uptake by the plant which, in the case of wheat, is from 4-5 leaf stage to anthesis. Net soil N mineralisation in a fallow/wheat rotation was investigated in field and pot experiments by Hart *et al.* (1979). Wheat significantly enhanced net soil mineralisation of N although the effect depended on the stage of development of wheat. Significant effects on mineralisation occurred only after sufficient root development and continued to the booting stage. They reported similar patterns of N uptake for wheat crops and their comparisons with fallow soil suggest that the crop during the most rapid growth phase enhances mineralisation. Dassk *et al.* (1993) reported that N release would occur even with the wide C:N ratios provided there was adequate time for decomposition. Microbial immobilisation initially was increased after the addition of organic matter when soil water content was both under field capacity and at 50% of field capacity. McTaggart and Smith (1993) argued that when decisions on cropping are normally made early, measurement of soil mineral N was not a practicable method for determining spring fertiliser application, because the soil mineral N will change during the season and that the measure of potentially mineralisable N appeared more promising in this regard. Robinson (1957-1958) in laboratory incubation studies with topsoil samples of the Kikuyu red loam soil have shown that active nitrification of the natural soil N stops at a soil water level just below the permanent wilting percentage.

Production of NO_3-N has often been used as an indicator of net mineralisation in soils (Cassman and Munns 1980, Hadas *et al.* 1989) because the NH_4-N concentration in aerated soils has been found to be small and constant during crop growth (Harmsen and Vanschreven 1955). Immobilisation of N associated with root decomposition would account for the absence of a significant amount of both NH_4-N and NO_3-N under pasture. It may not be possible to predict N availability and crop response precisely

given the dynamic nature of the N cycle and the variability imposed on it by the interaction of weather crop management and crop growth. Average rates of mineralisation after a long fallow under southern Australian conditions at a site of reasonably high fertility for a grain yield of about 2 t ha^{-1} would be sufficient,whereas just over 1 t ha^{-1} could be produced after a short fallow. Under high rainfall conditions rates of mineralisation of organic N and its vulnerability to leaching are high (McDonald 1989). Nitrate released from organic sources over summer and autumn, and leached into the soil profile in winter deep in the active root zone, may be taken up late in the growth season and thus may constitute a potential source of N for increasing the GPC (Ladd 1990).

2.3.2.4 Leaching

Leaching may be defined as the transport of N in water-soluble forms out of a defined soil volume, the root zone, into a subsoil (White 1988). Over 90% of soil N is not subject to leaching because it is bound in organic matter. However through the processes of mineralisation and nitrification organic-N may be transformed into NH_4-N and NO_3-N. The NH_4-N ion is relatively immobile in soils which have predominantly negative charge because it is retained on soil colloids by adsorption and fixation due to its positive charge. In contrast, the NO_3-N anion can be readily leached when water drains through the soil. There is potential for NO_3-N leaching to occur when ammonium fertiliser is used because during the nitrification process ammonium is changed to NO_3-N (Cameron and Scotter 1986). Nitrate leaching is an economic loss to the farmer if the NO_3-N is not recovered later in the season. Achieving high crop yields and high economic return on the investment by using fertiliser N but with little pollution is one of the challenges of current agriculture in other parts of the world where rainfall is high. In southern Australia, subsoil accumulation of nitrate (topsoil leaching) is common, but it usually remains within the root zone of crops (Greenland 1971) because rainfall is insufficient to cause leaching beyond the roots.

2.3.2.5 Nitrate in the soil solution and nitrate movement

The concentration of NO_3-N in the soil solution is determined by the rate of NO_3-N added by fertiliser, mineralisation, and nitrification relative to the rate of removal by plant uptake, denitrification, leaching and immobilisation (Cameron and Scotter 1986). Diminished amounts of NO_3-N in the upper soil layers and increased amounts in the lower layers indicate movement of the NO_3-N. When infiltration of precipitation or irrigation exceeds evapotranspiration large amount of NO_3-N can move well below 2 m depending on soil type (Pratt *et al.* 1972, MacGregor *et al.* 1974 , Schumann *et al.* 1975). For example, on an untilled Forman clay loam after 15 years of liberally fertilised maize, appreciable amounts of NO_3-N were found to at least the 10-m depth, which was equivalent to a movement of the NO_3-N front of 1.9 mm day^{-1} (MacGregor *et al.* 1974). Nitrate generally moves with the soil water and is absorbed by the roots when water is absorbed (Bray 1954). It must also be assumed that the balance of the accumulated nitrate in the subsoil was derived through leaching of the surface layers of soil. Summer rainfall (> 50 mm) can move nitrate down large cracks in the soil surface to a depth of 150 cm and below the rooting depth of crops (Kissel *et al.* 1974). Nitrate is readily carried to plant roots by mass flow (Russell 1980, Stevenson 1982). When the supply from mass flow is lower than the potential uptake, the process of diffusion begins, because the concentration of N at the root surface is lowered.

Two physical processes responsible for NO_3-N movement are diffusion and convection. When concentration gradients exist within a pore or between adjacent interconnected pores containing soil solution, molecular diffusion tends to flatten these gradients out. Thus, even if NO_3-N is present in a small soil pore not subject to significant viscous water flow, NO_3-N can be transported by diffusion into a large adjacent pore where it can be convected away. The concentration gradient that is established for a specific ion will depend on several factors: (a) the ion concentration in the soil solution; (b) the rate of ion uptake per unit of root surface; (c) the rate of ion movement to the root surface by mass-flow (d) the rate of ion diffusion along the surface of soil particles; (e) the rate of ion replenishment associated with other ions in

solution from ions held by the soil; and (f) the soil capacity to replenish the NO_3-N (Barber 1962).

Convection is the passive movement of NO_3-N dissolved in the soil water and is the main transport mechanism. Nye and Tinker (1977) reported that NO_3-N can diffuse about 10 mm over a day in moist soil. So on this scale convection and diffusion interact significantly, usually to enhance leaching. The geometric complexity of the soil pore space makes detailed quantitative description of leaching at a microscopic level impossible, except for highly idealised systems (*e.g.* Scotter 1978).

2.3.3 Factors affecting leaching losses

Leaching of mineral-N to the subsoil depends on the rate of mineralisation (which affects the quantity), the conditions that affected soil water, season and climate, soil properties, land use and soil management and rate of fertiliser.

2.3.3.1 Season and climate

In temperate agricultural systems, leaching occurs mostly during winter when both crop N uptake and evapotranspiration are low (Shaw 1962, Storrier 1965). Nitrate leaching is least likely to take place during the late part of the season when evapotranspiration usually exceeds precipitation, and plant uptake rates are high (Allison 1973).

Rainfall and temperature at the time of, and in the period following, N applications will have an important influence on the fate of the added N. Rainfall will determine the degree of leaching and distribution of N in the soil, and together with temperature will influence plant uptake and the biological processes affecting N transformation (Cuttle and Bournce, 1993). During summer, rain is generally used to satisfy the soil water deficit and leaching is usually minimal. A dry summer can result in an accumulation of soil NO_3-N due to lower crop uptake. An example of this is given by Strong and Cooper (1980). Amir *et al.* (1994) found that 15 to 38 kg ha^{-1} of NO_3-N measured for each 30 cm soil increment is not available for plant uptake. This plant

unavailable NO_3-N background in the soil can not be leached by repeated irrigation cycles of 100 mm each, or by heavy rains. Shuford *et al.* (1977) found that NO_3-N was leached from all depths down to 1 m in a well-drained silt loam following single application of 89 and 178 mm of water. The recovery of added NO_3-N down to 1.35 m was less than expected if uniform displacement had occurred suggesting that some may have moved by preferential flow below this depth. George *et al.* (1993) suggested that in tropical soil used for lowland rice crop, management during the dry season should be designed to limit NO_3-N build up so as to reduce NO_3-N that is prone to loss by denitrification during the dry to wet transition (Simpson 1962). Pasture production in the early spring may be expected to reflect the amount of NO_3-N leaching as found by Harmsen (1961) by becoming N-deficient after a wet winter but not after a dry one. In southern Australia, because rainfall is insufficient to cause leaching beyond the roots subsoil NO_3-N usually remains within the root zone of crops (Greenland 1971).

2.3.3.2 Soil properties

Biological, physical and chemical properties of soil are important influences on leaching. For example, if the rate of nitrification is rapid then there will be a large amount of NO_3-N available in the soil for leaching. Soil physical and chemical properties such as texture, cation and anion exchange capacity, also have an essential role in the amount of NO_3-N leached.

Biological

The release of inorganic N from soil organic matter by biological activity is recognised as essential for the N nutrition of non-leguminous plants grown in the field (Storrier 1962). A major source of NO_3-N in the soil is from mineralisation and nitrification of soil organic N. Large amounts of NO_3-N can be leached from unfertilised bare fallow soils. The amount of NO_3-N released, and thus potentially lost, will depend on environmental conditions such as soil pH, temperature, moisture status,

and aeration as well as total N status the C:N ratio. All of these factor can affect the activity of the microorganisms. Mineralisation of soil organic N is stimulated by cultivation and can result in subsequent leaching losses of NO_3-N (Cameron and Wild 1984). Immobilisation of fertiliser N can occur rapidly in moist temperate soils and this can lead to a large decrease in the amount of fertiliser derived NO_3-N which is leached. This was shown in a ^{15}N study in which 6% of the fertiliser N was leached from the normal lysimeters (103 mm rainfall) and 14% from the wetter lysimeters (186 mm rainfall). In contrast 76% of the applied bromide was leached from the wetter lysimeters (Mohammed *et al.* 1984). Application of fertiliser N may stimulate the mineralisation of soil organic N (Hauck and Bremner 1976) and thus lead to leaching of native soil nitrate. That is, due to immobilisation-mineralisation turnover, fertiliser will be immobilised but at the same time native soil organic N will be mineralised and possibly lost. In soils which are wet, denitrification may also occur and it is often difficult to apportion the total loss of N between gaseous losses and leaching. Nitrate within wet soil aggregates and in anaerobic zones in the soil potentially will be denitrified. Subsoil NO_3-N may be lost due to denitrification (Stewart *et al.* 1968, Meek *et al.* 1969). McGarity and Myers (1973) reported several significant drops in soil NO_3-N concentration due to denitrification in anaerobic conditions.

Physical

Soil structure and texture are the major factors determining hydraulic conductivity and water storage capacity. If hydraulic conductivity is low, rainfall will tend to cause surface runoff instead of leaching.

Soil water storage is important in two ways. Firstly, NO_3-N is leached to a depth approximately equal to the excess of rainfall over evapotranspiration divided by the volumetric water content of the soil at field capacity, provided movement through the soil is uniform. Secondly, if the available water holding capacity is high the amount of water required to rewet the root zone after a dry summer is also high (Cameron and Scotter 1986). A large amount of autumn-winter rainfall may be used to satisfy the soil water

deficit and does not lead to through drainage. Soils with different textures have different water contents at field capacity. Lower rates of leaching have been found to occur on clay soils and higher rates on poorly structured sandy soils (Ladd *et al.* 1981, Sommerfelt *et al.* 1982). Holford (1981) reported higher leaching losses on a red brown earth than on a black earth because the red-brown earth was more freely drained (Holford and Doyle 1978). Earthworms and roots can have overriding effects on solute leaching patterns because the macropores developed with root and earthworms (Wetselaar 1962, Wild 1972, Elrick and French 1966).

Chemical

Anions such as NO_3^- are excluded from the space closest to the colloid surfaces in soil which have a net negative charge. For this reason, a small proportion of the soil water does not participate in anion leaching and the effective pore volume is less (often up to 10-20%) than the soil water content (Wild 1981). Thomas and Swoboda (1970) reported that in some soils the phenomenon of anion exclusion cause a significantly faster rate of anion leaching. The positive charge on some soil colloids in acid soils is high and non-specific adsorption of NO_3-N can occur. In these situations the rate of NO_3-N leaching was decreased in this soil (Kinjo and Pratt 1971, Holland and During 1977).

2.3.3.3. Land use and soil management (tillage method, pasture, cropping system)

The amount of NO_3-N leached will be affected by soil management. For example, the use of different methods of cultivation, and crop rotation affect the amount of N in the soil and the movement of water. It is possible to improve the subsoil mineral N with good management.

Tillage method

Pores in the soil are affected by the method of tillage and planting. The continuity of the pores and the range of the pore size are likely to be different under ploughing compared with direct drilling (Ball 1981, Osborne 1984). These changes affect the flow pathways and hydraulic conductivity in the soil (Ehlers 1976, 1977) and ultimately affect leaching losses. There is usually a large number of continuous macropores open at the soil surface in direct drilling (Barnes and Ellis 1979). Sometimes a greater loss of NO_3-N has been observed from direct drill compared with conventional cultivation soil due to increased leaching (Tyler and Thomas 1977). Nitrate leaching was measured under no-till and conventional tillage irrigated maize on a sandy loam soil by Ritter *et al.* (1993) and was found to be slightly higher under conventional tillage than under no-till possibly because soil texture affected the nitrate leaching. Tillage method can also affect the rate of mineralisation by its effects on soil organic matter, soil water and soil temperature. Dowdell and Cannell (1975) concluded that leaching of NO_3-N in direct-drilled soil was small because the mineralisation was slow compared with ploughed soil. Under conventional tillage, more NO_3-N accumulated in the upper soil layers where most of the macropores were destroyed due to tillage activity, while the soil structure below the 60 cm depth remained undisturbed. In the no-till system, where macropores are allowed to develop and persist, the structure at the soil surface could be markedly different, but may be similar to that in conventional tillage at greater depths. This result was possibly due to higher rates of mineralisation and less leaching to the ground water under conventional tillage system (Varshney *et al.* 1993). The most important channel of N loss from field soil on cultivated agricultural lands is NO_3-N leaching (Haynes 1986).

Ladd (1990) argued that decreased soil temperature and increased soil water contents under no-tillage regimes will have contrasting effects on mineralisation rates, especially in soil in semi-arid climate regions and the effects will be modified by soil properties. The effect of tillage method on leaching of NO_3-N varies in different areas

because soil conditions and climate differ. It is possible to control some part of the leaching and finally subsoil mineral N by soil management.

Pasture

In extensively-grazed pastures, leaching losses are generally low (3-6 kg N ha^{-1} y^{-1}) (Crisp 1966, Bargh 1978). In a permanent growing pasture rapid immobilisation of NH_4-N in the rhizosphere and efficient plant uptake result in low concentration of NO_3-N in the soil (Huntjens 1971 a,b). Elliott and Clarke (1975) reported quick NO_3-N leaching in poor pastures in southern Australia. Leaching losses can be large, from pastures under intensive grazing, particularly where high fertiliser rates are used (Ball and Ryden 1984). Unirrigated pasture has a lower potential for NO_3-N leaching than irrigated pasture, due to higher stocking rates and the greater volume of through drainage (Burden 1982).

Cropping system

The amount of NO_3-N leached from soil is affected by the type of cropping system. Numerous studies have consistently reported higher NO_3-N leaching losses from arable crops than from pasture. In the wheat belt of south-eastern Australia, Storrier (1962) reported that N which had accumulated in the surface horizon after a period of fallow can be leached to the subsurface by heavy autumn and winter rains. The leaching losses can be large in rotations where grass or leguminous pasture is included for only a few years and then ploughed (Cameron and Wild 1984). The plant rooting habit exerts an important influence on the amount of NO_3-N leached from the soil profile because plant roots take up both NO_3-N and water. Diggle *et al.* (1990) reported that as the wheat grew increased transpiration of water would have slowed the rate of leaching, allowing the roots to catch the NO_3-N and the availability of N would have increased. In areas where leaching losses are high, use of deep rooting varieties of crops can be useful for N uptake (Myers 1983). Nitrogen application at the time of high

root activity also tends to lower fertiliser N losses by leaching and volatilisation processes (Ladd 1990).

2.4 Crop nitrogen requirements

Plant life on earth is not possible in the absence of N. Nitrogen stimulates root development and activity, and supports the uptake of other nutrients, is a component of the amino acids, of chlorophyll and enzymes. Nitrogen is essential for carbohydrate utilisation and the biosynthesis of proteins. Plant growth and root development are affected by N (Olson *et al.* 1964a, Holt and Fisher 1960, Kmoch *et al.* 1957, Keller and Smith 1967). Rooting depth as well as total root mass are enhanced by N availability.

2.4.1 Nitrogen uptake by the crop

The uptake of N is an essential part of the growth process, and if the uptake rate does not keep pace with the growth rate, the concentration of the N in the plant tissues must decrease, and the plant may become deficient. Nitrogen uptake by plants from the soil depends on both the availability of N in the soil and the demand by the plant. In the early part of the growing season, when tissue N concentration is high, total N uptake by plant involves a period of slow accumulation, followed by a rapid linear rate of accumulation that coincides with rapid growth (Tinker 1978, Pearson and Muirhead 1984). The N concentration of tissue decreases in the period of rapid growth. The rate of uptake during the rapid growth phase for field crops, can be extremely high (3-5 kg N ha^{-1} day^{-1}, Tinker 1978, Olson and Kurtz 1982). Late in the season, the rate of N accumulation decreases and continues at a decreased rate until maturity. When N fertiliser is applied early in the season net N uptake is generally completed by anthesis (Storrier 1962, Smith *et al.* 1989b). The basic scheme for N uptake, reduction, and movement in higher plants is shown in Fig.2.1.

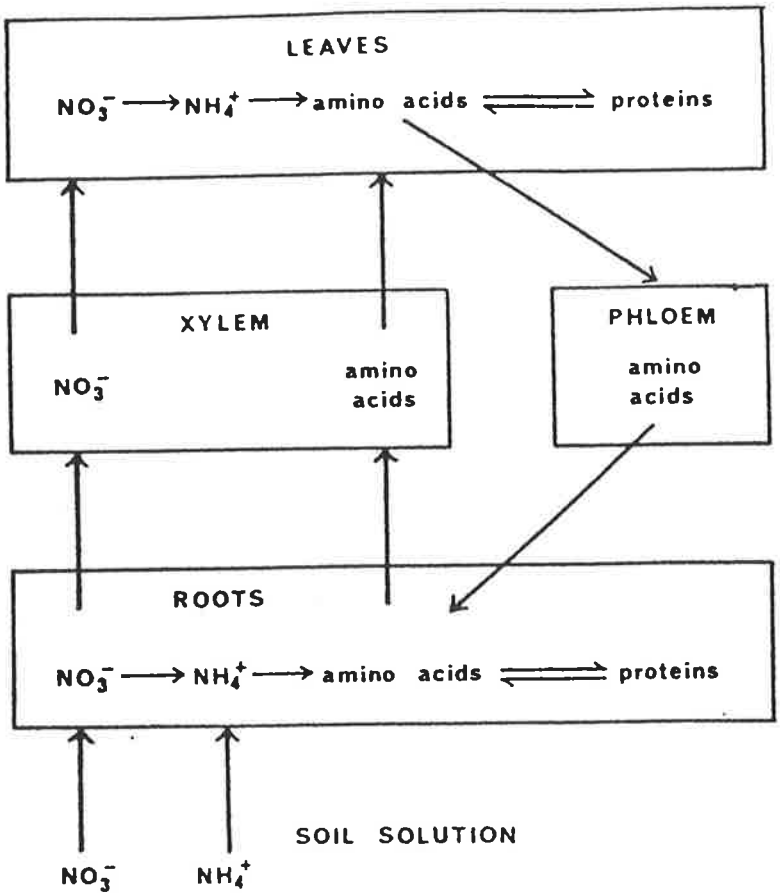

Fig.2.1. Basic scheme of nitrogen uptake and reduction and protein formation in higher plants (Haynes 1986)

Nitrate is mobile because the attraction between soil colloids and NO_3-N is negligible. A series of independent transformations occur during NO_3-N uptake by roots. Nitrate may be reduced and synthesised into amino acids by root tissues, stored in the roots, or transported across root cells and deposited in the xylem for movement into the shoots. Amino acids formed in the shoots can move back to roots or to grain via the phloem (Pate 1971, Pate *et al.* 1979). Mobilisation of products from vegetative to the reproductive tissue also begin when reproductive phase begins (Pate and Layzell 1981). For example, Mackown and van Sanford (1986) showed with wheat in USA that leaves, glumes, culm and roots contributed 40%, 22%, 22%, and 16% of remobilised N to the grain respectively. Nitrogen in the wheat grain could be derived entirely from the redistribution of N from vegetative organs (Simpson *et al.* 1983) although environmental contributions may influence the relative contribution of the different

plant parts to the grain. An important characteristic of N metabolism is the mobility of N within the plant. Grain yield and GPC are affected by translocation of N during grain development (Pearson and Miurhead 1984). For example, at anthesis wheat can contain about 80% of the N that is present in plant tops at maturity (Dalling *et al.* 1976, Austin *et al.* 1977).

During heading and anthesis, the total N uptake in plants increases but substantial losses can occur after this (Storrier 1962, Farquhar *et al.* 1980, Parton *et al.* 1988). Parton *et al.* (1988) reported that the most important mechanism for loss of N around anthesis is volatilisation of N from the plant. Errors involved in sampling through the loss of stem material and dead leaf may be another reason of the reported decrease in N at maturity.

2.4.2 Subsoil nitrogen uptake by root

As mentioned before, subsoil accumulation of NO_3-N is common in southern Australia, but it usually remains within the root zone of crops (Greenland 1971) because rainfall is insufficient to cause leaching beyond the roots. A better knowledge of the distribution of roots in soil is necessary to ascertain their effect on water and nutrient uptake by plants. In agriculture, it is usually considered an advantage for crop plants to have deep roots. In many cases, the supply of water under drought conditions may be safeguarded by the uptake of water from the subsoil. But the question also arises as to whether the roots in the deeper layers contribute to the mineral nutrition of the crop to any worthwhile extent. The majority of roots are in top 60-cm layer but in semiarid climates often all the water in this zone is used up by grain filling time and it is roots in the lower layer that facilitate the filling of heads (Hurd and Spratt 1975). In field experiments in South Australia, Schultz (1974) found an average of nearly 50% of the roots in the 0-15 cm layer. The surface soil is normally dry after September in South Australia when wheat is at the heading stage (Large 1954), and the ability of plants to extract water from the subsoil is important for final yield. To fully extract the water from subsoil, Wind (1961) calculated, from the theory of unsaturated water flow, that

root density has to be approximately 1 to 2 cm cm^{-3}. Roots near the surface are prone to desiccate and die; this would retard the ability of these rooting systems to make efficient use of rain from summer thunder storms (Russell 1973).

Roots in the subsoil have potential value in feeding the plant, provided plant nutrients are available. Their contribution to plant nutrition will further depend on the fertility of the subsoil layers, the water content of these layers and the amount of roots that have been able to develop in these regions. Uptake of N per unit of roots in contact with soil was higher in the deeper layers (Wiersum 1967). Any root in the subsoil can thus be considered as a potential absorbing unit for nutrient uptake. Fox and Lipps (1960) came to the conclusion that when the topsoil became dry, 3% of the roots of lucerne at 200 to 400 cm depth absorbed 62 % of the soil minerals taken up by the plant. Also Lieshout (1960) showed that for maize and oats the contribution in nutrition from the soil at 60 to 80 cm depth exceeded that of the percentage of roots, *viz* 1.7 % roots contributed 9.2 % of the uptake of phosphate.

Burns (1980) using an equation of Baldwin *et al.* (1973), estimated that most crops can continue growth normally with less than 15% of their roots involved in N uptake. This was explained by the fact that only a small proportion of the root system might have been involved in NO_3-N uptake, resulting in inflow rates considerably greater than those based on total root length. This finding is in line with speculation of Robinson *et al.* (1991) who supposed that a majority of a plant's root system may not be actively involved in NO_3-N uptake from the soil. Younger parts of the root system are most active in N uptake whereas the older parts retain functional importance mainly in long distance transport. Therefore, only small root length densities may be sufficient to utilize almost all the NO_3-N from soil. Small differences in NO_3-N uptake activity of different root sections were observed by Brady *et al.* (1993) with 14-day-old wheat plants. Uptake of ^{15}N over 24 h was 5, 5 and 7 µg per cm total root length in basal, middle and apical segment respectively. There is a lack of investigation with older wheat plants even though some field studies showed that during reproductive growth of maize (Wiesler and Horts 1993) and wheat (Kuhlmann *et al.* 1989) N uptake in the

topsoil ceased whereas uptake still continued in the subsoil. Mengel and Barber (1974) reported that mean N uptake per maize plant was 78 μmoles day^{-1}. The age of plant was 20 days and the plant mean root length was 4 m. Strebel and Duynisveld (1989) reported that during periods of high N uptake, the N inflow ranges between 0.26-2.07 μmol m^{-1} day^{-1} at various rootzone layers for cereal, and between 3.8-8.98 μmol m^{-1} day^{-1} for sugarbeet respectively. Growing of cultivars selected for high N uptake-capacity of the shoots combined with "high" root length densities in the subsoil may improve the utilisation of a high soil N supply and thus reduce the risk of NO_3-N leaching. Greater uptake after anthesis may also help maintain or improve GPC. The uptake has been considered seriously in terms of rate. Uptake rates have thus been expressed in terms of the weight, surface area and length of roots. In view of the different uses to which these values may be put, and the uncertainty which still surrounds the physiology of the root uptake process, none of these flow parameters can be considered to be correct, and the choice must depend upon the aim of the work.

Information on changes in the rate of N uptake per unit of plant root length (N flux) during the growth of the plant is important for evaluating the capacity of the soil to supply sufficient N to the root surface. N flux into the root can be expressed in several ways. Uptake per unit of root length is commonly used. To explain the relationship between root length density and NO_3-N utilisation further experimental efforts concerning different density of roots and uptake rates are necessary.

2.4.3 Nitrogen deficiency, sufficiency, toxicity

A number of variables associated with the transitory nature of N and plant growth complicate the definition of N sufficiency levels by plant analysis. Nitrogen concentration varies among different parts of a plant, and is affected by climate, stage of plant development, disease or pest attacks. Thus, any consideration of sufficiency of N will express a range for a specific species. Nitrogen concentration is maximum in a given genotype during early growth, and the N concentration generally declines as the plant ages. Deficiency of other nutrients that restrict crop growth may result from higher

plant N concentration. Conversely, supplying a deficient nutrient, which stimulates growth, may dilute the N concentration in the plant. The concentration of N in a given sample of crop tissue can be influenced by these variables acting alone or together. For this reason, the values presented in (Table 2.1) cover wide ranges and even wider ranges are possible under unusual conditions.

Table 2.1. Plant N concentrations associated with deficiency and excess in some important agricultural crops.

		N content at designated nutritional status of crop[A]				
Crop	Plant part	Deficient	Low	Sufficient	Possibly excessive	Reference
		%				
Lucerne *Medicago sativa* L.	Top 15 cm at early bloom	< 4.0	4.0-4.5	4.5-5.0	> 5.0	Jones (1967)
Maize *Zea mays* L.	Ear leaf at silk	< 2.25	2.26-2.75	2.76-3.5	> 3.5	Jones (1967)
Wheat *Triticum aestivum* L.	Total above ground plant at head emergence from the boot					Ward *et al.* (1973)
Winter		< 1.25	1.25-1.75	1.75-3.0	>3.0	
Spring		<1.5	1.5-2.0	2.0-3.0	>3.0	

[A] Ranges presented in many cases are adjustments or additions to original authors' values to fit the format employed here.

2.4.4. Amount, forms and distribution of nitrogen in plants

The quantity of N required by a crop is influenced by crop species and the dry matter production by the crop. Generally, larger quantities of N are found in forage legumes than in cereal crops (Table 2.2). A more detailed differentiation of N allocation

in plant parts has been determined for small grains (Boatwright and Haas 1961) and for maize and soybeans (Hanway and Weber 1971, Hanway 1962). For some of the grain crops, the total weight of roots below the soil surface have values in the range of 2000-5000 kg ha^{-1}. Generally, total N in roots of forage crops at harvest is about half of that in the above-ground components (Olson and Kurtz 1982).

Table 2.2 Approximate total N content and distribution in good yields of major harvested crops. (Derived in part from Anonymous 1972.)

Crop	Plant parts	Yield	Total N[A]
		kg ha^{-1}	
Lucerne	Total forage	18000	510
Maize	Grain	10000	150
	Stover	9000	80
Potatoes	Tubers	56000	170
	Vines	5000	115
Rice	Grain	7900	85
	Straw	10000	40
Sugar beets	Roots	68000	140
	Tops	36000	145
Wheat	Grain	5400	110
	Straw	6000	45

[A] Substantial variation from these values can occur depending on soil N status and fertilisation, *i.e.*, total N of the end product continues to increase with added N beyond that required for maximum yield.

2.4.5 Nitrate concentration in plant tissue as a means of studying the nitrogen status of wheat

The shoot NO_3-N test is a tool which aims to integrate both the current mineral N supplying power of the soil and current ability of wheat to absorb and translocate NO_3-N from the roots to the shoots. The NO_3-N concentration in plant tissue is considered to be a valuable means for assessing the N nutritional status of wheat plants because the NO_3-N concentration can be related to available soil N (Guettinger and Koehler 1963). Total N concentration is less sensitive than NO_3-N as an indicator of current nutrient status (El-Sheikhler and Broyer 1970, Ulrich and Hills 1973, Martin

and Matocha 1973). Another advantage of tissue NO_3-N is that total N analysis needs laboratory facilities while NO_3-N analysis can be done *in situ.* As variation in NO_3-N is a sensitive indicator of plant N status, measurements of its concentration can help in assessment of the interaction of N and water supply, and other factors such as diseases and pests, on yield of wheat in different rotations (Papastylianou and Puckridge 1983).

2.4.6 Environmental factors affecting the NO_3-N concentration in plant tissue

2.4.6.1 Nitrate in the plant

Water and mineral N contents of the soil affect NO_3-N concentrations in wheat plants in the field, and if stem NO_3-N falls below 2000 $\mu g\ g^{-1}$ at the boot stage visual symptoms of N deficiency may be observed (Gardner and Jackson 1976) and tiller survival reduced (Papastylianou and Puckridge 1981). In pot experiments, plant growth was restricted if stem NO_3-N was less than 5500, 1200 and 500 $\mu g\ g^{-1}$ at tillering, jointing and anthesis respectively (Papastylianou 1980). When the rate of absorption falls below the rate of growth, depletion of plant NO_3-N occurs (Terman and Allen 1978). When the temperature and light intensity are low, and plant growth limited accumulation of NO_3-N may occur in the plant.

2.4.6.2 Soil

The NO_3-N concentration in the plant is influenced by soil type which affects root growth and nutrient availability. Lovelac *et al.* (1968) found that bermuda grass grown on a sandy soil accumulated more NO_3-N during early growth than when grown on clay soil; the opposite was observed later in the season. Possibly roots in sandy soil grow faster and plants absorbed more NO_3-N early in the season but later the soil N was exhausted.

2.4.6.3 Water availability

Water stress is an environmental variable of importance in Australia which effects the growth rate and might be expected to alter the critical concentration of NO_3-N. Most studies on NO_3-N in plants have been done on non-irrigated crops and for this reason the effect of water supply on NO_3-N accumulation in plants has not been studied extensively. Some evidence indicates that NO_3-N accumulation in plants is increased by water stress (Mackenzie *et al.* 1963). This accumulation of NO_3-N occurs because the production of dry matter is influenced earlier than the uptake of NO_3-N under conditions of water stress (Wright and Davidson 1964, Darwinkel 1975). Under water stress, NO_3-N concentration may be decreased due to decreased N uptake (Wright and Trautman 1962). However the effect of soil water on NO_3-N concentration in the plants is small compared with the effect of N supply (Mackenzie *et al.* 1963).

2.4.6.4 Light

The diurnal variation in NO_3-N concentration in plants is largely due to changing light intensity (Hageman *et al.* 1961, Minotti and Stankey 1973, Papastylianou 1987). The effect of light on conversion of NO_3-N to organic forms of N is more than the effect on uptake (Kessler 1964, Beevers and Hageman 1969). Dry matter production will be affected by light intensity and light intensity will also influence NO_3-N accumulation. Nitrate reductase activity is affected by light intensity and conversion of NO_3-N to organic forms will be slow under low light (Hageman and Flesher 1960, Ziersel *et al.* 1963). The activity of nitrate reductase in the top leaves is higher than the bottom ones (Zierseral *et al.* 1963) due to shading and the senescence of the bottom leaves (Egmond and Breteler 1972, Darwinkel 1975). Self-shading by leaves and the production of non-photosynthetic tissues decrease the theoretical rate of C assimilation in the shoot system (Robinson 1986). Hageman *et al.* (1961) reported that NO_3-N concentration and NO_3-N reductase activity appear to be inversely related but Breniman *et al.* (1961) reported high concentrations of NO_3-N on a sunny day.

2.4.6.5 Temperature

The role of temperature on NO_3-N accumulation in the plants is not clear. George *et al.* (1971) reported that low temperature increased NO_3-N accumulation and decreased dry matter production while optimum temperature accelerated NO_3-N assimilation and growth resulting in low NO_3-N concentration in the plant. Temperature can also affect mineralisation of soil nitrogen. Maynard *et al.* (1976) concluded that an increase in mineralisation was the reason for higher NO_3-N concentration in summer grown compared with spring-grown lettuce. The effect of temperature on tissue NO_3-N is likely to reflect its effect on plant growth and on mineralisation; NO_3-N may increase or decrease with increased temperature depending on it relative response.

2.4.6.6 Nitrogen and other nutrients

Increasing N fertiliser application increases NO_3-N accumulation in plants (Murphy and Smith 1967, Harms and Tucker 1973, Terman *et al.* 1976, Gardner and Jackson 1976). Increasing the NO_3-N concentration in plants is also possible by increasing the soil N accretion by legumes (Hanway and Englehorn 1958), by manuring (Pratt *et al.* 1976) or generally by practices which increase N availability such as fallow (Kretschmer 1958). Plants may accumulate more NO_3-N when NO_3 forms of N fertilisers are used than after other N fertilisers (Crawford *et al.* 1961, Nowakowski 1961). However, the difference between forms of N fertiliser can quickly disappear in field situations depending on factors influencing microbial conversion. Hylton *et al.* (1964, 1970) reported that the concentration of NO_3-N in plant tissue does not increase linearly as the concentration of available soil N increases, but follows an asymptotic curve. In a range 50 to 400 kg ha^{-1} N fertiliser application, different species of vegetables (Brown and Smith 1967) reached a clear maximum of NO_3-N concentration. In contrast Crawford *et al.* (1961) found in an experiment with cereal that NO_3-N concentration increased up to 800 kg ha^{-1} N fertiliser application.

Other nutrients may affect the NO_3-N concentration in the plant tissue depending on the function of the element and its influence on uptake or assimilation of

NO_3-N. Hewitt and Smith (1975) reported that deficiencies of some elements such as molybdenum can cause accumulation of NO_3-N in the plant tissue because this element is necessary for NO_3-N metabolism. Moderate deficiencies of magnesium and phosphorus had no significant influence on NO_3-N accumulation in the plant (Barker and Maynard 1971, Brown and Smith 1966). Calcium and potassium deficiencies do not seem to influence NO_3-N accumulation in the plant (Barker and Maynard 1971, Brown and Smith 1966). Nutrients other than N are not expected to influence NO_3-N concentration in the plants in the wheat growing areas in Australia unless the area is known for a particular deficiency (Storrier 1975). Phosphorus is almost always supplied to cereal crops, but sulphur and potassium are not deficient (Storrier 1975).

2.5 Nitrogen supply: fertiliser

2.5.1 Residual nitrogen

During the past 30 years, hundreds of field experiments have been conducted throughout the world under a broad range of climate, soil and management conditions to determine the nature and size of crop yield responses to N fertilisation (Standford 1982). Results of such experiments in large part have provided the basis for making N fertiliser recommendations to farmers. The importance of residual mineral N as a source of plant-available N became increasingly evident with increasing use of N fertilisers, particularly in areas where the possibility of NO_3-N removal from the root zone by percolating water is minimal. Assessment of available soil N supply from residual NO_3-N measurements alone is inadequate if the capacity to mineralise soil organic N is different. MacGregor *et al.* (1974) in long-term N-rate experiments with maize in Minnesota suggest that an annual N application rate of 100-150 kg ha^{-1} might have resulted in significant NO_3-N accumulation within the root zone, during years of below-normal rainfall, in the course of 10-15 years of continuous cropping.

The important factors that need to be considered in assessing residual mineral N as a guide to N fertilisation are depth, scope, intensity and time of soil sampling. Depth,

scope and intensity of soil sampling are related to the lateral and vertical variability in NO_3-N distribution in the field (Biggar, 1978) and on effective rooting depth, and are not amenable to broad generalisations. In areas where residual soil NO_3-N measurements are used as a guide for N fertiliser recommendations the interpretation of these values in terms of N fertiliser requirement is usually based on (a) estimates of N needed for different yield levels, (b) expected yield and (c) the concentration of NO_3-N in the soil. Generally the availability of residual NO_3-N to the crop is considered to be about equal to that from applied fertiliser.

This type of calculation is being used for a number of crops in areas where residual NO_3-N is measured as a guide to N fertiliser use. Basically, N recommendations are estimated from the amount of residual NO_3-N in the root zone and N requirements of the crop for expected yield. Holford and Doyle (1992) have shown that measurement of soil N, usually as NO_3-N at various depths (to 90 cm) is an indicator of available N for predicting fertiliser responsiveness and requirements of crops. The soil analysis will give a general idea of the total N status of the particular site and the soil test cannot measure the N which will be available to the crop at important times during the coming growing season. The amount and distribution of inorganic N, primarily NO_3-N remaining in the soil profile at the end of growing season (residual N), is greatly affected by fertiliser management as well as by the soil water regime (Varshney *et al.* 1993).

2.5.2 Use of nitrogen

All nutrients increase the growth and yield of crops but in the main wheat-growing soils of southern Australia N has by far the largest effect. However the response to N is influenced strongly by the supply of phosphorus, potassium and trace element as well. For wheat, barley and other cereal crops N is the main key to yield. Crops that have insufficient N are stunted, yellowish and sickly-looking. Even a small amount of nitrogen given at the right time relieves these symptoms, and large amounts can increase the yield of grain by a factor of five or six on some occasions. Within the

range in which N increases yield the farmer will get roughly 20 kg of extra grain for each kg of fertiliser N that is supplied (Addiscott *et al* 1991). Yearly, world-wide biological N fixation on agricultural lands is equivalent to $89x10^6$ tons and industrial fertiliser N production is $49x10^6$ (National Research Council 1978). Atmospheric inputs from lightning and combustion would add to this total. In contrast, utilising a daily protein-N requirement of 4-6 g/capita per day, the annual protein-N uptake requires only $6\text{-}9x10^6$ tons N/year (Bolin and Arrhenius 1977), yet malnutrition is a fact of life in many parts of the world. The comparison results from inefficient food distribution, losses in the conversion of plant proteins to animal proteins, wastage during storage, low quality proteins, and the fact that production of food calories also depends on the plant N supply (Bolin and Arrhenius 1977). Sukhatme (1977) presents convincing arguments that, on average, per capita protein supply exceeds needs by about 60% for almost all of the developing countries; inadequate energy intake thus becomes a major factor in protein malnutrition. Food production must increase by the year 2000 to adequately feed the anticipated 6-7 billion population and this must be done on only 10% more arable land than present (Chancellor and Ross 1976). The additional lands and land currently used will likely require higher rates of N to sustain yields and inputs of N from biological N fixation and fertilizers will have to at least double (to about $280x10^6$ tons of N/year). The average rate of N application in Australia is low (about 2-3 kg N ha^{-1}) although under intensive cropping rates 25-50 kg N ha^{-1} are commonly applied to wheat. Economics of wheat production effect the use of N fertiliser and has tended to increase when wheat prices are high and decrease when wheat prices are low or fertiliser prices are high (McDonald 1989). However, there has been a general trend for higher rates of N fertiliser to be applied to cereal crops across southern Australia. Long-term alternatives include utilising more legume and cereal grains directly for human consumption, relying less on animal protein (Pimentel *et al.* 1975, Kaul 1977, Sukhatme 1977), and increasing the efficiency of use of our N resources.

2.5.3 Forms of nitrogen application

The fact that all kinds of fertiliser N are normally transformed to NO_3-N in the soil means that the initial form is not overly important for crops in most situations. For most crops and soils, the choice of the N carrier is more economic than agronomic. The ranking of anhydrous NH_3 as the leading carrier is due to its being the least expensive form on the market rather than to its superior characteristics as a plant nutrient. Some N compounds, such as KNO_3, tend to increase pH, while others such as $(NH_4)_2SO_4$, can decrease soil pH greatly. A generally accepted factor has been that 1.8 kg of effective lime is required to neutralise each kg of NH_4-N applied in fertiliser (Olson and Kurtz 1982).

The form of N fertiliser applied will influence the N lost from leaching. NH_4-N is less mobile than NO_3-N in the soils. Lower leaching losses are often reported to occur from ammonium salts or urea than from nitrate salts (Myers 1978, Bauder and Montgomery 1979). Before significant leaching losses of NH_4-N fertiliser can occur the NH_4-N must undergo nitrification. Rate and time of nitrification relative to the rate and time of plant uptake are important in determining leaching losses from NH_4-N based fertilisers. To decrease the NO_3-N leached from the soil, nitrification inhibitors have sometimes been used (Owens 1981). The aim of using a nitrification inhibitor is to decrease the rate of nitrification, thereby reducing the amount of NO_3-N availability for leaching.

2.5.4 Amounts of nitrogen fertiliser applied

The amount of fertiliser N required by a crop will be a net amount resulting from several debits and credits resulting from the dynamics of N in soils. Residual fertiliser NO_3-N in the soil along with non-fertiliser sources, such as decomposition of soil organic matter and crop residues, would be credits. In some cases, contributions would also come from symbiotic N_2 fixation and from animal manures. Crop removals and losses from denitrification, leaching and erosion would be deducted and a balance

obtained. The net result of these gains and losses would be useful for estimating the amount of fertiliser N to be applied (Olson and Kurtz 1982).

Additions of fertiliser N, which are often essential to obtain high crop yields, commonly increase leaching losses. When high fertiliser rates are combined with heavy irrigation regimes or high rainfall on light-textured soils, leaching losses of NO_3-N can be large. When high rates of N are required, one method of reducing the leaching loss is to split the application (Gupta *et al.* 1982). In maize production in the USA, the best method for controlling NO_3-N leaching is proper nitrogen application rates for realistic yield goals. A soil NO_3-N test should be used to determine N sidedressing requirements and nitrogen should be applied in split application (Ritter *et al.* 1993). Split applications of N fertiliser are not commonly used in southern Australia, because often the rates used dont justify more than a single application.

The use of N fertiliser on wheat crops in Australia has increased (McDonald 1989) but the total amount is still small and variable (10000 t of N fertiliser per year, *i.e.* 20-25% of Australia's total consumption of N fertiliser). The average rate of N application is 2-3 kg N ha^{-1} in all dryland wheat, but much higher rates (up to 70 kg N ha^{-1}) are used in high rainfall areas. This low level of N application maybe due to farmers regarding N as an inessential element in wheat production (McDonald 1989). To maintain yields and GPC of wheat, application of N fertiliser is becoming more important. The crop rotation, soil type, rainfall and expected yield will affect the rates of N used. Generally, in southern Australia application rates of less than 25 kg ha^{-1} are being commonly used (McDonald 1992), and this is generally applied at sowing.

2.5.5 Time of nitrogen application

The time of application of N fertiliser is important because the cereal crop has different needs for N at different stages of growth. The relation between the time of application of N fertilisers to wheat and the yield response produced has been the subject of a large number of field experiments in many countries. As N fertilisers are expensive, it is important to use the N efficiently by matching the N supply to the needs

of the crop, both in terms of amount and timing of nutrient supply. The efficient uptake and utilisation of N by the crop also limits losses of N through processes such as leaching (Mason *et al.* 1972) which can be environmentally hazardous.

Many of the yield responses to N fertiliser are achieved by increasing the number of grain-bearing ears per unit area, *i.e.* by increasing tillering (Feyter and Cossens 1977, Halse *et al.* 1969, Spiertz and De Vos 1983, Darwinkel 1983). Maximum effects on tiller formation and spikelet initiation are brought about by a source of N at the beginning of tillering (Darwinkel 1983) while Langer and Liew (1973) showed that spikelet numbers were increased only by early application of N, not later than the double ridge stage. Nitrogen applied some weeks before the critical stages of growth is usually much more effective than N applied at or just a few days before those stages, because wheat plants do not immediately take up a large proportion of the N suddenly offered to them. An adequate supply of N is therefore essential in the early stage of crop growth (Mason 1991).

Responses to later applications of N fertiliser can often be brought about by promoting tiller survival and leaf area duration (Spiertz and De Vos 1983). Profitable responses can sometimes be obtained from very late applications of N (Mason 1986b) but usually the response from earlier applications are far more profitable. This need for an early supply of available N has to be balanced against the likelihood of leaching. Recovery of autumn-applied fertiliser N is low (11-40%) and between 40% and 80% of the fertiliser N is lost mainly by leaching (Powlson *et al* . 1986b) although this will depend on soil type, rainfall and form of nitrogen present at the time of leaching rains. However, in some situations losses of nitrate can occur from leaching before the crop develops an effective rooting system in the subsoil (Mason *et al.* 1972). Other factors, such as volatilisation losses of ammonia from top-dressed urea may affect the decision on timing of application (Simpson and Freney 1974, Gasser 1964, Nommik 1973).

Rainfall may affect the response of field crops to N fertiliser in two ways: if the rainfall is less than average the water supply available to the crop may become a limiting factor to growth so that additional N in the soil can not be utilised even if it is

taken up by the crop. Excessive rainfall may leach the N applied away from those regions of the soil from which it can be absorbed by the roots of the crop.

Nitrogen fertiliser, particularly as a late application, tends to increase GPC (Hunter and Stanford 1973, Hamid and Sarvar 1976, Strong 1982, Smith *et al.* 1989a, Randall *et al.* 1990). Late application of N fertiliser may therefore, overcome low GPC. However, in semiarid environments the efficiency of post-anthesis application of N may be limited by the drying of the topsoil. Each additional 40 kg ha^{-1} N produced a mean increase of 1.1% in GPC (Cooper and Blakeney 1990).

Strong (1982) found that the time of N application determines the appropriate method of application. For example, after anthesis foliar application rather than solid fertiliser was the more effective method. In a comparison between three rates of N (25, 50, 100 kg ha^{-1}) applied at booting, anthesis and 16 days after anthesis, the only significant difference in GPC was a small increase when 100 kg N ha^{-1} was applied 16 days after anthesis. Cooper and Blakeney (1990) also found a high recovery of N only 3 weeks after it was applied to the soil surface (ammonium nitrate). A foliar spray of urea also increased GPC which indicates rapid N uptake. The 2.9% increase in GPC that was obtained in this experiment was appreciable, because in the field the range of GPC commonly encountered is 9-15%. This result is supported by the data of Recous *et al.* (1988) and Smith *et al.* (1989a) who reported that when N is applied late in the growing season, the plants can compete with the soil organisms for the available N and that gaseous N losses are low. Finney *et al.* (1957) showed that when N was applied as a spray application at the boot stage more N was assimilated into grain than after anthesis. However, application before anthesis produced a grain yield response, thereby reducing its effectiveness in increasing GPC. In contrast, application at anthesis or later had the singular effect of increasing GPC. Thus a fertilisation strategy in which the N application is split between sowing and anthesis would more likely result in grain of a higher protein content than the strategy in which the entire amount of N is applied prior to booting. Strong (1982) also indicated some flexibility in the time of N application for maximum protein increase.

Increasing the application of N at sowing may fail to increase the supply of N to the grain (Strong 1981) because the fertiliser N may be lost before it can be assimilated; at least 6 weeks' growth is required before the crop can accumulate much of the N applied at sowing (Muirhead *et al.* 1985, Smith *et al.* 1989). More efficient use of fertiliser N may result when the application coincides with the period of rapid plant uptake (Keeney 1982). High rates of N uptake have been reported when fertiliser application was delayed until anthesis (Spiertz and Ellen 1978, Smith *et al.* 1989a). In addition, fertilisation prior to anthesis was effective in increasing grain protein (Strong 1982, 1986) and improving the baking quality of the grain.

In South Australia, Elliott *et al.* (1985) found that split applications of N were desirable where heavy winter rainfall caused temporary N deficiency during tillering. Generally, most N fertiliser is applied at sowing in the higher rainfall areas but there may be an additional topdressing to deficient crops. Variable responses to delayed N application have been reported (Littler 1963, Elliott *et al.* 1985, Randall *et al.* 1990). The later the time of application, the greater the risk that the added N will not increase grain yield, even though some vegetative response may be obtained (Smith *et al.* 1989a). Much of the variability in yield response with post-sowing applications of N is related to variability in rainfall. In southern Australia, late application of various N fertiliser rates gave 65 to 75 % of the yield responses to those obtained with sowing applications (Russell 1968c). There were also some indications that the form of N applied was less critical in the autumn than the spring. However, little is known of the efficiency of use or fate of subsoil mineral N after anthesis. Additional research is needed on this subject.

2.5.6 Depth of nitrogen application

In the cereal-growing areas of southern Australia, the cultivated and fertilised topsoil contains the bulk of the labile forms of plant nutrients. When the topsoil dries out and the nutrients therein become less available and roots less active, plants may suffer from nutrient shortage. Applying fertilisers in the subsoil is one possible way of overcoming nutrient shortage caused by topsoil drying (Alston 1980). If the N supply is

low, uptake of mineral N at depth (below 30 cm) would be necessary for maximum N uptake and crop production (Taylor *et al.*1988). Sharma and Chaudhary (1984) reported that deep placement of fertiliser N in coarse textured soil resulted in its more uniform distribution in the root zone, more extensive root proliferation and enhanced sub-soil water utilisation. It should ensure a greater N uptake by roots as they would encounter a N rich zone. Daigger and Sander (1976) showed that dry matter production was not significantly affected by depth of N placement and as depth of placement increased N uptake was significantly decreased. The relatively high NO_3-N content in the soil profile at this location may have had a confounding effect on the results. Nitrogen uptake by wheat when N was surface-applied was about 62 kg ha^{-1} compared with about 49 kg ha^{-1} when N was placed at a 150 cm depth. However, unpublished studies of Strong on wheat experiments in south-eastern Queensland have shown that soil NO_3-N (0- 60 cm) can account for up to 40% of variance in yield response (Holford and Doyle 1992).

Strong and Cooper (1980) showed that deep placement of N fertiliser was an important strategy for increasing yield, especially when there was a prolonged dry period after anthesis. Alston (1980) concluded that deep placement of N, where the nutrient content of the topsoil is high and soil remains wet during the early part of the season, can not be recommended. Deep placement of N fertiliser had different results in southern Australia (Alston 1980) and in southern Queensland (Strong and Cooper 1980) because the experiments were conducted under different experimental conditions: on a deep, cracking clay after fallow in a summer rainfall area in Queensland and on a red brown earth in a winter rainfall area in South Australia. However, Storrier (1962) found that N uptake stopped after anthesis and little subsoil N was used while Strong and Cooper (1980) found post-anthesis uptake of N during the dry year was increased by deep placement. It is likely that the pattern of rainfall (winter dominant in southern Australia and summer dominant in Queensland) was an important factor that affected the results. Further research to identify where deep placement will increase the yield and GPC is necessary.

2.5.7 Efficiency of nitrogen use

Nitrogen fertiliser should receive more care in its overall management than any other plant nutrients because of the transitory nature of N in soil, its tendency for loss from the soil, and its potential for becoming a pollutant of air and water. It is necessary to have some indices to assess of the efficiency of N fertiliser application and utilisation by crops over a range of environments and cropping practices. In cereal crops, the efficiency of N can be defined in terms of three efficiency parameters (Craswell and Godwin 1984, Harmsen 1984):

(i) Agronomic efficiency (kg kg^{-1})=$(Y_F-Y_C)/F$

(ii) Apparent recovery (%)= 100x $(N_F-N_C)/F$

(iii) Physiological efficiency (kg kg^{-1})=$(Y_F-Y_C)/(N_F-N_C)$

where Y_F and Y_C are the grain yields of the fertilised and unfertilised crops respectively, N_F and N_C are the N contained in the grain and straw (kg ha^{-1}) of the fertilised and unfertilised crops respectively, and F is the amount of fertiliser N applied (kg ha^{-1}). These parameters are normally based on measurements of N uptake in the above-ground plant parts and the calculation of apparent recovery depends on the assumption that both the fertilised and unfertilised crops absorb the same amount of soil N.

Both physiological and agronomic efficiencies are based on grain yield. The physiological efficiency reflects the ability of crops to utilise N in the plant for the synthesis of grain yield, while the apparent recovery reflects the efficiency of the crop in obtaining fertiliser N from the soil.

Agronomic efficiency is the product of the physiological efficiency and the apparent recovery and thus estimates the overall efficiency of the system. Increases in either or both the physiological efficiency and apparent recovery will increase the agronomic efficiency (Novoa and Loomis 1981, Craswell and Godwin 1984). With increasing rates of fertiliser applied, the agronomic efficiency generally decreases

(Harmsen *et al.* 1983). The ratio of grain produced to the total N absorbed by the above-ground plant parts (grain plus straw) at maturity is named the physiological efficiency of uptake N which is another index for measuring the efficiency of N utilisation. The physiological efficiency of N uptake of Novoa and Loomis (1981) differs from the physiological efficiency (see (iii) above). Crop variety and environmental conditions during the growing season can affect the physiological efficiency. The efficiency of cereal genotypes to convert N to grain yield are significantly different. The efficiency of fertiliser application can be assessed by the apparent recovery which is a more suitable indicator than the agronomic efficiency which includes a crop factor (physiological efficiency). When plant growth and N uptake are closely related, any factor that affects plant growth will influence physiological efficiency. Physiological efficiency will be affected by environmental stresses, which are important determinants of the partitioning of crop dry matter between roots, straw and grain. Alessi *et al.* (1979) suggested that the physiological efficiency varies between plant genotype, as demonstrated by a comparison of tall and short wheat varieties. Improved varieties have played an important role in not only increasing crop yields but also improving N fertiliser efficiency (Tinker and Widdowson 1982).

2.5.8 Apparent recovery

Apparent recovery is influenced by climate through effects on losses of N, the availability of N and through effects on crop growth (Craswell and Godwin 1984). The apparent recovery of N is influenced by the redistribution of N from roots to shoots especially when N uptake in the aerial portion of the crop is normally considered in the calculation of recovery (Campbell *et al.* 1977). Different climatic factors affect the amount of volatile N loss from plant tops (Wetselaar and Farquhar 1980) and the amount of N lost via root exudation (Russell 1977). These losses, with losses through environmentally influenced senescence, will affect both physiological efficiency and recovery efficiency.

2.5.9 The effect of nitrogen fertiliser on GPC

A positive effect of N fertiliser on crop quality is its increase in the GPC. All cultivars do not respond similarly to N fertiliser. High N fertility levels in drier regions is a hazard for cereals because N can cause over stimulation of vegetative growth. Excessive vegetative growth uses available water at the expense of grain yield, but if grain does form, the protein percentages of shrivelled kernels are higher than normal. As time of N application is delayed, providing sufficient rainfall is received to carry fertiliser N to the root system, GPC tends to become progressively higher. Nitrogen application may be delayed with foliar treatments so that protein, but not yield is increased (Finney *et al.* 1957). Spraying of urea (1 to 12%) after anthesis was more efficient than at heading or at anthesis for increasing the rate of accumulation of protein in grain (Sadaphal and Das 1966). Application of economic rates of N for maize just before anthesis have also been more effective for maximizing protein yields than have earlier treatments. A large N supply that stimulates heavy vegetative growth early is not especially efficient in producing strong proteinaceous materials in the maize grain. Beachell *et al.* (1972) and Dedatta *et al.* (1972) reported that fertiliser N applied to rice during the growing season rather than before, or at planting, enhances total N uptake and utilisation for GPC. For maximum protein production some of residual NO_3-N must exist in the lower rooting zone and be utilized late during the growing season. Since water is extracted from the upper rooting zone early in the season applied fertiliser N is likely to remain in the surface soil throughout the year of application. Nitrogen uptake late from the lower rooting zone by maize will be channelled more directly to grain with less immobilisation in vegetative parts. Smika and Greb (1973) reported that not only is availability of N from various sources important for protein but also residual soil NO_3-N, available water to 1.5 m at sowing, maximum air temperature for the 15 to 20 day period before maturity, and precipitation during 40 to 45 days before maturity are important factors related to GPC.

2.5.10 Yield and protein response to nitrogen fertiliser

Nitrogen fertiliser has been, and still is used to supplement soil N for increasing yield. The use of supplemental N is not always associated with increased quality (Deckard *et al.* 1986). Negative correlations between GPC and overall yield are often reported (Halloran 1981, Loffler and Busch 1982, Loffler *et al.* 1985, Cox *et al.* 1985, Stoddard and Marshall 1990, Jenner *et al.* 1991).

Yield and protein response to N supply is strongly influenced by the environmental conditions, especially the amount and timing of water available to the crop and the soil N. A generalised grain yield and GPC response to increasing N supply are shown in Fig 2.2.

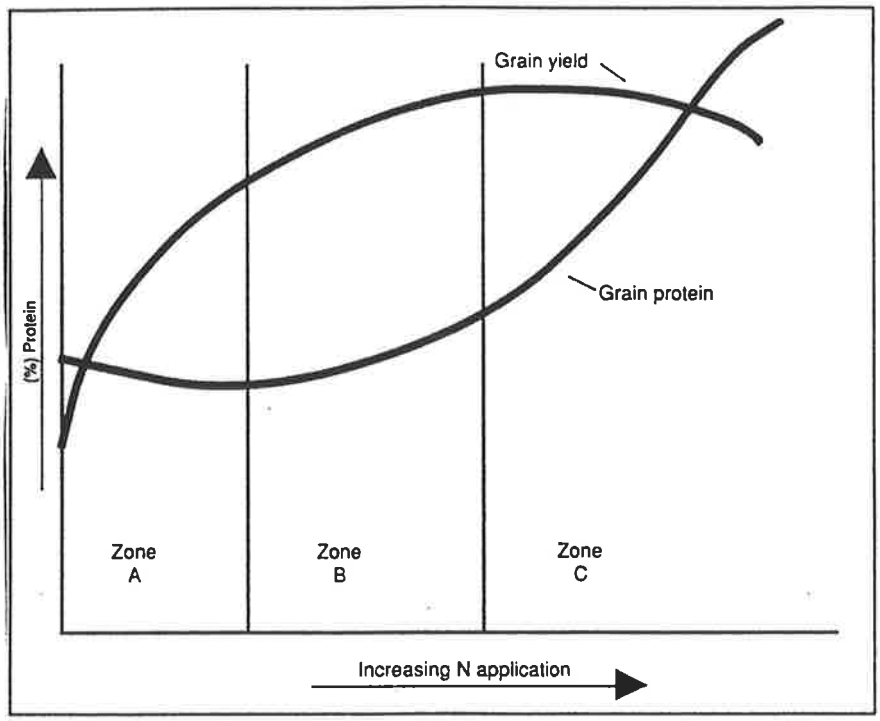

Fig. 2. 2. General relationship between grain yield and GPC and N application for rainfed wheat (from Perry and Hillman 1991).

The relationship between yield and GPC changes from negative to positive according to the soil N level. Part A Fig 2. 2 shows that GPC may fall or remain unchanged under conditions that favour a large response to N (*e.g.* low soil N levels, or favourable water conditions). In this part of the general response there may be a negative relationship between yield and GPC, or GPC may differ little over a range of yields. Eventually, GPC will begin to increase along with grain yield and there will be a positive correlation between yield and GPC (Part B, Fig 2.2). When grain yield is limited by factors other than N (*e.g.* water) or at high rates of N, the yield response is low or some time negative while GPC continues to increase (Part C, Fig 2.2). There may be a negative correlation between grain yield and GPC in this case.

Among cultivars grown under the same conditions, the relationship between grain yield and GPC is frequently negative (Part A and C, Fig 2.2). The apparent negative relationship between grain yield and GPC may be explained by a number of factors. Negative relation between yield and protein may be effected by dilution and energy constraints (Stoddard and Marshall 1990). The energy required for protein synthesis is about twice the energy for starch synthesis (Penning de Vries *et al.* 1974). Protein may be produced at the cost of yield under conditions of limiting energy. Thus, under equal amounts of photosynthate, low carbohydrate-high protein cultivars were predicted to produce less grain yield than high carbohydrate-low protein cultivars (Bhatia and Robson 1976). Higher energy costs of protein synthesis can be another reason for negative correlations between GPC and yield (Jenner *et al.* 1991). Dilution is a more powerful and more consistent cause for the negative relationship of yield to protein.

Starch and protein deposition proceed simultaneously and GPC changes within a fairly narrow range of ±2% during development under good growing conditions (Bechtel *et al.* 1982). Under poor conditions yield is depressed and GPC elevated through drought and high temperature effects. The availability of sucrose which a grain may convert to starch decreases under dry conditions (Brooks *et al.* 1982). The conversion of sucrose to starch is also suppressed by high temperature (Bhullar and

Jenner 1986). In the Australian wheat-belt, the water deficits and high temperatures during grain filling are common and prevent the dilution of protein by starch.

Breeding methods used for improving crop cultivars are another explanation for the negative relationship between yield and GPC. These methods have resulted in the inadvertent improvement of harvest index without improvement of biomass (Kramer 1978). At anthesis, more than two-thirds of N used to synthesis grain protein will be in the plant. Changing the plant genetically or environmentally to increase or decrease harvest index may affect the remobilisation of this N and subsequently GPC.

Donovan *et al.* (1977) reported that a high protein variety of wheat have a lower grain weight than a low protein variety. The difference in GPC and weight were due to different levels of carbohydrates other than starch in the low protein variety. However the relationship between grain size and GPC is variable. Nitrogen can affect kernel weight and GPC directly or influence grain number which tends to confound the correlation between GPC and grain weight.

2.6 Grain protein concentration in southern Australia

Low GPC is an important problem in southern Australia. The Department of Agriculture of South Australia (1990) reported that in the past 10 years the average GPC of wheat has dropped from 12 % to 10.5 %. Dyson and Fewings (1990) have assessed the decrease in GPC in Australian wheat through the 1980s as being the result of the following.

1. More intensive cropping.
2. Overestimation of N added by legume pasture rotation particularly to support the increased protein removal.
3. Attaining higher yields and thus, by dilution, lower protein percentage by combating weeds and diseases and by more timely sowing.
4. The more widespread growing of higher yielding cultivars with low GPC.

The amount of N supply and N demands change in different climates. As pointed out by McDonald (1989), when the amount of available N was high relative to

the amount of available water, premature ripening of wheat crops and decreased grain yields occurred. When high rates of available N lead to decreased grain yield, yields may be negatively related to GPC.

Ladd (1990) reported that cultivar and environmental conditions influence the effect of N on grain yields and GPC of wheat crops. Availability of water, mineralisation of organic N and location within the soil profiles of inorganic N from all sources have effects on the wheat growing of southern Australia. Until the 1989/90 season, farmers in South Australia were more concerned about increasing yield than about GPC of wheat (Dyson and Fewings 1990). However, under a new payment system where farmers are paid on the basis of protein as well ($2 to $3 extra per tonne for the production of higher protein Australian Standard White wheat) it is hoped that growers will take up the challenge to increase the GPC. This can be achieved concurrently with an increase in yield: the key is to improve soil fertility, especially N fertility.

2.6.1 The effect of nitrogen deficiency on GPC of wheat

Nitrogen deficiency causes low GPC and low yields. Wheat gets its N primarily from the mineralisation of soil organic matter, but also from added fertilisers, manures and from legumes (Campbell and Paul 1978). Russell (1963) showed in his 52 field experiments (undertaken in South Australia between 1956-61) that N deficiency in wheat resulted in low grain protein. This has since been confirmed in other N fertiliser experiments conducted in the 1980s (Reuter and Dyson 1990). Russell (1963) also suggested that GPC <11.7% (<2.3% grain N) were indicative of soil N stress. Nitrogen deficiency has been reported by McDonald (1989), Reuter (1989), Xu *et al.* (1991 and 1992), Xu and Elliott (1993) to be a major, widespread factor limiting cereal productivity and quality in the cereal zone of southern Australia. Some research has been done on detecting and correcting N deficiency in wheat. A report produced by the Department of Agriculture of South Australia (1985) summarised the important points as follows.

1. Climate has an essential role in wheat farming and a minimum of 250 mm annual rain is needed. When the rainfall is low and temperatures are high the grain yield is decreased.
2. At the sites where large grain yield increases were achieved, the most profitable N application rates were between 40 and 60 kg N ha^{-1}. These rates are higher than used commercially.
3. Nitrogen applied during early tillering (7 weeks from sowing) or as a split application was beneficial at most locations. Nitrogen applied during tillering by increasing the number of ear bearing per plant improved the yield.
4. The type of rotation is important and has an effect on the amount of N harvested in grain.The type of N fertiliser is not important for grain yield except in the case of urea when applied with or close to the seed is detrimental to plant emergence and N applied at more than 40 kg N ha^{-1} can depress plant emergence and decrease plant yield.

In the early part of the 20th century deficiencies in available soil N in southern Australia were in part overcome by the use of fallowing which promoted the mineralisation of organic N, and more recently by the adoption of broader rotations involving legume-based pastures. Russell (1968a, 1968b) quantified the factors influencing the response of wheat to applied N. The amount of NO_3-N at sowing and the time of sowing were the major soil and cultural factors involved although the length of fallow and the total soil water were also significantly related to yield and protein response to N. In southern Australia, late application of various N fertilisers gave 65 to 75% of the yield responses obtained with sowing application (Russell 1968c). Climate dominated the responses and, in particular, there was a positive effect due to winter rainfall and a negative effect due to high temperatures in the latter part of the growing season. A negative GPC response to N fertiliser occurred with increased winter rainfall, August temperatures and rainfall and by high temperatures in the later part of the growing season.

2.6.2 Grain protein concentration of wheat as affected by subsoil mineral nitrogen.

Subsoil mineral N may be a useful N reserve for a field crop when N in the surface layer is unavailable or not accessible because the topsoil is dry. In the wheat growing areas of southern Australia the topsoil, which contains a large amount of labile form of plant nutrients , can dry out by the time that wheat reaches ear emergence. The plant may suffer from N deficiency later in the season on a consequence. Changes in the nutrient supply late in the season affects the nutrient concentration in plants more than yield (Alston 1976, 1979, 1980). The subsoil mineral N can be considered as a N reserve late in the season.

Leaching of NO_3-N from the topsoil is an important factor which increases the concentration of NO_3-N in the subsoil (Kissel *et al.* 1974, Ladd 1990, Weier and Macrae 1993). Storrier (1962) reported that N which had accumulated in the surface horizon of soil in the wheat belt of south-eastern Australia after a period of fallow can be leached from the surface by heavy autumn and winter rains. However, leaching after cropping was not detected in any of these studies. The lower rainfall and higher evapo-transpiration during spring and summer will prevent the development of the saturated soil conditions necessary for leaching.

The low concentration of mineral N present in the surface horizon at the critical stages of wheat growth (ear-emergence to anthesis) may indicate a temporary N deficiency (Storrier 1962). Craswell and Strong (1976) found that more applied N was lost from applications to the topsoil than from application to the subsoil.

Kuhlmann *et al.* (1989) found that N uptake after anthesis of winter wheat mainly occurred from the subsoil layers, whereas N uptake during the vegetative growth was restricted to the top soil. Smika and Greb (1973) showed that the GPC was higher when high NO_3-N concentrations were found in the soil below the 60 cm depth compared with large amount of NO_3-N in the top 30 cm of the soil.

In considering the quantity of nutrients the subsoil can contribute to the crop a number of factors have to be taken into account. The most important are the water

content and nutrient status of the subsoil and root density (Wiersum 1967). Strong and Cooper (1980) reported that by maturity, depth of placement had a significant effect on uptake, with more N being taken up from the deep than from the shallow placement. They also showed that N applications caused an increase of less than 1% in GPC. However regardless of soil, the application of N before sowing would be a better practice because it increases the chance for fertiliser to be leached into subsoil where it is available even during prolonged drought. There is little information available however to indicate the effect of subsoil mineral N on the GPC of wheat in southern Australia. Depth, amount and time of mineral N application are important factors related to GPC of wheat.

2.7 Conclusion

This review has shown that a legume-pasture rotation is the traditional land use in southern Australia and N input for cereal production was derived from the legume-based pasture. Cropping has increased and pastures have been replaced with grain legumes, and N deficiency is a major factor limiting yield and GPC of wheat. Some of the N produced from decomposition of organic residual (mineralisation) or added as fertiliser leached to and moved through the subsoil. Subsoil accumulation of NO_3-N is common in southern Australia and NO_3-N usually remains within the root zone of crops. Late in the season, when the available water in the topsoil is not enough for the plant, the plant may use subsoil water. Ladd (1990) stated that subsoil mineral N may be taken up by wheat late in the season and may increase the GPC of wheat. The subsoil mineral N is affected by mineralisation, leaching, time and amount of N application. To optimise the use of subsoil mineral N and water under conditions of limited water in the topsoil, primary attention must be given to the efficient use of subsoil N and water. This may be accomplished in two ways:

(i) storing maximum amounts of mineral N in the subsoil by improved farming practices and

(ii) by making maximum use of subsoil mineral N and water through improved crop management practices.

Despite considerable work on fertiliser responses in Australia, there have been few experiments which have specifically examined the effect of subsoil mineral N on GPC of wheat. Understanding the behaviour of mineral N in the soil profile and the ability of wheat to take up N from the subsoil during the post anthesis period and its effect on GPC will be useful for solving the problem of low grain GPC in southern Australia. In view of this, the present study was planned with the following aims:

(i) to examine the recovery of N from the subsoil by wheat

(ii) to compare pre and post anthesis N application and

(iii) to study short term N uptake by the wheat at different rooting densities.

CHAPTER 3

GENERAL MATERIALS AND METHODS

In this chapter, the materials and methods commonly used in this study are described. Further details related to specific experiments are presented in subsequent chapters.

3.1 Soil

Two soil types were used in the experiments, a John Innes potting soil and a red brown earth obtained from the surface soil (0-10 cm depth) at the Waite Agricultural Research Institute. The John Innes soil had no N added to it when being made up. The characteristics of the soils are given in Table 3.1.

Table 3.1 Some physical and chemical characteristics of the soils used in this study

Soil	F.C.[A] (kg kg^{-1})	pH (1:5 H_2O)	Total N (mg kg^{-1})	Extractable P (mg kg^{-1})[B]	Organic C (mg kg^{-1})	NO_3-N (mg kg^{-1})
John Innes sand	0.05	6.8	200	10	4200	3
Red-brown earth loam	0.12	6.0	800	56	1000	20

[A] Field Capacity [B] Colwell (1963)

3.2 The size of the pot

Most experiments employed PVC pots 105 cm deep and 11 cm in diameter. The bases of pots were closed. The soil was packed to a bulk density of 1.3 g cm^{-3} to a depth of 100 cm in the pots. A polyethylene tube, 70 cm long and 2.5 cm diameter, was inserted in the middle of the soil to a depth of 60 cm to permit addition of fertiliser and water to the subsoil.

3.3 Nutrients for plant growth

The pot experiments were designed to ensure that nutrients other than N did not limit plant growth. Each pot was supplied with a mineral nutrient solution containing 100 mg $CaH_4(PO_4)_2.H_2O$, 10 mg $CuSO_4.5H_2O$, 10 mg $ZnSO_4.7H_2O$, 20 mg $MgSO_4.7H_2O$.

3.4 Watering the plants

Measured quantities of water were added to the surface of the soil two or three times weekly to maintain the soil water near field capacity. A more accurate determination of the amount of water necessary to bring the soil water to near field capacity was achieved by weighing the pots twice per week. The pots were also weighed every week to enable water use to be calculated. Water use was determined from the water content in the soil at the beginning and the end of the experiment and the amount of water added during the experiment.

3.5 Separating roots from soil

The soil from each pot was separated into depths of 0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm. A subsample of soil was taken from each layer to determine water content, and NO_3-N concentration. Roots were separated from the remaining soil samples. Soil containing plant roots was placed on a 2 mm sieve and soil particles were carefully separated from roots by washing with tap water. The roots were placed in a plastic container containing deionized water to remove the remaining soil particles and organic debris, before they were prepared for determination of root length.

3.6 Drying plant materials

At each harvest of plants, shoots and roots were separated. After the fresh weight of the roots were recorded, the plant material was dried in a oven for 24 hours at 80° C and weighed to determine dry weight. At the last harvest, the plant was separated into grain and straw.

3.7 Determination of root length

The total length of root in each sample was estimated by the line intercept method of Tennant (1975). A black filter paper was placed on the bottom of the Buchner funnel (10 cm diameter), water containing the subsample roots poured in and the roots positioned over a filter paper to make observation easy. When necessary, the root material was teased apart or cut into smaller pieces. After stock the water the root material was dried. Grid squares (0.5 cm) were placed on the filter paper containing the root. Counts of the intercepts of the roots with the vertical and horizontal grid lines were made with the aid of a magnifying glass. All counts were accumulated on a hand tally counter. Complete counts were converted to length measurements using the modified formula inclusive of the grid unit:

Root length (R)=11/14 x Number of intercepts (N) x Grid unit.

The 11/14 of the equation was combined with the grid unit to give a length conversion factor. For the 0.5cm spacing it was 0.3928. The modified formula therefore took the easily managed form:

Root length (R) = Number of intercepts (N) x Length conversion factor (0.3928).

The accuracy of this technique of root length measurement was checked by measuring segments of a known length of fine cotton thread.

3.8 Determination of total nitrogen concentration in plants

Total N was determined by a micro-Kjeldahl method (Bremner 1965). Plant material (250 mg) was placed in a 50 ml digestion tube and 4 ml of sulphuric acid, one digest tablet (1.5 g potassium sulphate K_2SO_4 and 0.007 g Selenium) and 2 ml hydrogen peroxide were added and allowed to stand overnight in a fume hood. Tubes with the plant material were put into an aluminium digestion block, which was programmed as follows:

Step	Temperature (°C)	Ramp (minute)	Time (minute)
1	150	10	20
2	250	10	20
3	330	10	120

After the digestion the digests were diluted to 50 ml with deionised water and aliquot of solution were distilled according to the procedure described by McKenzie and Wallace (1954). The tip of the condenser was immersed into 5 ml of boric acid plus indicator solution (mixture of methyl red and methyl blue). An aliquot of 5 ml of digest was placed in a distillation apparatus 10 ml of 10 M NaOH was added and distillation started. When about 10 ml had distilled over, the flask containing the indicator solution was lowered and a further 15 ml was distilled over. The distillate was titrated with 0.01 M H_2SO_4 to a lilac end point (E.P.). Total N in the sample was calculated as follows:

$$\%N = \frac{0.14 \times \text{ml acid to reach E.P.}}{\text{mg sample}} \times \frac{\text{Dilution}}{\text{Aliquot}} \times 100$$

3.9 Determination of nitrate in the soil

The concentration of NO_3-N in the soil was determined by the method of Best (1976). Twenty g portions of fresh soil < 2 mm were weighed into extraction bottles, 100 ml 2M KCl was added to each bottle and the soil extracted for one hour on an end-over-end shaker. The extract was filtered through Whatman no. 42 filter paper and 0.6 ml of the soil extract was pipetted into 50 ml tube and mixed thoroughly with 1.2 ml of $CuSO_4$, 0.8 ml hydrazine and 1.2 ml buffer reagent. The mixture was incubated at 37° C for 30 min cooled for 30 min and 1.2 ml colour reagent was then added and the solution mixed. Solutions of known NO_3-N concentration were developed in the same way as the soil samples. The absorbance of the solution and the standards was measured at 520 nm in a 1 cm cuvette and the concentration of NO_3-N was determined from the calibration graph.

3.10 Determination of nitrate in the plant tissue

The NO_3-N concentration in the plant shoots was assessed by the salicylic acid method (Cataldo *et al.* 1975). Shoot material of wheat, dried at 80° C for 24 h, was ground to pass a 40 mess screen. Ground tissue (100 mg) was suspended in 10 ml water and placed in a 45° C oven for 30 min. After cooling, the extract was filtered through Whatman No.1 filter paper. A 0.2 ml sample of the plant extract was pipetted into a 50 ml Erlenmeyer flask and mixed thoroughly with 0.4 ml of 5% (w/v) salicylic acid in concentrated H_2SO_4 (98.07 %). After cooling for 20 min, 19 ml 2M NaOH was added and the solution mixed and allowed to cool to room temperature. Solution of known NO_3-N concentration were developed in the same way as filtered samples. The absorbance of the solution was then determined at 410 nm in a 1 cm cuvette and NO_3-N level determined from the calibration graph.

3.11 Analysis of the data

Analysis of the data were done using GENSTAT 5 software (Giles *et al.* 1994). The standard experimental design was a Randomised Complete Block Design (RCBD) and analysis of variance (ANOVA) was used to assess the effects of the treatments. Treatment means were compared using the protected LSD (Steel and Torrie 1960) procedure operating at the 5% and 1% levels of significance.

CHAPTER 4

EFFECT OF NITROGEN FERTILISER PLACEMENT ON GPC OF WHEAT UNDER DIFFERENT WATER REGIMES.

4.1 Introduction

Maintaining an adequate concentration of grain protein is of economic importance in wheat production. As farmers strive for higher yields, the N available to the crop needs to be increased to maintain grain quality standards. To achieve this, wheat crops must exploit the available soil N effectively to maintain an adequate supply of N throughout the year. Nitrogen supplied as fertiliser during autumn and winter or the flush of N from mineralisation of organic matter early in the growing season may benefit yield, but unless the supply of N to the grain is sustained during grain filling, the GPC of the crop may be low.

In the cereal-growing areas of southern Australia, the cultivated and fertilised topsoil contains most of the labile forms of N. When the topsoil dries in spring and summer and the N become less available and roots less active, plants may suffer from a shortage of N which may adversely affect yield and GPC. Experiments have been conducted which showed that when fertiliser was added to the subsoil the nutrient shortage caused by topsoil drying was overcome (Alston 1980, Strong and Cooper 1980) but commercial application of this technique is limited. Subsoil mineral N may be a useful N source for crops when N in the surface soil is unavailable or not accessible because the topsoil is dry (Gass *et al.* 1971).

In southern Australia, subsoil accumulation of NO_3-N is common, and the NO_3-N usually remains within the root zone of crops (Storrier 1965, Greenland 1971) because rainfall is generally insufficient to cause leaching beyond the roots. The NO_3-N can be taken up by the wheat late in the season and Ladd (1990) suggests that late season uptake of N from the subsoil can play an important role in determining GPC of wheat.

There is considerable evidence that uptake of N late in the season (at or after anthesis) can increase GPC (*e.g.* Strong 1982, 1986) and that, in some environments substantial uptake from the subsoil does occur at this time. The main factors which appear to determine the amount of subsoil N taken up are the amount of mineral N, the soil water content and the amount of root growth in the subsoil. Storrier (1962) in southern NSW, found that N uptake stopped after anthesis and little subsoil N was used; however Strong and Cooper (1980) found that post-anthesis uptake of N during a dry year was increased when the N uptake was increased by deep placement of fertiliser. Sharma and Chaudhary (1984) reported that deep placement of fertiliser N in a coarse textured soil resulted in its more uniform distribution in the root zone, more extensive root proliferation and enhanced subsoil water utilisation than surface placement of N. Kuhlmann *et al.* (1989) found that N taken up after anthesis of winter wheat in northern Germany and Rothamsted (England) mainly occurred from the subsoil layers, whereas N uptake during the vegetative growth was restricted to the topsoil. Smika and Greb (1973) in an experiment on the Central Great Plains of U.S.A. showed that GPC was higher when high concentrations of NO_3-N were found in the subsoil. These studies, though demonstrating the importance of the availability of subsoil N to GPC, provide relatively little information on the efficiency with which wheat plants can utilise N from the subsoil or the rate at which this can be extracted from the soil. Nitrogen in the grain comes from two sources: post-anthesis uptake and remobilisation and transport of N from the vegetative parts of the plant to the grain. Many studies on GPC have been conducted under conditions when post-anthesis uptake is low and remobilisation accounts for most of the N in the grain at maturity. Fewer studies have examined the importance of subsoil N availability on GPC. Therefore 3 greenhouse experiments were conducted to examine the effects of the availability of N, the time of increased N availability and the soil water content on GPC in wheat.

4.2. Materials and methods

The experiments employed long pots (Plate 4.1) as described in Chapter 3, Section 3.2.

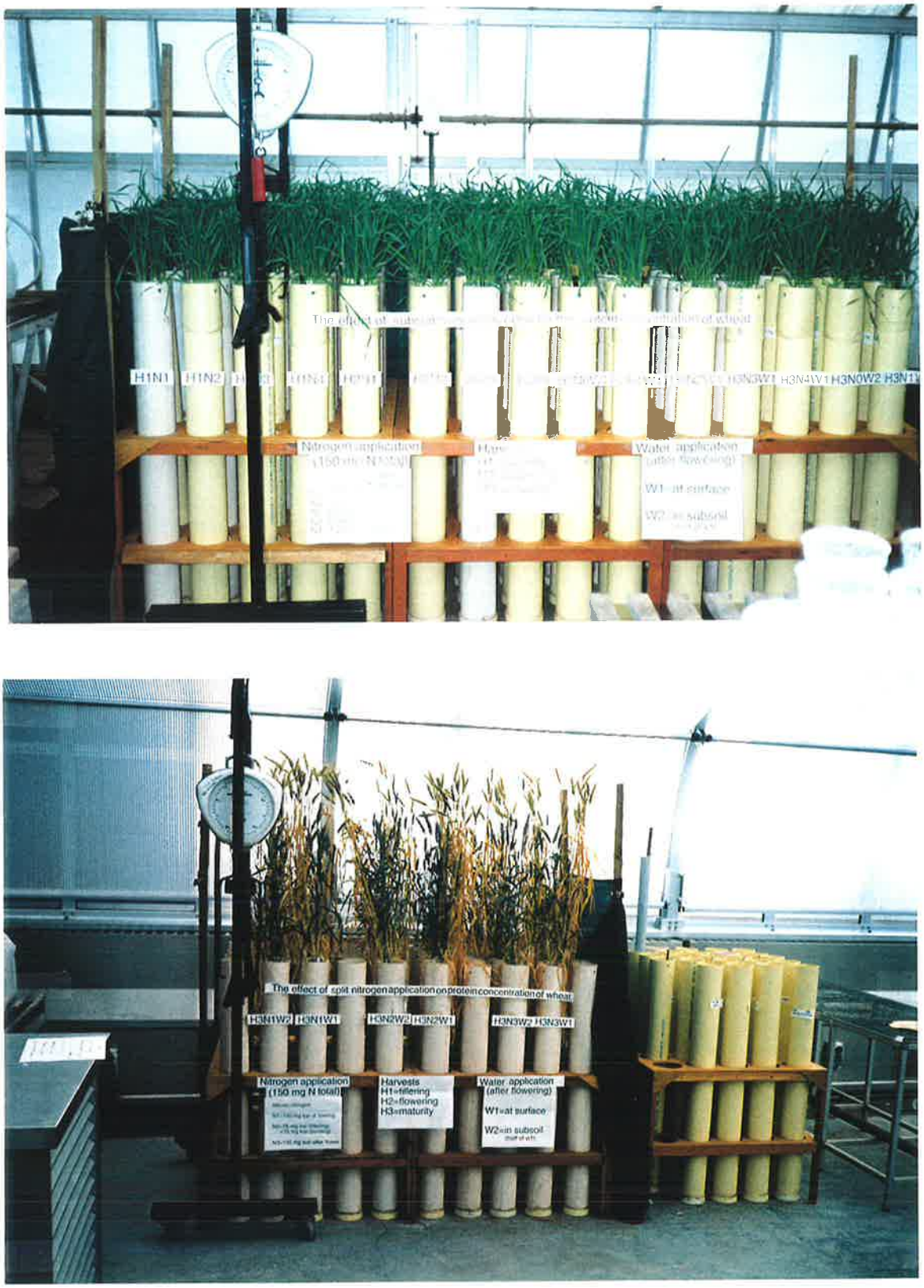

Plate 4.1 The pots used in the greenhouse to examine the effects of subsoil N on GPC of wheat in Experiments 1, 2, 3 and 4.

4.2.1. Experiment 1

The first experiment examined the ability of wheat plants to take up subsoil mineral N and its subsequent effect on GPC. A 'pulse' of N was applied to the subsoil one week after anthesis and its fate was examined over the subsequent period. The experiment had a randomised complete block design with 3 replications. The factors tested were 2 N treatments and 8 harvest dates, arranged in a factorial combination.

Wheat (cv. Molineux) was grown in a sandy soil (sand 90%, silt 6%, clay 4%). The chemical characteristics of soil have been described in Chapter 3. Six wheat seeds were sown per pot on 1 April 1994 at a depth of 1.5 cm, and a basal fertiliser solution (Chapter 3) plus 20 mg N as KNO_3, was added to the surface of the soil three weeks later. Three days after emergence, the plants were thinned to three seedlings per pot. Shadecloth was placed around the experiment at a height of two-thirds that of the plants to reduce edge effects and prevent the excessive tillering and growth that commonly occurs with plants grown in spaced pots. The mean daily minimum and maximum air temperatures in the greenhouse ranged from 18° C and 25° C, respectively, in April to 17° C and 20° C in September. The pots were weighed once per week. A measured amount of water was added to the surface of the soil two or three times weekly to maintain the soil water near field capacity. Signs of N deficiency appeared during the experiment, thus on 8 May, 24 May, 17 June, 5 July and 19 July a solution of KNO_3 containing 20, 20, 10, 10 and 5 mg N, respectively, was added to the surface soil in all pots. The post-anthesis N treatments (0 and 150 mg N) were applied in solution to the subsoil on 23 July, two weeks after anthesis, by adding 40 ml of 0.27 M KNO_3 to the treated pots and an equivalent amount of water was added to each control pot.

Plants were harvested at tillering (15 May), stem elongation (1 June), anthesis (9 July), two weeks after anthesis (23 July), two days (25 July) and seven days (30 July) after applying N, at the dough stage (22 August) and at maturity (14 September). The plants were cut at soil level, dried at 80° C, weighed and ground. At the last two harvests, the plant material was separated into grain and straw. Total N was determined in a sulphuric acid digest of the plant material by a micro-Kjeldahl method (Bremner

1965), and the NO_3-N concentration in the plant shoots was assessed by the salicylic acid method (Cataldo *et al.* 1975).

The soil from each pot was separated into depths of 0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm. A subsample of soil was taken from each layer to determine water content, and NO_3-N concentration by the method of Best (1976). Roots were separated from the remaining soil samples by flotation and root lengths and root dry weight were measured. The root lengths were measured by the modified line intercept method of Tennant (1975).

4.2.2 Experiment 2

The second pot experiment considered the effects of depth, time and amount of N application, as well as soil water regime on N uptake and GPC. All of these factors can affect the availability of N in the soil and hence the GPC. In this experiment, a post-anthesis N application was compared with a treatment where the N was placed either in the topsoil or in the subsoil during the early part of the season under different water treatments. This experiment was designed to simulate possible conditions in the field *i.e.* subsoil N derived from decomposition of organic material or from fertiliser application early in the season (leaching). The experiment had a randomised complete block design with 3 replications. Factors tested were 5 N treatments, 3 harvest dates and two water regimes arranged in factorial combination. Wheat (cv. Molineux) was again used in this experiment. Because the sandy soil in previous experiment was very low in N, and tended to cause N deficiency to develop quickly it was decided to use a loamy soil which had a higher supply of available N in this second experiment. The chemical characteristics of the soil have been described in Chapter 3. The N treatments were: (N0) no N ; (N1) 150 mg per pot as KNO_3 placed in the topsoil at sowing; (N2) 75 mg placed in the topsoil and 75 mg in the subsoil at sowing; (N3) 150 mg placed in the subsoil at sowing and; (N4) 75 mg placed in the topsoil at sowing and 75 mg placed in the subsoil one week after anthesis. The topsoil applications were applied to the surface of the soil which was then lightly cultivated. The plants were harvested at tillering, anthesis and

maturity. The plants that did not receive N (N0) were harvested only at maturity. The two water treatments, introduced when the wheat was at anthesis, were surface irrigation sufficient to keep water stress low in the plants (W1), and no surface water, but subsoil irrigation at 60 cm depth (W2). Six wheat seeds, were sown per pot on 15 November 1994 at a depth of 1.5 cm, and a basal fertiliser solution as described in Chapter 3 was added to the surface of the soil one week later. Because the amount of soil phosphorus in this experiment was high the amount of phosphorus in the basal fertiliser solution was reduced from 100 mg to 50 mg. The mean daily minimum and maximum air temperatures in the greenhouse ranged from 20° C and 23° C respectively for November to 25° C and 27° C for February. The pots were weighed 3 times per week and water was added to maintain the soil water content near field capacity. W2 simulated post-anthesis drought with topsoil drying. The pots with the subsoil irrigation treatment (W2) received half the quantity of water that would have been needed to bring all the soil to field capacity. The measurements on growth and plant and soil N in the second pot experiment were similar to the first pot experiment.

4.2.3. Experiment 3

Diminished amounts of NO_3-N in the upper soil layers and increased amounts in the lower layers indicate that movement NO_3-N has occurred within the soil profile. When infiltration of precipitation or irrigation exceeds evapotranspiration large amounts of NO_3-N can move well below 2 m depending on some soil types (Pratt *et al.* 1972, MacGregor *et al.* 1974 , Schumann *et al.* 1975). In the field, where most of the NO_3-N is in the surface, it can be assumed that the majority of the accumulated NO_3-N in the subsoil is derived through leaching of the surface layers of soil.

If the recovery of subsoil N is high, the application of N at the start of the growing season would be a good practice because it increases the chance for fertiliser to be leached into subsoil where it is available even during prolonged drought. In the current experiments the plants are watered frequently, a situation that is conducive to leaching. To follow the leaching of N from the topsoil in Experiment 2, an additional

series of pots, in which 150 mg N was applied at sowing (N1), were established and watered as for the well-watered treatment (*i.e.* W1). Between sowing and maturity nine harvests were made and the concentration of soil NO_3-N, amount of soil water and root growth measured down the profile. This experiment was designed to quantify the downward movement of N in the soil during the growing season. The soil used was the same as that used for the second experiment. There were 27 pots, 9 harvests, and 3 replicates at each harvest. Wheat (cv. Molineux) was sown on 15 November 1994. The N (150 mg N per pot as potassium nitrate) was placed at the topsoil at sowing. Plants were harvested at the 3 leaf (1 Dec), and 6 leaf (8 Dec) stage, at tillering (20 Dec), stem elongation (31 Dec), anthesis (11 Jan), one week after anthesis (17 Jan), 2 week after anthesis (23 Jan), dough stage (31 Jan) and maturity (15 Feb). The root length was measured only at tillering, anthesis and maturity. The management of the experiment and measurement of the plant and soil was the same as second experiment.

4.3 Results

4.3.1. Experiment 1

4.3.1.1 Dry matter yield

Shoot dry weight increased during the growing season. Nitrogen increased the dry matter production of both roots and shoots (Table 4.1). The effect was particularly evident in root growth where without additional N, root weight decreased during the post anthesis period whereas the additional N maintained root growth. Adding N to the subsoil two weeks after anthesis significantly increased the yield of grain and straw (Table 4.2).

Table 4.1. Dry weight and concentration of nitrate and total nitrogen in the plant (Experiment 1)

Time of harvest[A]	Dry weight (g per pot)		Nitrogen (%)		NO_3-N (mg kg^{-1})	Total N (mg per pot)
	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
			Before adding nitrogen			
15 May	1.12	1.33	3.06	1.31	1325	53
1 June	2.27	1.95	2.63	1.19	1064	85
9 July	7.98	3.42	1.29	0.93	554	139
23 July	9.73	3.00	1.08	0.93	589	138
l.s.d. (P = 0.05)	0.79	1.24	0.15	0.24	276	21
			After adding nitrogen			
			Control (0 nitrogen)			
25 July	9.81	4.05	0.93	0.86	793	133
30 July	10.44	4.05	0.92	0.80	740	139
22 Aug	14.81	3.86	0.83	0.88	925	167
14 Sept	16.05	3.59	0.78	0.76	380	155
			Nitrogen (150 mg per pot)			
25 July	9.71	3.25	1.06	0.98	1130	145
30 July	10.74	4.34	1.04	0.97	825	162
22 Aug	15.59	5.51	1.22	0.86	1141	251
14 Sept	18.72	6.18	1.23	0.64	429	276
l.s.d. (P = 0.05)	1.57	0.99	0.05	0.11	147	19

[A] The growth stage at the date of harvest was tillering (15 May), stem elongation (1 June), anthesis (9 July), 2 weeks after anthesis (23 July), 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.) and maturity (14 Sept.).

Table 4. 2. Effect of nitrogen treatments on the dry matter yield, grain protein concentration and apparent N recovery (Experiment 1).

Nitrogen applied	Dry matter yield (g per pot)		Nitrogen (%)	Protein (%)	Apparent N recovery (%)
(mg per pot)	Grain	Straw	Straw	Grain	Shoots
			22 Aug		
0	3.95	10.86	0.60	8.4	-
150	4.36	11.24	0.91	11.1	45
			14 Sept		
0	6.64	9.41	0.36	7.8	-
150	8.57	10.16	0.45	12.4	72
l.s.d. (P = 0.05)	1.37	1.04	0.12	0.6	18

4.3.1.2. Nitrate concentration in the shoot

The NO_3-N concentration in the shoots generally decreased with plant growth. The decrease was most pronounced between dough stage (22 August) and maturity (14 September, Table 4.1). Two weeks after anthesis (23 July), the nitrate solution was added to the subsoil and two days later the concentration of NO_3-N in the plant increased and subsequently decreased (Table 4.1). The NO_3-N concentration also increased in the control presumably due to the utilization of NO_3-N below 80 cm in the soil.

4.3.1.3. Total nitrogen and grain protein concentration in the plant

Total N concentration in the shoots at tillering (15 May) was high and the concentration declined with plant maturation (Table 4. 1). During the week after adding N to the subsoil, the concentration of total N in the shoots stopped decreasing and had increased again by the dough stage (22 Aug.) From July 30 onwards, the system receiving subsoil N reflected this treatment in the total plant N (Table 4. 1).

The content of total N in the plant increased during the growing season (Table 4.1). The GPC was increased significantly by applying N to the subsoil after anthesis (Table 4.2).

4.3.1.4. Recovery of nitrogen and remobilisation to the grain

Two days after applying the N, 99% of the N could be accounted for in the plant and soil, but over the next month recovery diminished to 60-80% (Table 4.3). Initially little of the added N was taken up by the plants; for example of the 150 mg added to the subsoil, only an extra 12 mg was found in the plants after two days (Table 4.1) and uptake occurred slowly over the next six weeks. By maturity 121 mg of extra N was measured in plants to which the subsoil N was applied. This was equivalent to an apparent recovery in the root and shoots of 80%. By maturity, most of the N was translocated to the grain. The addition of N after anthesis reduced the amount of N remobilised from the straw from 73 mg per pot to 51 mg per pot ($P<0.05$) and it reduced the apparent contribution of remobilised N to grain from 81% to 27% ($P<0.05$) (Table 4.4).

Table 4.3. Effect of nitrogen treatments on the apparent recovery of nitrogen (Experiment 1).

Time of Harvest	Difference between treatment and control pots: Plant total N (mg per pot)	Soil nitrate-N (mg per pot)	Total N (plant+soil) (mg per pot)	Recovery [A] (%)
25 July	12	136	148	99
30 July	23	83	106	71
22 Aug	84	4	88	59
14 Sept	121	0	121	81

[A] Recovery has been calculated as the sum of the increase in plant total nitrogen and soil nitrate in the fertilised pot, compared to the non-fertilised (control) pot divided by the amount of applied nitrogen.

Table 4.4. Post-anthesis nitrogen balance in the plant (Experiment 1)

Nitrogen (mg per pot)	Increase in (root + shoot)N (mg per pot)	Increase in grain N (mg per pot)	Nitrogen [A] remobilised (mg per pot)	N remobilised N in grain (%)
0	17	90	73	81
150	136	187	51	27
l.s.d. (P=0.05)	42	47	7	30

[A] calculated from differences between increase in (root+shoot) total N and grain total N.

4.3.1.5 Root growth

Root distribution followed the expected pattern, being high in the surface soil and decreasing with depth (Table 4.5). Roots grew slowly before tillering (15 May), but root length density increased rapidly after 1 June (stem elongation) until 23 July (2 weeks after anthesis) especially in the surface layers. The roots did not extend below 80 cm until stem elongation (1 June). Adding N to the subsoil just after anthesis resulted in an increase in root length in the subsoil that was apparent by the dough stage (22

Aug). The root length density in the topsoil decreased 3 weeks after anthesis (30 July). Root dry weight followed a similar trend to root length during the growing season but dry weight was more affected than length by the addition of N to the subsoil (Table 4.6).

Table 4. 5. Root length density in the soil profile during the growing season (Experiment 1)

Depth (cm)	Root length density (cm cm^{-3})			
		Before adding nitrogen		
	15 May[A]	1 June	9 July	23 July
0-10	5.04	9.94	15.5	18.36
10-20	2.67	3.69	4.71	4.35
20-40	1.47	2.03	2.22	2.73
40-60	1.62	1.85	2.01	2.51
60-80	0.63	1.38	1.70	1.88
80-100	0.00	0.25	1.26	0.96
l.s.d. (P = 0.05)		1.68		
		After adding nitrogen		
	25 July	30 July	22 Aug	14 Sept
		Control (0 nitrogen)		
0-10	16.40	19.79	17.66	11.87
10-20	4.73	6.45	5.15	4.68
20-40	4.04	2.28	3.51	3.38
40-60	2.91	2.23	2.80	2.88
60-80	0.98	1.85	1.53	1.44
80-100	0.16	0.34	0.92	0.07
		Nitrogen (150 mg per pot)		
0-10	17.55	19.29	16.15	12.08
10-20	5.42	4.56	6.86	5.46
20-40	3.63	3.20	3.80	3.32
40-60	2.58	2.30	3.56	3.70
60-80	1.73	1.88	2.47	2.97
80-100	0.38	0.30	0.27	0.79
l.s.d. (P = 0.05)		1.7		

[A] The growth stage at the date of harvest was tillering (15 May), stem elongation (1 June), anthesis (9 July), 2 weeks after anthesis (23 July), 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.) and maturity (14 Sept.).

l.s.d applies to interaction between depth and harvest.

Table 4. 6 Root dry weight in the soil profile during the growing season (Experiment 1).

Depth (cm)	Root dry weight (g)			
		Before adding nitrogen		
	15 May[A]	1 June	9 July	23 July
0-10	0.52	0.84	1.49	1.27
10-20	0.13	0.19	0.29	0.27
20-40	0.19	0.23	0.28	0.40
40-60	0.28	0.25	0.32	0.45
60-80	0.21	0.36	0.45	0.66
80-100	0.00	0.14	0.58	0.56
l.s.d. (P = 0.05)		0.27		
		After adding nitrogen		
	25 July	30 July	22 Aug	14 Sept
		Control (0 nitrogen)		
0-10	1.44	1.85	1.46	1.22
10-20	0.34	0.42	0.35	0.41
20-40	0.62	0.52	0.52	0.52
40-60	0.93	0.60	0.53	0.64
60-80	0.58	0.79	0.56	0.72
80-100	0.14	0.29	0.44	0.07
		Nitrogen (150 mg per pot)		
0-10	1.04	1.52	1.39	1.34
10-20	0.30	0.29	0.59	0.44
20-40	0.46	0.56	0.52	0.56
40-60	0.50	0.59	1.11	1.41
60-80	0.71	1.12	1.76	2.01
80-100	0.25	0.26	0.15	0.43
l.s.d. (P = 0.05)		0.39		

[A]The growth stage at the date of harvest was tillering (15 May), stem elongation (1 June), anthesis (9 July), 2 weeks after anthesis (23 July), 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.) and maturity (14 Sept.).

l.s.d. applies to interaction between depth and harvest.

4.3.1.6 Root activity (uptake of NO_3-N by roots)

Root activity below 60 cm was estimated from loss of NO_3-N in the subsoil (60-100 cm) between 2 days after adding N until maturity and the mean root length or root dry weight during this period of time. There was a substantial increase in the N uptake when N was applied. The rate of N uptake per gram or per cm of root by the N-treated plants was greater than the control (Table 4.7).

Table 4.7. Estimation of root activity (NO_3-N uptake) in the subsoil (depth 60-100 cm) two days after adding N (Experiment 1).

Harvest[A]	Average root dry weight (g)	Average root length (cm)	NO_3-N uptake (mg)	NO_3-N uptake (mg $g^{-1}d^{-1}$)	NO_3-N uptake (µg $cm^{-1}d^{-1}$)
			Control (0 nitrogen)		
25 July - 30 July	0.90	3164	-5.2	-	-
30 July - 22 Aug	1.04	4408	12.3	0.5	0.1
22 Aug - 14 Sept	0.90	3762	-5.1	-	-
			Nitrogen (150 mg per pot)		
25 July - 30 July	1.17	4075	33.9	4.1	1.1
30 July - 22 Aug	1.65	4674	83.2	2.2	0.8
22 Aug - 14 Sept	2.17	6175	6.4	0.1	0.04

[A] The growth stage at the date of harvest was 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.) and maturity (14 Sept.).

4.3.1.7 Soil water and water use

Applying N increased water use due to an increase in plant growth, but the efficiency of water use was also increased (Table 4.8). There was greater use of water from the subsoil when N was added.

Table 4.8. Effect of the nitrogen treatments on the soil water content at maturity, amount of water used and efficiency of water use (Experiment 1).

Nitrogen (mg per pot)	Soil water content (mL per pot)	Total water use (mL per pot)	Water use efficiency (g L^{-1})[A]
		22 Aug	
0	540	4410	0.896
150	473	4549	0.958
		14 Sept	
0	504	4896	1.360
150	480	5342	1.600

[A] Water use efficiency was calculated for the grain dry weight.

4.3.1.8 Soil nitrate

Soil NO_3-N concentrations were low (<5 mg kg^{-1}) in the top 80 cm prior to anthesis. At tillering (15 May) and stem elongation (1 June) the concentration of NO_3-N increased with depth and there was accumulation of NO_3-N at the bottom of the pot which is attributed to leaching (Fig 4.1). Between stem elongation (1 June) and anthesis (9 July) the concentration of NO_3-N at 80-100 cm decreased as the root growth in these layers increased. As expected there was a large increase in the concentration of NO_3-N when the nitrate solution (150 mg N per pot) was added to the subsoil (60 cm) two weeks after anthesis. The concentration of NO_3-N declined within one week (30 July) of adding the N. The N uptake by plants from soil during this period was high (Fig 4.1) and by the last two harvests (22 Aug and 14 Sept) the subsoil contained only a trace amount of NO_3-N.

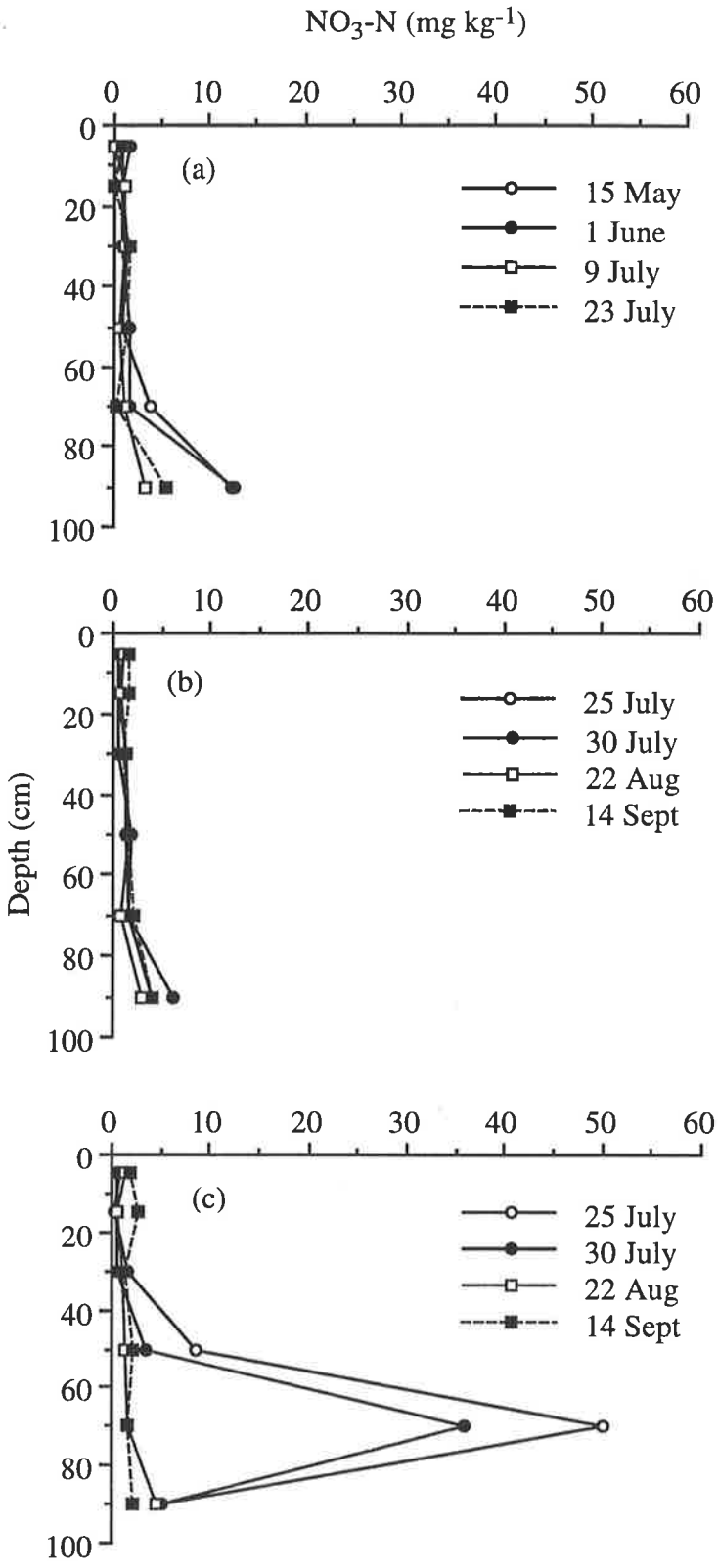

Fig. 4.1. Concentration of NO_3 -N in the soil profile before adding N (a) and after adding N, (b) control, (c) N treatment. The initial concentration of NO_3-N in the soil was 3 mg kg^{-1} (Experiment 1).

4.3.2 Experiment 2

In most cases N added to wheat is applied as a single application before or near to sowing. Therefore in this experiment, N1 rather than N0 can be regarded as the appropriate control. The inclusion of N0 was to allow the N responsiveness of the different water treatments to be assessed.

4.3.2.1 Dry matter and grain yield

Nitrogen increased shoot dry matter at maturity, but there was no significant difference in the effect between N treatments. The interaction between N treatment and water was not significant (Table 4.9). Similarly, the grain yield of plants treated with N was higher than for the N0 treatment but neither the time nor method of N placement affected the grain yield significantly (Table 4.10). Reducing the supply of water after anthesis restricted grain yield compared with surface irrigation (12.9 g per pot compared with 13.8 g per pot) but the difference was not significant at $P \leq 0.05$. The post-anthesis water regime had no affect on the response to N.

4.3.2.2 Nitrate concentration in the shoot

The NO_3-N concentration in the tops of the plants decreased during the season (Table 4.9). At the tillering stage, the concentration of NO_3-N in the plant that received 150 mg N in the subsoil at sowing (N3) was low compared with the other N treatments but differences between N treatments were small at other times. Subsoil irrigation resulted in a significant increase in the concentration of NO_3-N in the plant shoots at maturity (Table 4.9).

Table 4. 9. Dry weight, concentration of nitrate and total nitrogen in the plant (Experiment 2)

Nitrogen treatment	Dry weight (g per pot)		Nitrogen (%)		NO_3-N (mg kg^{-1})	Total N (mg per pot)
	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
			Tillering			
N1[A]	3.20	2.20	3.75	1.64	9167	184
N2	2.80	1.80	3.62	1.60	8708	152
N3	2.70	2.20	3.32	1.40	7208	142
N4	2.80	1.80	3.36	1.60	8750	147
			Anthesis			
N1	13.60	5.20	1.94	1.16	2847	358
N2	12.95	5.40	2.07	1.12	2582	362
N3	14.50	6.30	1.90	1.06	2277	373
N4	13.40	6.40	1.85	0.95	2115	336
l.s.d.[B] (P=0.05)	n.s.	0.86	0.20	0.11	921	n.s.
			Maturity			
			Surface irrigation (W1)			
N0	27.20	5.24	1.22	1.04	529	400
N1	33.80	5.55	1.35	1.14	988	554
N2	35.00	6.40	1.31	1.10	833	557
N3	33.80	4.84	1.36	1.04	750	529
N4	33.50	5.35	1.37	1.04	817	541
			Maturity			
			Subsoil irrigation (W2)			
N0	25.60	4.04	1.32	1.14	792	402
N1	32.30	5.69	1.37	1.02	1688	554
N2	32.50	5.22	1.38	1.02	1313	543
N3	32.30	5.56	1.45	1.00	1604	574
N4	30.50	5.67	1.40	0.98	1583	530
l.s.d.[C] (P = 0.05)	n.s.	1.04	n.s.	0.09	308	n.s.

[A] N0 = no N, N1 = 150 mg N topsoil at sowing, N2 = 75 mg N topsoil and 75 mg N subsoil at sowing, N3 = 150 mg N subsoil at sowing, N4 = 75 mg N topsoil at sowing and 75 mg N subsoil after anthesis.

[B] l.s.d. applies to the interaction between nitrogen and harvest.

[C] l.s.d. applies to the interaction between nitrogen and water treatment.

Table 4.10. Effect of nitrogen treatments on the dry matter yield, grain protein concentration and apparent N recovery at maturity (Experiment 2).

Nitrogen treatment	Dry matter yield (g per pot) Grain	Straw	Nitrogen (%) Straw	Protein (%) Grain	Apparent N recovery(%) Shoots
N0[A]	11.70	14.70	0.64	11.8	-
N1	13.70	19.40	0.75	12.7	77
N2	13.90	19.80	0.70	12.9	80
N3	14.00	19.10	0.77	13.0	85
N4	13.50	18.60	0.75	13.0	73
l.s.d. (P=0.05)	1.70	3.00	n.s.	0.70	n.s.

[A] N0 = no N, N1 = 150 mg N topsoil at sowing, N2 = 75 mg N topsoil and 75 mg N subsoil at sowing, N3 = 150 mg N subsoil at sowing, N4 = 75 mg N topsoil at sowing and 75 mg N subsoil after anthesis.

4.3.2.3 Total nitrogen and grain protein concentration in the plant

Total N concentration in the shoots at tillering was high but the concentration decreased as the plants matured (Table 4.9). At tillering, N concentration in shoots was greater for N1 and N2 than for N3 and N4 and at anthesis it was greater for N2. At tillering, the N concentration of the root where 150 mg was placed in the subsoil at sowing (N3) was low compared with other N treatments. However, from anthesis onwards, N4 tended to result in the lowest root N concentration and where subsoil irrigation was used, N0 had the highest root N concentration. Total N in the plant (shoot and root) increased during the growing season. At maturity all N treatments increased plant N compared to N0, but there was no difference due to N placement or water treatment (Table 4.9).

The content of the N in the plants that received 150 mg N in subsoil at sowing (N3) under subsoil irrigation was higher than with the other treatments. All N treatments significantly increased the GPC compared with N0 (Table 4.10). The GPC was significantly higher (13.1%) with the limited subsoil irrigation than with surface irrigation (12.3%).

4.3.2.4 Post-anthesis nitrogen remobilisation

The different N and water treatments had no significant effect on the amount of N remobilised from the straw. The apparent contribution of remobilised N to grain was highest in the plant that received 150 mg N subsoil at sowing (N3) under surface irrigation but the differences between treatments were not significant at $P \leq 0.05$ (Table 4.11)

Table 4.11. Post-anthesis nitrogen balance in the plant (Experiment 2)

Nitrogen (mg per pot)	Increase in (root+shoot)N (mg per pot)	Increase in grain N (mg per pot)	Nitrogen [A] remobilised (mg per pot)	N remobilised N in grain (%)
		Surface irrigation (W1)		
N1[B]	195.4	315.1	119.6	38.0
N2	195.1	305.1	110.1	36.2
N3	155.7	315.5	159.8	50.3
N4	204.9	314.0	109.0	35.1
		Subsoil irrigation (W2)		
N1	195.3	292.5	97.2	33.3
N2	180.9	321.7	140.8	44.7
N3	202.0	318.9	116.9	36.8
N4	194.3	297.6	103.2	34.5
l.s.d. (P=0.05)	n.s.	n.s.	n.s.	n.s.

[A] calculated from differences between increase in (root+shoot) total N and grain total N

[B] N1= 150 mg N topsoil at sowing, N2 = 75 mg N topsoil and 75 mg N subsoil (60 cm) at sowing, N3 = 150 mg N subsoil at sowing, N4 = 75 mg N topsoil at sowing and 75 mg N subsoil after anthesis.

4.3.2.5 Root growth

Roots grew slowly until tillering, then root length density increased sharply from tillering to anthesis, especially in the surface layers (Table 4.12). Neither the addition of N nor its placement affected root length density.

Table 4. 12. Root length density and root dry weight in the soil profile[A] (Experiment 2).

	Harvest		
Depth (cm)	Tillering	Anthesis	Maturity
	Root length density (cm cm^{-3})		
0-10	7.10	14.49	9.46
10-20	4.50	12.43	8.55
20-40	3.37	6.85	6.34
40-60	1.98	4.65	3.92
60-80	0.21	1.52	2.31
80-100	0.05	1.16	2.30
l.s.d.(P=0.05)		1.36	
	Root dry weight (g)		
0-10	0.63	1.75	1.38
10-20	0.36	0.92	0.88
20-40	0.51	1.41	1.20
40-60	0.39	0.99	0.89
60-80	0.08	0.42	0.57
80-100	0.01	0.30	0.67
l.s.d.(P=0.05)		0.15	

[A] Values have been averaged over all N treatments because the effect of N was not significant.

l.s.d applies to the interaction between depth and harvest.

Root dry weight increased until anthesis, then decreased sharply in the top 10 cm of soil (Table 4.12). However root dry weight in the 60 to 100 cm depth increased up to

maturity. The root dry weight of the plants treated with no N (N0) or with 75 mg in the topsoil and 75 mg in the subsoil at sowing (N2) under the subsoil irrigation regime was slightly lower at maturity compared with the plants under surface irrigation (Table 4.13).

Table 4.13. The effect of nitrogen and water treatments on root dry weight (g) at maturity (Experiment 2).

	Nitrogen treatment				
Water treatment	N0[A]	N1	N2	N3	N4
Surface irrigation	0.87	0.93	1.07	0.83	0.91
Subsoil irrigation	0.67	0.95	0.87	0.93	0.95
l.s.d.(P=0.05)			0.16		

[A] N0 = no N, N1 = 150 mg N topsoil at sowing, N2 = 75 mg N topsoil and 75 mg N subsoil (60 cm) at sowing, N3 = 150 mg N subsoil at sowing, N4 = 75 mg N topsoil at sowing and 75 mg N subsoil after anthesis.

4.3.2.6 Soil water and water use

The N-treated plants used similar amounts of water, and more than the unfertilised control plants. However, water use efficiency, though tending to be higher where N was applied, was not significantly ($P< 0.05$) affected (Table 4.14). Total water use by wheat was greater, while water use efficiency was poorer with surface irrigation compared with subsoil irrigation.

Table 4.14. Effect of the nitrogen and water treatments on the soil water content at the end of the experiment, amount of water used and efficiency of water use (Experiment 2).

Nitrogen treatment	Soil water content (mL per pot)	Total water use (mL per pot)	Water use efficiency (g L^{-1})[A]
N0[B]	480	7489	1.57
N1	404	7855	1.74
N2	384	7912	1.77
N3	426	7741	1.81
N4	342	7844	1.72
l.s.d.(P=0.05)	62	215	n.s.
Water treatment			
Surface irrigation	422	8389	1.65
Subsoil irrigation	392	7148	1.80
l.s.d. (P=0.05)	n.s.	136	0.13

[A] Water use efficiency was calculated for the weight of grain

[B] N0 = no N, N1 = 150 mg N topsoil at sowing, N2 = 75 mg N topsoil and 75 mg N subsoil (60 cm) at sowing, N3 = 150 mg N subsoil at sowing, N4 = 75 mg N topsoil at sowing and 75 mg N subsoil after anthesis.

4.3.2.7 Soil nitrate

As expected there was a large amount of NO_3-N in the topsoil at tillering for treatments that added 150 mg N to the topsoil at sowing and in the subsoil of pots that received 150 mg N in the subsoil at sowing (Fig 4. 2). By anthesis, the concentration of NO_3-N had decreased in the topsoil but there was still a considerable amount of NO_3-N in the subsoil. At anthesis, the concentration of NO_3-N in the 60-100 cm depth for the pots that received 75 mg N in the topsoil and 75 mg N in the subsoil (N2) or those receiving 150 mg N in the subsoil at sowing (N3) were similar.

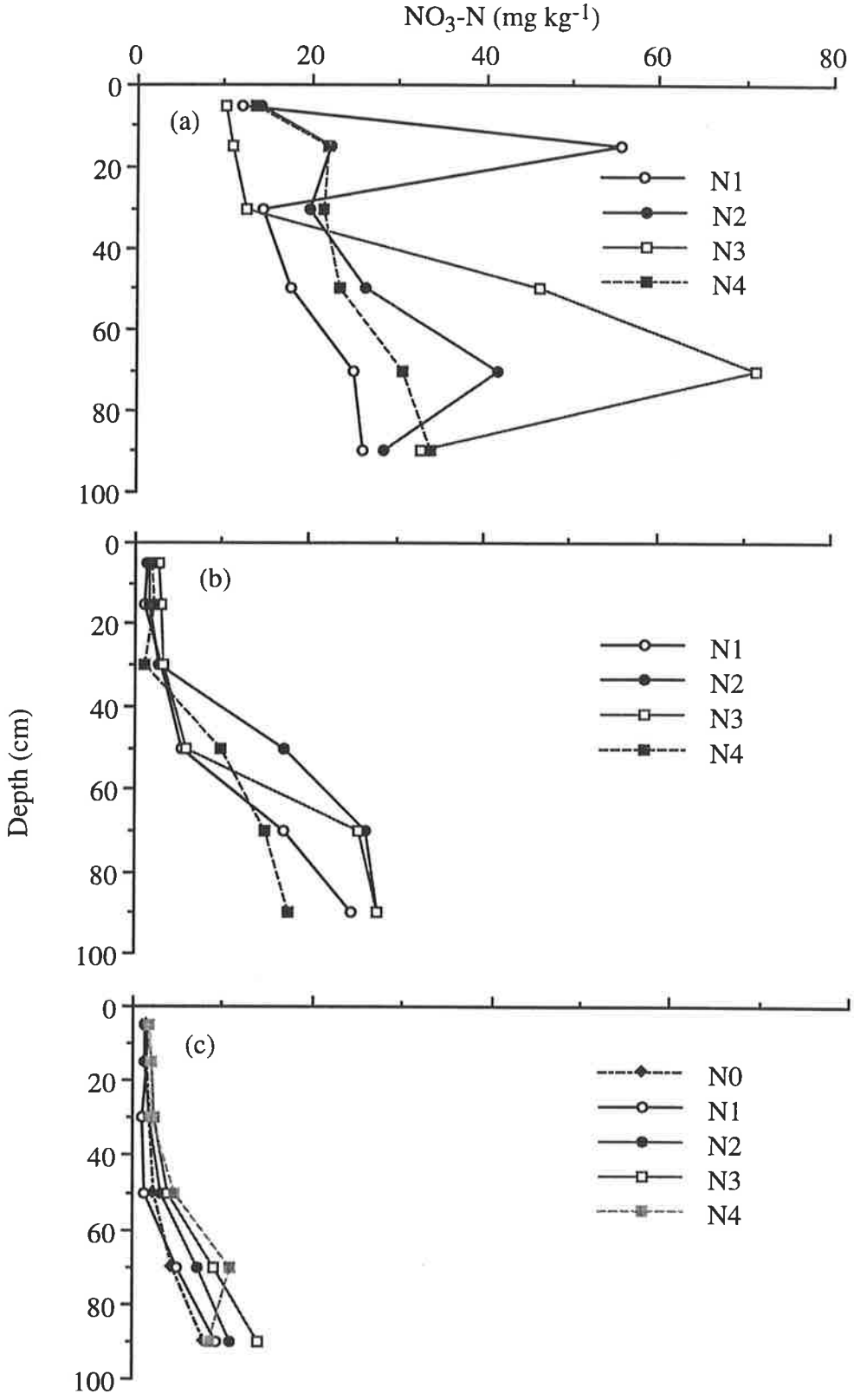

Fig.4.2. Concentration of NO_3-N in the soil profile at (a) tillering, (b) anthesis and (c) maturity. N0 = no N, N1 = 150 mg N topsoil at sowing, N2 = 75 mg N topsoil and 75 mg N subsoil at sowing, N3 = 150 mg N subsoil at sowing, N4 = 75 mg topsoil at sowing and 75 mg N subsoil after anthesis. The initial concentration of NO_3-N in the soil was 20 mg kg^{-1} (Experiment 2).

4.3.3 Experiment 3

4.3.3.1 Dry matter yield

Shoot dry weight increased throughout the experiment (Table 4.15). In contrast, root growth increased up until anthesis (11 Jan) after which root dry weight remained constant (Table 4.15).

Table 4.15 Dry weight and concentration of nitrate and total nitrogen in the plant (Experiment 3)

Time of	Dry weight (g per pot)		Nitrogen (%)		Nitrate-N (mg kg^{-1})	Total N (mg per pot)
Harvest [A]	Shoot	Root	Shoot	Root	Shoot	Plant
1 Dec	0.15	0.10	4.06	1.54	10833	8.9
8 Dec	0.33	0.28	4.06	1.54	9675	21.3
20 Dec	3.19	2.19	3.75	1.64	9167	184.3
31 Dec	6.65	2.99	2.95	1.42	6832	283.0
11 Jan	13.62	5.18	1.94	1.16	2847	358.3
17 Jan	15.47	5.37	1.88	1.12	3497	404.7
23 Jan	21.18	6.19	1.44	0.99	2277	414.7
31 Jan	24.63	4.96	1.50	1.06	1875	467.3
15 Feb	33.83	5.52	1.36	1.14	988	554.0
l.s.d. (P = 0.05)	3.40	1.44	0.43	0.26	879	79.5

[A] The stages of growth at the time of harvest was 3 leaf (1 Dec), 6 leaf (8 Dec), tillering (20 Dec), stem elongation (31 Dec), anthesis (11 Jan), one week after anthesis (17 Jan), 2 weeks after anthesis (23 Jan), dough stage (31 Jan) and maturity (15 Feb).

4.3.3.2 Nitrate concentration in the wheat

The NO_3-N concentration in the tops of the plants (Table 4.15) was high at the first harvest and then decreased. There was a little increase in NO_3-N concentration at 17 Jan. There was a close relation ($r^2 = 0.99$) between shoot NO_3-N and total N over the period of growth (Fig. 4.3).

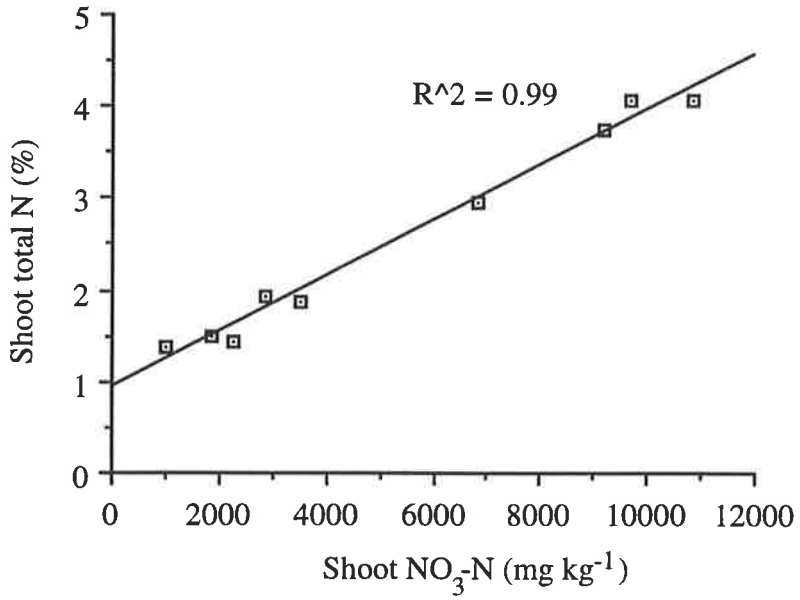

Fig. 4.3. Correlation between shoot NO_3-N and shoot total N (Experiment 3).

4.3.3.3 Total nitrogen concentration in the plant

Total N concentration in the shoots at 3 and 6 leaves was high (Table 4.15) but the concentration fell as the plants aged. Root total N concentration was lower than shoot total N concentration, root total N increased slightly at tillering (20 Dec) and then decreased (Table 4.15). The concentration of total N in the root fell during the growing season but the decline was not as great as that measured in the shoot. The amount of total N in the plant at the first and second harvests was low (Table 4.15), but it increased from tillering (20 Dec) until maturity (15 Feb).

4.3.3.4 Nitrogen uptake by the plant

Total N uptake during the first and second harvest (1 Dec to 8 Dec) was low but it was increased sharply between the second and third harvests (8 Dec-20 Dec). During this period the rate of N uptake by the root was high (Table 4.16). The rate of N uptake per unit root length or dry weight from tillering until anthesis (20 Dec -11 Jan) was higher than that from anthesis until maturity (11 Jan-15 Feb) (Table 4.17). Most of the NO_3-N in the soil disappeared prior to 8-20 December which corresponded to the period of greatest plant N uptake (Table 4.16).

Table 4.16. Soil NO_3-N change and the rate of nitrogen uptake by wheat during the growing season (Experiment 3).

Time periods harvest[A]	Average root dry weight (g)	Soil NO_3-N change (mg per pot)	Plant total N uptake[B] (mg per pot)	Plant total N uptake (mg g^{-1}root d^{-1})
1 Dec - 8 Dec	0.19	+ 35.10	12.38	8.14
8 Dec - 20 Dec	1.24	- 126.70	163.10	9.99
20 Dec - 31Dec	2.60	-71.31	98.67	3.16
31 Dec - 11 Jan	4.10	-87.35	75.33	1.53
11 Jan - 17 Jan	5.28	-15.49	46.34	1.25
17 Jan - 23 Jan	5.80	-30.46	10.00	0.25
23 Jan -31 Jan	5.58	-25.00	52.66	1.05
31 Jan - 15 Feb	5.24	-13.60	86.67	1.03

[A] The stages of growth at the time of harvest was 3 leaf (1 Dec), 6 leaf (8 Dec), tillering (20 Dec), stem elongation (31 Dec), anthesis (11 Jan), one week after anthesis (17 Jan), 2 weeks after anthesis (23 Jan), dough stage (31 Jan) and maturity (15 Feb).

[B] Total N uptake calculated as the increase in total N in roots and shoot.

Table 4. 17. The rate of nitrogen uptake by wheat during the growing season.

(Experiment 3)

Time periods [A]	Average root dry weight (g)	Average root length (cm)	Plant total N uptake (mg per pot)	Total N uptake mg g^{-1}root d^{-1}	Total N uptake µg cm^{-1}root d^{-1}
20 Dec-11 Jan	3.40	36750	174	2.33	0.22
11 Jan-15 Feb	5.5	47585	196	1.02	0.12

[A] The stages of growth at the time of harvest was tillering (20 Dec), anthesis (11 Jan), and maturity (15 Feb).

4.3.3.5 Root growth

Roots grew slowly until tillering and were confined to the top 60 cm, but after that root growth occurred below 80 cm (Fig 4.4). Significant amounts of root growth at the base of the pot were not measured until stem elongation (31 Dec). Root dry weight increased until the sixth harvest (17 Jan) especially in the top 10 cm of soil but it decreased thereafter.

4.3.3.6 Soil water

The water content of soil varied with depth during the season (Fig 4.5). At the first harvest (1 Dec) there was no change in the soil water content. At the second harvest (8 Dec) there was water depletion from the soil surface and water increased with depth. Water depletion at the third harvest (20 Dec) was high. The water content in the soil was very low at the last harvest (15 Feb).

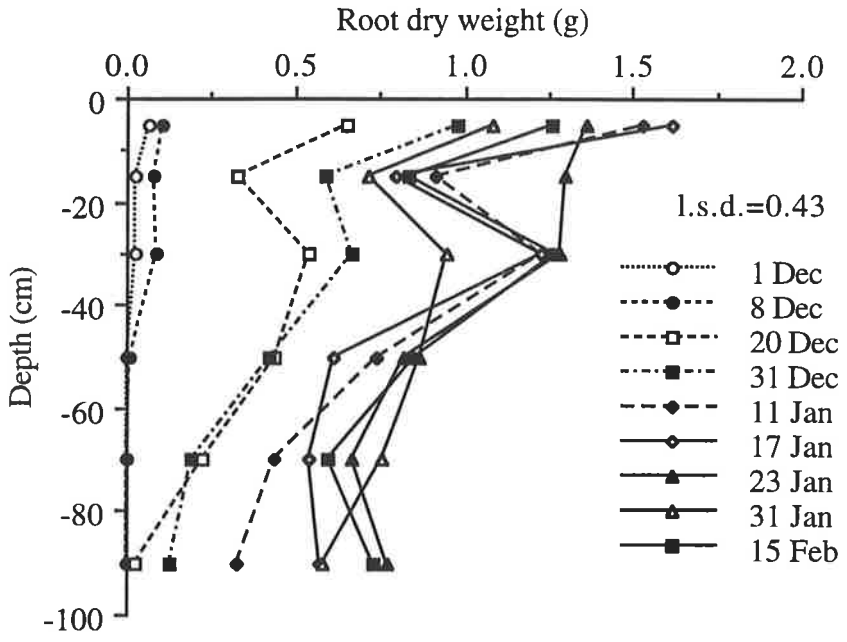

Fig 4.4. Root dry weight in the soil profile (Experiment 3).

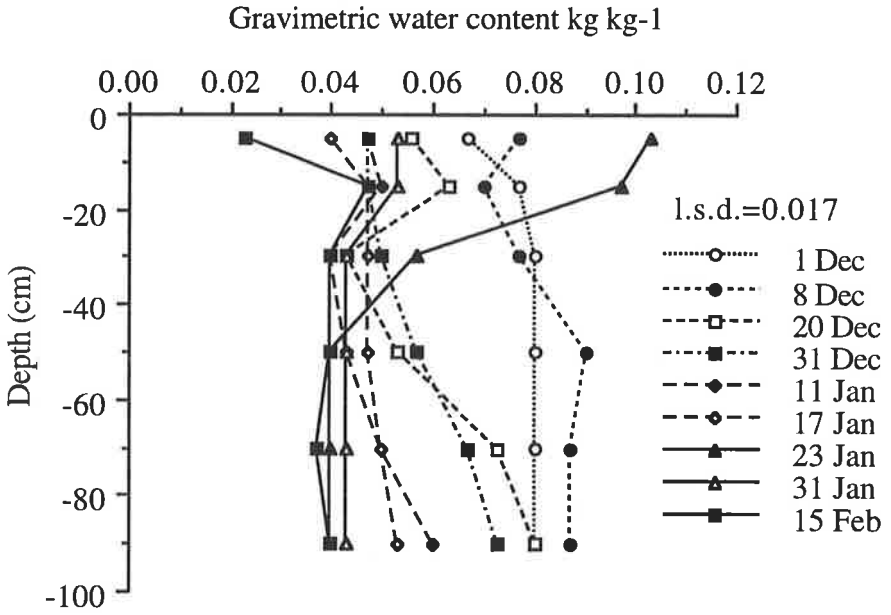

Fig 4.5. Gravimetric water content in the soil profile (Experiment 3).

4.3.3.7 Soil nitrate nitrogen and its downward movement in the soil

The concentration of NO_3-N and its distribution in the soil changed during the growing season. At the first and second harvests the concentration of NO_3-N in the surface of the soil was high (Fig 4.6, Table 4.18). At the third harvest (20 Dec) there was a decrease in concentration of NO_3-N in the topsoil, some part of it due to uptake by plant. In the initial stages of plant growth (1 Dec until 20 Dec), much of the NO_3-N moved from the surface to 20 cm. The amount of water added during this period was 820 ml per pot. The measurements of NO_3-N over growing season in the soil with the N1 treatment indicated an accumulation of NO_3-N in the deeper layer (mostly 20 cm depth) prior to anthesis (Fig 4.6, Table 4.19). There was little NO_3-N available in the topsoil at stem elongation (31 Dec), and the plants relied on NO_3-N in the subsoil thereafter.

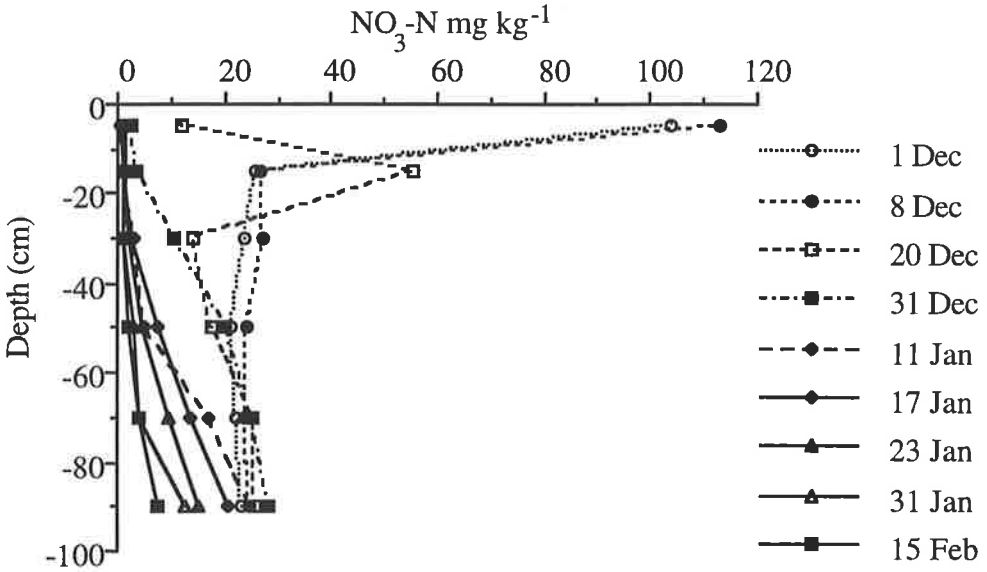

Fig 4.6. Concentration of NO_3-N in the soil profile (Experiment 3).

Table 4.18 Concentration of NO_3-N (mg kg^{-1}) in the soil profile (Experiment 3)

		Harvest time[A]	
Depth (cm)	1 Dec	8 Dec	20 Dec
0-10	103.8	112.9	12.0
10-20	25.5	26.9	55.6
20-40	23.8	27.0	14.4
40-60	21.2	23.9	17.8
60-80	22.1	23.9	25.0
80-100	23.4	24.7	25.9
		Harvest time	
	31 Dec	11 Jan	17 Jan
0-10	2.3	1.3	0.7
10-20	3.4	1.0	0.9
20-40	10.7	2.8	2.4
40-60	20.4	5.3	7.5
60-80	25.0	17.1	13.6
80-100	28.4	24.9	20.5
		Harvest time	
	23 Jan	31 Jan	15 Feb
0-10	1.0	0.9	1.3
10-20	1.0	1.0	1.6
20-40	2.0	1.1	1.1
40-60	4.7	2.8	1.9
60-80	9.4	4.2	4.0
80-100	14.9	12.6	7.6
l.s.d. (P =0.05)		10.3	

[A] The stages of growth at the time of harvest was 3 leaf (1 Dec), 6 leaf (8 Dec), tillering (20 Dec), stem elongation (31 Dec), anthesis (11 Jan), one week after anthesis (17 Jan), 2 weeks after anthesis (23 Jan), dough stage (31 Jan) and maturity (15 Feb).

Table 4. 19. The change of soil NO3-N (mg) in different layers during the growing season (Experiment 3).

Depth (cm)	Dec 1-8	Dec 8-20	Dec 20-31	Dec-Jan 31-11	Jan 11-17	Jan 17-23	Jan 23-31	Jan-Feb 31-15
0-10	+ 10.9	- 121.1	-11.7	- 1.14	- 0.70	+ 0.27	- 0.11	+ 0.56
10-20	+ 1.6	+ 34.6	-62.6	- 2.89	- 0.14	+ 0.12	- 0.08	+ 0.76
20-40	+ 8.0	- 30.6	-8.8	- 18.80	- 1.08	-0.94	- 2.11	- 0.19
40-60	+ 7.0	- 15.3	+6.0	- 36.05	+ 5.40	- 6.75	- 4.58	- 2.16
60-80	+ 4.4	+ 2.64	0.0	- 18.98	- 8.48	- 10.04	-12.47	- 0.43
80-100	+ 3.2	+ 3.1	+ 5.8	- 8.46	- 10.49	- 13.4	- 5.66	- 11.9
Total NO_3-N	+ 35.1	- 126.7	- 71.31	- 87.35	-15.49	-30.5	- 25.0	- 13.6

[A] The stages of growth at the time of harvest was 3 leaf (1 Dec), 6 leaf (8 Dec), tillering (20 Dec), stem elongation (31 Dec), anthesis (11 Jan), one week after anthesis (17 Jan), 2 weeks after anthesis (23 Jan), dough stage (31 Jan) and maturity (15 Feb).

4.4 Discussion

The experiments (Experiments 1 and 2) examined N responses by wheat with quite different N status. Measurement of plant NO_3-N concentration in Experiment 1 showed that the values were low enough to restrict plant growth. Papastylianou (1980) reported plant growth in pot experiments was restricted when the stem NO_3-N concentrations at tillering, jointing and anthesis fell below 5500, 1200 and 500 mg kg^{-1} respectively. Consequently, there was a large increase in grain yield and a significant increase in GPC following the addition of N. The use of a loamy soil with greater N availability in Experiments 2 and 3 improved growth and yield compared with Experiment 1 and the plant NO_3-N concentrations at tillering (7000-9000 mg kg^{-1}) were well above the critical levels. However, despite this, yield and GPC were again significantly increased by addition of N, although the yield response was not as great as obtained in Experiment 1. The timing and the initial placement of N in Experiment 2 had no effect on yield nor on the GPC response. Furthermore responses to the different

N treatments were not affected by restricting the supply of water during the post-anthesis period. The only N treatment in which no N was applied to the subsoil was N1. However when the fate of this N was followed during the pre-anthesis period in Experiment 3, it was found that some NO_3-N was leached into the subsoil (Fig 4.6). It was also observed that very little soil NO_3-N located below 60 cm was used prior to anthesis except with when all the N was applied in subsoil at sowing (N3). However following the depletion of soil NO_3-N in the surface layers, plants relied on this subsoil NO_3-N to sustain growth (Fig 4.2). When all the N was supplied to the subsoil at sowing (N3), the plant exploited this reserve without any detrimental effect on grain yield or GPC. When applied N was split between the topsoil and subsoil at sowing (N2), there was greater use of topsoil N prior to anthesis, but a greater reliance on the subsoil N reserves after anthesis when the topsoil concentration became very low (Fig.4.2). This pattern of uptake has been reported elsewhere under field conditions (*e.g.* Kuhlmann *et al.* 1989). The preferential use of topsoil N initially is probably associated with the greater root length densities in the topsoil layers. The results from Experiments 1 and 2 support the suggestion by Ladd (1990) that some subsoil mineral N make be taken up late in the season and contribute to GPC.

The other source of N used for grain filling is that remobilised from the non-grain parts of the plant. Under dryland conditions, when the availability of post-anthesis soil N is low, remobilisation is the major source of N for grain filling. Increasing applied N during the post-anthesis period substantially reduced the amount of N remobilised in the plant and its contribution to grain N yield. In Experiment 2, the effect of applying all the N to the topsoil at sowing (N1) was comparable either to applying the N to the topsoil and subsoil at sowing (N2) or to applying the N only to the subsoil at sowing (N3) or to delaying the application of some of the N until after anthesis (N4). This suggests that as long as the N is not leached from the root zone, application of N to the topsoil and subsoil has an equivalent effect.

An important factor that influences the ability of plants to exploit N from the subsoil is the root length density (Wiersum 1967). Root length density declines with

depth and often rooting in the subsoil is sparse. The ability of plants to take up N from the subsoil may be limited if the root length density is very low. However, the importance of this factor will depend on how transient is the increased availability of N. Localised, high concentrations of N in the soil can increase the proliferation and branching of wheat roots, which in turn can increase N uptake from the soil (Passioura and Wetselaar 1972). Immediately after applying N to the subsoil in Experiment 1, the recovery of N was low; 2 days after applying N only 12 mg N per pot was recovered in the roots and shoots, and 7 days after application 23 mg N was recovered per pot (calculated from Table 4.1). However, over the time to maturity there was a steady increase in the amount of N recovered so that by maturity the recovery was 120 mg per pot.

When N was added to the subsoil, root growth below 60 cm continued to increase up to maturity and paralleled the continued uptake in N from below this depth (Table 4.7), suggesting that the exploitation of subsoil N was associated with active root growth. However, the rate of NO_3-N uptake per g root dry matter (or per cm root) was also greater than the control treatment, so the initial increase in the root length density alone did not account for the increase in soil NO_3-N uptake. It appears that the initial uptake of NO_3-N from the soil was limited by the amount of root growth in the zone of N application, and although there was an increase in the rate of uptake per unit root growth, complete exploitation of the NO_3-N depended on the stimulation of root growth in the subsoil. The root length densities measured in the experiment were generally greater than those measured in the field, and the use of a non-draining pot ensured that NO_3-N was not leached from the pot. This resulted in a steady uptake of N by the wheat. For example, the total N content of roots in Experiment 3 increased up to 11 Jan (anthesis) and remained constant thereafter, whereas shoot total N content continued to increase. There was no evidence of N mobilised from roots. Increased shoot N must have been due to continued N uptake from soil, whereas in the field, uptake generally slows down after heading (Campbell *et al.* 1977). These results (Experiments 1, 2 and 3) probably represent an ideal situation of relatively high root length density and no

movement of NO_3-N beyond the roots. If the ability of roots to respond to N is limited by soil chemical or physical properties, or if the soil texture allows the NO_3-N to be leached beyond the root zone, the ability of the plant to exploit subsoil N may be poor. These results imply that growth and distribution of roots in the soil play major roles in the post-anthesis N economy of plants.

The increased root growth in response to N was also associated with greater water use. A number of studies in southern Australia have shown the existence of significant amounts of subsoil water under cereal crops at maturity, even though roots are present in the subsoil (Schultz 1971, Walter and Barley 1974). Moreover, the water use efficiency of the plants in Experiment 1 was improved by addition of N. Thus it appears that the presence of substantial reserves of N in the subsoil and its subsequent effect on root growth is not only beneficial to GPC but may lead to better exploitation of soil water reserves and improved water use efficiency.

4.5 Conclusion

From this study, it can be concluded that the ability of plant roots to recover N from the subsoil is high but it occursover a number of weeks. The results indicated that a transient increase in subsoil N increased root growth and N uptake per unit root length and thereby increased shoot N and GPC. Nitrogen placement increased the grain yield and GPC of wheat compared with the control (N0 treatment). Water use and water use efficiency were increased by N application. Further research is needed to examine the effect of split N application on GPC of wheat.

CHAPTER 5

THE EFFECT OF SPLIT NITROGEN APPLICATION ON GPC OF WHEAT UNDER DIFFERENT WATER REGIMES.

5.1 Introduction

The cereal crop has different needs for N at different stages of growth. It is important to use the N fertiliser efficiently by matching the N supply to the needs of the crop, both in terms of amount and timing of nutrient supply because N fertilisers are expensive. Losses of N through processes such as leaching, which can be environmentally hazardous, can be limited by the efficient uptake and utilisation of N by the crop (Mason *et al.* 1972). A large number of field experiments in many countries has examined the relation between the time of application of N fertilisers to wheat and the yield and protein response produced.

Late application of N fertiliser tends to increase GPC (Hunter and Stanford 1973, Hamid and Sarvar 1976, Strong 1982, Strong 1986, Recous *et al.* 1988, Smith *etal.* 1989a, Cooper and Blakeney 1990). When N is applied before anthesis (at boot) more N is assimilated into grain than when N is applied after anthesis (Finney *et al* . 1957). However, application at anthesis or later had the singular effect of increasing GPC. In contrast, application before anthesis produced a grain yield response, thereby reducing its effectiveness to increase GPC. Thus a fertilisation strategy in which the N application is split between sowing and anthesis would more likely result in grain of a higher protein concentration than the strategy in which the entire application is made at sowing.

Strong (1981) reported that increasing the application of N at sowing may fail to increase the supply of N to the grain because the fertiliser N may be lost before it can be assimilated; at least 6 weeks' growth is required before the crop can accumulate much of the N applied at sowing (Muirhead *et al.* 1985, Smith *et al.* 1989). More efficient use of fertiliser N may result when the application coincides with the period of rapid plant

uptake (Keeney 1982). High rates of N uptake have been reported when fertiliser application was delayed until anthesis (Spiertz and Ellen 1978, Smith *et al.* 1989a).

The balance between pre and post anthesis N uptake can be affected by the placement of N in the soil. Storrier (1962) found that uptake of N applied to the surface stopped after anthesis and little subsoil N was used, while post-anthesis uptake of N during a dry year was increased by deep placement (Strong and Copper 1980). Timing of N application is one way of increasing N for maximum yield. Elliott *et al.* (1985) reported that split N applications were desirable in South Australia where heavy winter rainfall caused temporary N deficiency during tillering if all N applied at sowing. It is possible to improve GPC of wheat by applying N fertiliser late at the boot stage (Scott 1981). In previous pot experiment Experiment 1, (Chapter 4) placing N at a depth of 60 cm two weeks after anthesis produced higher GPC compared with the control. The result from Experiment 2 (Chapter 4) suggested that, as long as the N was not leached from the root zone, topsoil N application at sowing and subsoil N application after anthesis had equivalent effects on GPC. However, it is clear that both depth and timing of N can also influence the yield and protein responses of wheat. The experiment reported here compared a treatment where the N was placed in the topsoil at sowing or applied in the topsoil at tillering and booting with a single post-anthesis N under different water regimes. The experiment tests the hypothesis that split N application has an equivalent effect as subsoil N application on GPC.

5.2 Materials and methods-Experiment 4

Wheat (cv. Molineux) was grown in sandy loam in pots 105 cm deep and 11 cm in diameter the base of pots were closed. The soil was a red-brown earth and had a pH (1:5 soil:water) of 5.8, contained 800 mg kg^{-1} total N, 24 mg kg^{-1} NO_3-N and 55 mg kg^{-1} $NaHCO_3$-extractable phosphorus (Colwell 1963). The experiment had a factorial design with 3 replications. The treatment factors (4 N levels, 3 harvests and 2 water regimes) were randomized in each replicate. The N treatments were: no N (N0), 150 mg N per pot as KNO_3 placed at the topsoil at sowing (N1), 75 mg N placement at the top at

tillering and 75 mg N in topsoil at booting (N2), 150 mg N per pot placed in the subsoil after anthesis (N3). The harvest stages were at tillering, anthesis and maturity. The two water treatments, introduced when the wheat was at anthesis were surface irrigation sufficient to keep water stress low in the plants (W1), and no surface watering, but subsoil irrigation at 60 cm depth (W2). The pots with the subsoil irrigation treatment (W2) received half the quantity of water that was needed for all the soil to reach field capacity. W2 simulated post-anthesis drought with topsoil drying. The management of the experiment was the same as for the Experiment 2 described in Chapter 4. Six wheat seeds were sown per pot on 1 May 1995 at a depth of 1.5 cm, and a basal fertilizer solution containing 50 mg phosphorus as $CaH_4(PO_4)_2.H_2O$, 10 mg copper as $CuSO_4.5H_2O$, 10 mg zinc as $ZnSO_4.7H_2O$ and 20 mg magnesium as $MgSO_4.7H_2O$ was added to the surface of the soil one week later. The N fertiliser treatments were also applied in solution. The pots were arranged in a greenhouse in three blocks. Three days after emergence, the plants were thinned to leave three plants per pot. Shadecloth was maintained around the experiment at a height of two-thirds that of the plants to prevent the excessive tillering and growth that commonly occurs with plants in spaced pots and to reduce edge effects. The mean daily minimum and maximum air temperatures in the greenhouse ranged from 20° C and 25° C respectively for May to 18 and 22 for October. For all pots prior to anthesis, and with the well watered treatment thereafter, a measured amount water was added to the surface of the soil two or three times weekly to maintain the soil water near field capacity. The pots were weighed every week to allow water use to be calculated. The measurements of the soil and plants were the same as these described for the Experiment 2 in Chapter 4.

5.3 Results

5.3.1 Dry matter production and grain yield

There was no significant difference between N treatments in root or shoot dry matter production during the experiment (Table 5.1), but at maturity there was a significant reduction in shoot and root dry weight with restricted watering (Table 5.2).

Table 5. 1. Dry weight and concentration of nitrate and total nitrogen in the plant

Nitrogen treatment	Dry weight (g per pot) Shoot	Root	Nitrogen (%) Shoot	Root	Nitrate-N (mg kg^{-1}) Shoot	Total N (mg per pot) Shoot+Root
			Tillering			
N0[A]	0.96	0.99	4.15	1.73	10542	65.6
N1	1.21	0.97	4.01	1.55	12880	78.9
N2	1.30	0.88	3.83	1.57	12037	79.2
N3	1.31	0.89	3.81	1.50	11500	78.0
l.s.d.[B] (P=0.05)	n.s.	n.s.	n.s.	n.s.	866	n.s.
			Anthesis			
N0	12.44	2.89	2.11	1.45	5788	376
N1	14.17	3.19	2.13	1.60	6632	441
N2	11.13	3.03	2.31	1.60	8318	385
N3	12.16	2.86	2.11	1.49	6402	365
l.s.d [B]	n.s.	n.s.	n.s.	n.s.	954.5	n.s.
l.s.d.[C] (P=0.05)	n.s.	n.s.	0.22	n.s.	n.s.	n.s.
			Maturity[D]			
N0	29.26	3.96	1.49	1.17	2960	562
N1	32.91	4.36	1.61	1.18	3390	688
N2	32.52	4.06	1.67	1.25	3800	716
N3	29.53	3.69	1.69	1.28	3890	651
l.s.d.[B] (P=0.05)	n.s.	n.s.	0.09	0.08	n.s.	70.1

[A] N0 = no N, N1= 150 mg N topsoil at sowing, N2= 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3= 150 mg N subsoil (60 cm) after anthesis

[B] l.s.d. applies to comparison between fertiliser treatments.

[C] l.s.d. applies to interaction between nitrogen and harvest.

[D] The value are averaged over water treatments.

Table 5. 2. Dry weight and concentration of nitrate and total nitrogen in the plant at maturity

Water treatment	Dry weight (g per pot)		Nitrogen (%)		Nitrate-N (mg kg^{-1})	Total N (mg per pot)
	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
Surface irrigation	33.26	4.52	1.55	1.15	2870	660
Subsoil irrigation	28.84	3.51	1.69	1.29	4150	649
l.s.d. (P=0.05)	2.77	0.59	0.06	0.058	619.3	n.s.

Applying the N at sowing or split application at tillering and booting improved the grain yield by about 10% compared with the control, but the effect was not significant at $P \leq 0.05$ (Table 5.3). Reduced watering after anthesis restricted the growth and the yield of the grain (Table 5.4).

Table 5. 3. Effect of the nitrogen treatments on the dry matter yield, concentration of nitrogen and grain protein at maturity.

Nitrogen treatment	Dry matter yield (g per pot)		Nitrogen (%)	Protein (%)	Apparent N recovery(%)
	Grain	Straw	Straw	Grain	Shoot
N0[A]	11.98	17.23	0.86	13.7	-
N1	13.27	19.64	1.02	14.3	64
N2	13.32	19.20	1.06	14.6	73
N3	11.45	18.08	1.09	15.0	42
l.s.d. (P=0.05)	n.s.	n.s.	0.08	0.88	n.s.

[A] N0 = no N, N1= 150 mg N topsoil at sowing, N2= 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3= 150 mg N subsoil (60 cm) after anthesis.

Table 5. 4. Effect of the water treatments on the dry matter yield, concentration of nitrogen and grain protein at maturity.

Water treatment	Dry matter yield (g per pot)		Nitrogen (%)		Protein (%)
	Grain	Straw	Grain	Straw	Grain
Surface irrigation	13.65	19.59	2.42	0.94	13.8
Subsoil irrigation	11.36	17.49	2.63	1.07	15.0
l.s.d. (P=0.05)	1.35	1.75	0.11	0.05	0.62

5.3.2 Nitrate concentration in the shoot

The plants did not show any N deficiency during the growing season because the N status of the soil was high. The NO_3-N concentration in the tops of the plants decreased during the season (Table 5.1). At the tillering stage, the concentration of NO_3-N in the plant that received 150 mg N in topsoil at sowing (N1) was significantly greater than with the N0 and N3 treatments where no N was applied. At anthesis, the concentration of NO_3-N in the plant treated with 75 mg N at tillering and 75 mg N at booting (N2) was higher than that in another treatments. By maturity, these differences had disappeared. At maturity shoot NO_3-N concentration was higher with subsoil irrigation than with surface irrigation (Table 5.2).

5.3.3 Total nitrogen concentration in the plant

Total N concentration in the shoots and roots, but particularly in the shoots was high at tillering but the concentration fell as the plants aged (Table 5.1). At anthesis, plants that received 75 mg N at the boot stage (N2) had a significantly higher N concentration than the other treatments, even that which received 150 mg N at sowing. Shoot total N at maturity significantly was increased by adding N. At maturity, the concentration of total N in the plants grown with subsoil irrigation was significantly higher than in plants grown under well watered conditions (Table 5.2).

Total N concentration in roots decreased during the growing season (Table 5.1). At maturity, the concentration of total N in the roots treated with 150 mg per pot placed in the subsoil after anthesis (N3) was high compared with the control (Table 5.1).

At the final harvest, there was a significant difference in plant N content between the N treatments. The content of the N in the plants that received 75 mg N in the topsoil at tillering and 75 mg N in topsoil at booting (N2) was higher than with the other treatments but it was only significantly greater than the control. The difference in concentration of total N in the plants under surface and subsoil irrigation is related to the reduced growth because total N uptake was not significantly different between the two water treatments (Table 5.2).

5.3.4 Grain protein concentration

Split N application at tillering and booting (N2) significantly increased the GPC of wheat compared with the control, and the effect was comparable to subsoil N application after anthesis (N3) (Table 5.3). The GPC of the plant was significantly higher with subsoil irrigation compared with surface irrigation (Table 5.4).

5.3.5 Recovery of nitrogen and remobilisation to the grain

The apparent N recovery by the plants was slightly improved by applying 75 mg N at tillering and 75 mg N at booting but the difference was not significant (at $P \leq 0.05$) compared with other N treatment (Table 5.3). The N treatments had no significant effect on the amount of N remobilised from the straw (Table 5.5). The apparent contribution of remobilised N to grain was highest in the control plant and the plants that received 150 mg N in the topsoil at sowing (N1) but the differences between treatments were not significant at $P \leq 0.05$ (Table 5.5). This could be due to the variability of the data.

Table 5. 5. Post-anthesis nitrogen balance in the plant (Experiment 4)

Nitrogen (mg per pot)	Increase in N (Root + Shoot) (mg per pot)	Increase in N (Grain) (mg per pot)	Nitrogen remobilised (mg per pot)	N remobilised N in grain (%)
		Surface irrigation (W1)		
N0[A]	198	294.7	96	33
N1	261	357.3	96	25
N2	307	337.7	31	5
N3	304	329.7	25	8
		Subsoil irrigation (W2)		
N0	174	278.0	104	38
N1	233	299.7	67	24
N2	355	342.7	-12	-6
N3	267	272.7	6	0.3
l.s.d. (p=0.05)[B]	148.8	65.63	n.s.	n.s.

[A] N0 = no N, N1= 150 mg N topsoil at sowing, N2 = 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3 = 150 mg N subsoil after anthesis.

[B] l.s.d. applies to interaction between nitrogen and water treatment.

5.3.6 Root growth

The length and distribution of roots in the soil at the different stages of plant growth are shown in Table 5.6. Until tillering, roots grew slowly and there were few roots below 60 cm. Root length density had increased by anthesis, especially in the surface layers. Root length in the subsoil (60-80 cm) had further increased by maturity (Table 5.6).

Root dry weight increased until maturity especially in the plants kept well watered (Table 5.7). At maturity, the root dry weight of the plants that received 150 mg N in the topsoil at sowing (N1) or 75 mg topsoil at tillering and 75 mg topsoil at booting (N2) increased in the subsoil under subsoil irrigation regime compared with surface irrigation.

Table 5. 6 Root length density (cm cm^{-3}) in the soil profile during the season.

Depth (cm)	Nitrogen			
	N0[A]	N1	N2	N3
	Tillering			
0-10	1.66	2.42	1.48	0.99
10-20	1.11	0.61	0.67	0.45
20-40	0.75	0.37	0.69	0.85
40-60	0.43	0.34	0.53	0.86
60-80	0.13	0.07	0.11	0.19
80-100	0.00	0.00	0.00	0.00
	Anthesis			
0-10	3.93	3.64	3.16	3.45
10-20	2.70	2.70	2.50	2.14
20-40	2.12	2.68	1.86	2.42
40-60	1.89	1.98	1.62	2.07
60-80	3.40	2.99	2.08	2.94
80-100	1.28	1.56	1.41	1.74
l.s.d.(P=0.05)	1.32			
	Maturity			
	Surface irrigation (W1)			
0-10	5.37	7.10	6.39	5.53
10-20	4.32	6.19	3.79	3.61
20-40	3.91	2.63	3.96	4.08
40-60	3.48	2.99	3.08	2.79
60-80	4.52	3.64	4.41	4.25
80-100	4.98	3.17	3.22	2.37
	Maturity			
	Subsoil irrigation (W2)			
0-10	2.60	3.80	2.46	2.33
10-20	2.12	2.15	2.35	2.98
20-40	1.36	2.63	2.51	1.56
40-60	1.93	2.95	2.27	1.94
60-80	3.56	4.79	4.71	5.01
80-100	3.15	3.11	2.96	2.17
l.s.d.(P=0.05)	1.70			

[A] N0 = no N, N1= 150 mg N topsoil at sowing, N2 = 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3 = 150 mg N subsoil (60 cm) after anthesis.

Table 5.7. Root dry weight (g) in the soil profile during the season.

Depth (cm)	Nitrogen treatment N0[A]	N1	N2	N3
		Tillering		
0-10	0.27	0.36	0.26	0.28
10-20	0.23	0.17	0.16	0.06
20-40	0.24	0.19	0.23	0.19
40-60	0.17	0.21	0.20	0.25
60-80	0.08	0.05	0.03	0.10
80-100	0.00	0.00	0.00	0.00
		Anthesis		
0-10	0.64	0.50	0.42	0.48
10-20	0.25	0.30	0.25	0.24
20-40	0.42	0.82	0.45	0.48
40-60	0.41	0.48	0.46	0.42
60-80	0.81	0.62	1.03	0.71
80-100	0.36	0.47	0.42	0.53
l.s.d.(P=0.05)		0.34		
		Maturity		
		Surface irrigation (W1)		
0-10	0.63	0.95	0.92	0.77
10-20	0.45	0.63	0.42	0.54
20-40	0.76	0.57	0.82	0.82
40-60	0.56	0.64	0.61	0.71
60-80	1.09	0.78	0.80	0.98
80-100	1.37	0.81	0.85	0.67
		Maturity		
		Subsoil irrigation (W2)		
0-10	0.45	0.74	0.49	0.47
10-20	0.28	0.24	0.29	0.23
20-40	0.31	0.44	0.53	0.36
40-60	0.43	0.70	0.53	0.44
60-80	0.79	1.41	1.14	0.84
80-100	0.80	0.88	0.70	0.53
l.s.d.(P= 0.05)		0.31		

[A] N0 = no N, N1= 150 mg N topsoil at sowing, N2 = 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3= 150 mg N subsoil (60 cm) after anthesis.

5.3.7 Soil water and water use

As expected the water content of soil varied with depth during the season (Table 5.8).

Table 5. 8. Gravimetric water content (kg kg^{-1}) in the soil profile.

	Nitrogen treatment			
Depth (cm)	N0	N1	N2	N3
		Tillering		
0-10	0.134	0.124	0.113	0.108
10-20	0.132	0.128	0.116	0.112
20-40	0.122	0.118	0.100	0.097
40-60	0.109	0.104	0.093	0.083
60-80	0.083	0.065	0.067	0.073
80-100	0.040	0.022	0.025	0.023
		Anthesis		
0-10	0.088	0.084	0.088	0.086
10-20	0.074	0.084	0.087	0.088
20-40	0.073	0.069	0.076	0.081
40-60	0.054	0.047	0.064	0.067
60-80	0.042	0.041	0.058	0.051
80-100	0.032	0.037	0.056	0.042
l.s.d.(P=0.05)		0.015		
		Maturity		
		Surface irrigation (W1)		
0-10	0.013	0.028	0.022	0.026
10-20	0.025	0.026	0.028	0.031
20-40	0.027	0.026	0.029	0.036
40-60	0.027	0.025	0.031	0.035
60-80	0.028	0.027	0.029	0.027
80-100	0.019	0.026	0.029	0.026
		Maturity		
		Subsoil irrigation (W2)		
0-10	0.020	0.016	0.019	0.020
10-20	0.028	0.026	0.026	0.025
20-40	0.034	0.029	0.028	0.033
40-60	0.037	0.028	0.028	0.035
60-80	0.045	0.028	0.028	0.036
80-100	0.040	0.030	0.029	0.036
l.s.d.(P=0.05)		0.012		

There was a significant differences in gravimetric water content between sections of the soil profile. The water content decreased with depth at tillering and anthesis stage. As expected at the last harvest (Table 5.8) the topsoil water content of the pot was low compared with subsoil.

Table 5.9 shows the effect of N and water treatments on the water content of the soil at the end of the experiment and water use over the whole growing season. As expected, the total amount of water used by the wheat was greater under the surface irrigation than subsoil irrigation. There was an interaction between N and water treatment with respect to water use efficiency. This indicated that the effect of N on water use efficiency differed with water treatments.

Table 5. 9. Effect of the water and nitrogen treatments on the soil water content at the end of the experiment, amount of water used and efficiency of water use over the whole growing season.

Nitrogen treatment	Soil water content (mL per pot)	Total water use (L per pot)	Water use efficiency (g L^{-1})[A]
	Surface irrigation (W1)		
N0[B]	287	11.05	1.19
N1	315	11.10	1.33
N2	344	10.98	1.23
N3	369	11.04	1.19
	Subsoil irrigation (W2)		
N0	429	8.24	1.32
N1	326	9.91	1.18
N2	325	9.74	1.35
N3	392	8.49	1.13
l.s.d.[C] (P=0.05)	ns	ns	0.18

[A] Water use efficiency was calculated for the weight of grain.

[B] N0 = no N, N1= 150 mg N topsoil at sowing, N2= 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3= 150 mg N subsoil (60 cm) after anthesis

[C] l.s.d. applies to interaction between nitrogen and water treatment.

After anthesis plants under the surface irrigation regimes with 150 mg N topsoil at sowing (N1) used more water than all other treatments. Under subsoil irrigation the water use efficiency by plants treated with 75 mg N at tillering and 75 mg N at booting (N2) was higher than that by plants treated with N1 and N3 (Table 5.10).

Table 5. 10. Effect of the water and nitrogen treatments on the soil water content at the end of the experiment, amount of water used and efficiency of water use after anthesis.

Nitrogen treatment	Soil water content (ml per pot)	Total water use (L per pot)	Water use efficiency (g L^{-1})[A]
	Surface irrigation (W1)		
N0[B]	287	5.81	2.27
N1	315	6.26	2.36
N2	344	5.67	2.37
N3	369	5.77	2.35
	Subsoil irrigation (W2)		
N0	429	4.01	2.74
N1	326	4.50	2.59
N2	325	4.48	2.93
N3	392	3.82	2.52
l.s.d.[C] (p=0.05)	ns	0.50	0.23

[A] Water use efficiency was calculated for the weight of grain.

[B] N0 = no N, N1= 150 mg N topsoil at sowing, N2= 75 mg N topsoil at tillering and 75 mg N topsoil at booting, N3= 150 mg N subsoil (60 cm) after anthesis

[C] l.s.d. applies to water treatment.

5.3.8 Soil nitrate

The concentration of NO_3-N and its distribution in the soil changed during the growing season. At tillering, after the addition of N, there was a considerable amount of NO_3-N in the topsoil of the pot that received 150 mg N in the topsoil at sowing (N1) or 75 mg in the topsoil at tillering and 75 mg at booting (N2). The concentration of NO_3-N in the bottom of the pot was high with all of the N treatments (even with the N0 treatment) at tillering due to leaching. (Fig 5.1).

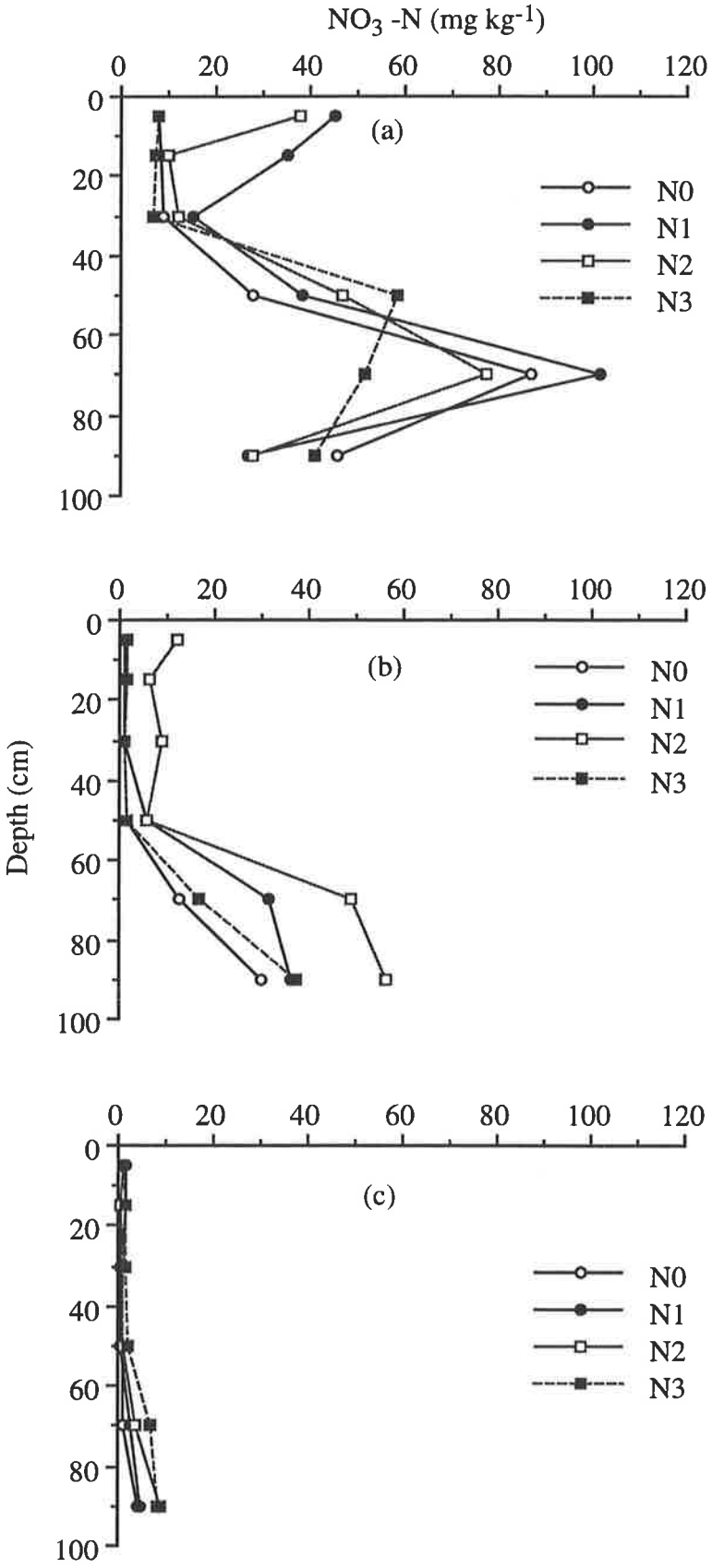

Fig 5.1. Concentration of NO_3 -N in the soil profile (a) tillering, (b) anthesis, (c) maturity The initial concentration of NO_3-N in the soil was 24 mg kg^{-1} (Experiment 4).

At anthesis (soil sample taken before the addition of N one week after anthesis) the concentration of NO_3-N had decreased in the topsoil but there was still a considerable amount of NO_3-N in the subsoil especially in the pots that received 75 mg N at tillering and 75 mg N at booting (N2). Irrespective of N treatment the concentration of NO_3-N had decreased in the subsoil by maturity due to uptake by the plants.

5.4 Discussion

The experiment showed that NO_3-N concentration was higher in the plants under subsoil irrigation than surface irrigation (Table 5.1). There is some evidence to indicate that NO_3-N accumulation in plants is increased by water stress (Gilbert *et al.* 1946, Hanway and Englehorn 1958, MacKenzie *et al.* 1963). This accumulation of NO_3-N occurs because the production of dry matter is influenced earlier than the uptake of NO_3-N under water stress (Wright and Davidson 1964, Darwinkel 1975). Papastylianou (1980) reported that plant growth in pot experiments was restricted if stem NO_3-N was less than 5500, 1200 and 500 mg kg^{-1} at tillering, jointing and anthesis respectively. The concentrations of NO_3-N in the shoots were much higher than the values reported by Papastylianou, indicating that plants were well supplied with N. The concentrations of NO_3-N in the plants were higher than those in Experiment 2, reported in Chapter 4.

Split N application at tillering and booting or subsoil N application after anthesis significantly increased the GPC compared with the control. There was an accumulation of NO_3-N in the subsoil at anthesis with all of the N treatments, including the N0 treatment. The high accumulation of NO_3-N in the subsoil at anthesis when N was split between tillering and booting (N2) was one possible reason for the high GPC observed with this treatment. The results from this experiment support the results presented in Chapter 4 that subsoil mineral N may be taken up late in the season and contribute to GPC. Subsoil N application in this experiment increased only the GPC (Table 5.3) while in Experiment 1 (Table 4.2 Chapter 4) subsoil N application not only increased GPC but also increased grain yield. The low level of N in the soil in that experiment was one

possible reason because when the N status of soil is high the application of N will effect the GPC and when the soil N is low, N application can effect both GPC and grain yield. Applying all the N to the topsoil at sowing (N1) improved grain yield and GPC but the effect was not significant compared with the control. The effect of split N application (N2) was comparable to subsoil N application after anthesis (N3) suggesting that as long as the N is not leached from the root zone it has an equivalent effect as subsoil N application.

This result is in agreement with the result of Smika and Grabouski (1976) who reported that in dryland farming, enhanced GPC and yield of winter wheat were associated with NO_3-N in the deeper portions of the rooting zone after water had been depleted from upper zone prior to the grain filling stage. Smika and Greb (1973) reported that, when high NO_3-N concentrations were found in the soil below the 60 cm depth, the GPC was high.

The subsoil irrigation decreased the grain yield but increased the GPC compared with surface irrigation (Table 5.4). Campbell *et al.* (1981) reported that the effect of water stress on GPC was mainly through its influence on grain yield (dilution effect). The GPC increased with drought (Salter and Goode 1967, Brooks *et al.* 1982). Results from this experiment also demonstrated the effects of post anthesis water stress on grain N concentration. GPC increased with water stress, but the grain N yield decreased. In other words, despite less N being transported to the grain, water stress increased the proportion of N in the grain. Nicolas *et al.* (1985) reported GPC was significantly higher under drought than under well-watered conditions. However, the grain N yield per ear and protein content per grain were lower under drought, indicating that the higher GPC was due to their smaller size. Drought also reduces the sink size of the grains (Nicolas *et al.* 1985). As well, high protein cultivars of wheat were found to have a lower grain weights than a low protein cultivars (Donovan *et al.* 1977). Jenner *et al.* (1991) suggested the response in the wheat grain to developing water stress is similar to the response to elevated temperature. Perry and Hillman (1991) reported that when grain yield is limited by factors other than N (*e.g.* water) or at high rates of N, the yield

response is low or sometimes negative while GPC continues to increase. Negative correlations between GPC and overall yield are often reported (Halloran 1981, Loffler *et al.* 1985, Cox *et al.* 1985, Stoddard and Marshall 1990, Jenner *et al.* 1991).

Root length was measured on three occasions and the results (Table 5.6) show an increase in measured root length from anthesis to maturity especially in the subsoil. Generally the root length density in this experiment was lower than that in the Experiment (Table 4.12 Chapter 4) especially in the topsoil at tillering. It was one possible reason for greater NO_3-N leaching and accumulation of NO_3-N in the subsoil at anthesis (Fig 5.1) compared with Experiment 2 (Fig 4.2. Chapter 4). Walter and Barley (1974) found that in field experiment in South Australia root length density at a depth of 100 cm is 1 cm cm^{-3}, which is much less than in our experiment. This may explain why large amounts of soil water remain unexploited at crop maturity (Schultz 1971). It is of interest to consider how the subsoil N affected root distribution in the soil and water use by the plants because water supply is an important factor influencing the yield of cereal crops in southern Australia. Walter and Barley (1974) reported that soil within the rooting zone may contain appreciable amounts of water at the end of the season. Greater use of this could be induced by fertiliser placement to grow more extensive root systems as confirmed by the experiment reported here. Passioura and Wetselaar (1972) also showed that a higher amount of N at a particular depth increased the proliferation and branching of wheat roots. A greater N uptake by roots would occur if the roots encounter a N rich zone. The result of this experiment showed some evidence of increased root dry weight in the subsoil of the pot that received N under subsoil irrigation regime. The effect of the two surface N application (N1 and N2) on root dry weight was more than that with subsoil N application (N3) in this experiment (Table 5.7). The plant dry weight and grain yield was slightly (but not significantly) greater in these treatments compared with N3. It may be that N1 and N2 treatment stimulated growth slightly more than N3, which promoted better root growth.

The soil water data showed that water use by plants that received all of the N at sowing was high compared with the other N treatments but the difference was not

significant (Table 5.9). Post anthesis water use showed that subsoil irrigation increased water use efficiency in the plant that received N at tillering and booting (Table 5.10). The water content of the soil is very important to N uptake by wheat because if the topsoil or subsoil contains enough mineral N but water is not available, the plant cannot take up the N. Low soil water in the soil root zone may change the availability of NO_3-N in the soil profile.

The experiment showed that NO_3-N was available in the soil profile early in the season and was gradually taken up by the plant, and at anthesis there was considerable amount of NO_3-N in the subsoil. Slow development of the plant root system up to tillering (Table 5.6) probably contributed to the low uptake of NO_3-N from subsoil. At anthesis the concentration of NO_3-N in the subsoil decreased because the plant root length had increased below 80 cm and NO_3-N was taken up by the plant roots. The results confirm investigations of Kuhlmann *et al.* (1989), who found that N uptake during the vegetative growth of winter wheat occurred mainly from the upper soil layers, whereas N uptake after anthesis was restricted to the subsoil.

5.5 Conclusion

From this study it can be concluded that the split N application significantly increased the GPC compared with the control (Table 5.3). The effect of split N application on GPC was comparable to subsoil N application after anthesis. The N fertiliser to increase the GPC must be balanced with the amount of soil water available. Subsoil irrigation decreased the grain yield but increased the GPC. Roots in the subsoil are certainly of potential value in feeding the plant. Their contribution to plant nutrition will further depend on the fertility of the subsoil layers, the water content of these layers and the amount of roots that have developed in these regions. The amount of N taken up by the roots as a function of soil depth and other factors which may affect N uptake from the subsoil can affect the GPC of wheat. Further research to explain the relationship between root length density and NO_3-N utilisation is necessary.

CHAPTER 6

SHORT TERM N UPTAKE BY WHEAT AT DIFFERENT ROOTING DENSITIES.

6.1. Introduction

As mentioned in Chapter 2 (Section 2.3.3.1), the predominantly winter rainfall in the cereal growing areas of southern Australia can leach nitrogen (N) into the subsoil. This N may be utilized late in the season and contribute to the GPC of wheat (Ladd 1990). Results of Experiment 1 (Chapter 4) suggested that short-term uptake of subsoil NO_3-N after anthesis is mainly dependent on total root length although NO_3-N uptake per unit root length may also increase. Data of Wiersum (1967) suggest that the potential for uptake of N by roots in the subsoil is high. However, the root length density in the subsoil of wheat crops in southern Australia is often low (Schultz 1971) and it may not be sufficient to utilise subsoil N effectively, or it may limit the ability of the plant to take advantage of transient increases of available N from mineralisation.

Growing cultivars selected for high N uptake-capacity of the shoots combined with high root length densities in the subsoil may improve the utilisation of a high supply of N and thus decrease the risk of NO_3-N leaching from the soil. Much of the work on NO_3-N uptake shows that plant growth rate is a major factor determining uptake of N from the soil. For example, Kuhlmann and Barraclough (1987) found that uptake of N per unit root decreased as the plants aged due to reduction of the relative growth rate and decreased in shoot demand. A similar result was found in Experiment 3 (Chapter 4). Changes in plant demand for nutrients are usually linked with changes in internal concentration and often result from alteration in the supply of nutrients to the plant (Burns 1980). The relative uptake rate of nutrients is equal to the relative growth rate of the plant in situations where plant nutrient composition is constant. The potential uptake rate of a nutrient is set by its concentration in the soil solution and the amount of root present.

After anthesis, the rate of senescence increases, and the growth rate and photosynthesis rates of the crop decline. Therefore, the ability of a crop to use N from the subsoil may be limited by two factors: the low root length density and the low growth rate. To examine the interaction between root length density, NO_3-N supply and photoassimilate production two greenhouse experiments were conducted in which the short-term (48 hour) N uptake by the wheat was measured. Different plant densities were used with different levels of plant demand imposed by the use of different shading treatments. Short-term studies were used to examine the direct effects of N supply and photosynthesis without causing substantial changes in plant dry matter. Studies of longer duration would have resulted in the N and shading treatments affecting growth. Seedlings were used as a model system because root length densities were smaller and more manageable and it was easier to impose shading treatments. One of the problems of altering plant number to produce different root lengths was that we immediately altered shoot growth (altered demand for N). Shading and leaf area reduction were attempts to reduce demand without altering root length too much.

6.2 Materials and methods

The experiments used PVC pots 23 cm deep and 11 cm in diameter (Plate 6.1). The bases of pots were closed. The soil (2.4 kg) was packed to a bulk density of 1.3 g cm^{-3} to a depth of 20 cm in the pots.

6.2.1 Experiment 5

The experiment had a randomised complete block design with 3 replications (blocks). The treatment factors (4 N levels, 2 rates of sowing and 2 shade treatments) were randomized in each block. The N treatments, which were applied at tillering (4 weeks after sowing) were: 0, 50, 100, and 150 mg N per pot as potassium nitrate. The two shade treatments (non-shaded and shaded) were introduced when the N was applied. Shaded plants were covered with a rectangular frame (40x40x100 cm) covered by shade cloth which decreased the light to about 5% of that received by the non-shaded plants.

Plate 6.1 The pots used in the greenhouse to examine the short term N uptake by wheat at different rooting densities under different shade treatments in Experiments 5 and 6.

Wheat (cv. Molineux) was grown in a sandy soil (sand 90%, silt 6%, clay 4%) in pots as mentioned above. Polyethylene beads were added to the surface of each pot as a mulch to minimise evaporation from the surface of the soil. The soil was the same as described in Chapter 3 and contained 280 mg kg^{-1} total N and 2 mg kg^{-1} NO_3-N.

Three or nine wheat seeds, were sown per pot on 1 Dec 1995 at a depth of 1.5 cm, and a basal fertilizer solution containing 10 mg N as KNO_3, 25 mg phosphorus as $CaH_4(PO_4)_2.H_2O$, 5 mg copper as $CuSO_4.5H_2O$, 5 mg zinc as $ZnSO_4.7H_2O$ and 10 mg magnesium as $MgSO_4$ $.7H_2O$ was added to the surface of the soil one week later. The N fertiliser treatments were also applied in solution. The mean daily minimum and maximum air temperatures in the greenhouse ranged from 20 to 25° C. The mean daily light intensity in the greenhouse ranged from 135 to 513 μE m^{-2} s^{-1} in the non-shaded and from 5 to 31 μE m^{-2} s^{-1} for the shaded treatment. Measured amounts of water were added to the surface of the soil three times weekly to maintain the soil water near field capacity: the pots were weighed, and total water use calculated.

Plants were harvested at tillering (4 weeks after sowing) immediately before adding N treatments, and 2 days later. The plants were cut at soil level, dried at 80° C, weighed and ground. Total N was determined in a sulphuric acid digest of the plant material by a micro Kjeldahl method (Bremner 1965), and the NO_3-N concentration in the plant shoots was assessed by the salicylic acid method (Cataldo *et al.* 1975). A subsample of soil was taken from each pot to determine water content. Roots were separated from the remaining soil samples by flotation and root lengths were measured by the modified line intercept method of Tennant (1975).

6.2.2 Experiment 6

The materials and methods in this experiment were the same as Experiment 5. The plant numbers and N treatments were also the same, but there were 3 shade treatments (shaded, non shaded and leaves half covered). The date of sowing was 7 February 1996. In this experiment the illuminated leaf area (LA) was reduced non-

destructively by covering the half of each leaf (closest to the stem) with aluminium foil at the time that the N treatments were imposed. The LA was reduced to the same level as the low plant density without greatly altering the root length. The mean daily light intensity in the greenhouse ranged from 279 to 404 μE m^{-2} s^{-1} in the non-shaded and from 22 to 28 μE m^{-2} s^{-1} with the shaded treatment. Plants were harvested at tillering (4 weeks after sowing) immediately before adding N treatments, and 2 days later.

6.3. Results

6.3.1 Experiment 5

6.3.1.1 Dry matter yield

Both total shoot and root dry weight per pot were higher at the higher plant density (Table 6.1).

Table 6.1. Effect of plant density on dry weight, concentration of nitrate and total nitrogen in the plant at two harvests (Experiment 5).

Plant density	Dry weight (g per pot)		Nitrogen (%)		Nitrate (mg kg^{-1})	Total N (mg per pot)
Plants per pot	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
			Before nitrogen and shade treatment (Harvest 1)			
3	0.36	0.47	2.52	1.00	1169	14.1
9	0.56	1.10	2.33	0.82	1246	22.7
			After nitrogen and shade treatment (Harvest 2)[A]			
3	0.37	0.55	3.18	1.25	2948	19.4
9	0.57	0.86	2.77	1.00	2522	25.8
l.s.d. (P=0.05)	0.02	0.10	0.09	0.18	108	1.3

[A] The data presented for harvest 2 are means for N, shaded and non shaded treatments.

Shoot and root dry weights were decreased by shading but were not significantly affected by N treatments (Table 6.2). The interaction between shading and N was significant with respect to root dry weight: non-shaded plants that did not receive N had significantly higher root weights than the shaded plants (Table 6.2).

Table 6.2. Dry weight, concentration of nitrate and total nitrogen in the plant under different nitrogen and shade treatments at harvest 2. Values have been averaged over low and high plant densities. (Experiment 5)

Nitrogen treatment	Dry weight (g per pot)		Nitrogen (%)		Nitrate (mg kg^{-1})	Total N (mg per pot)
mg per pot	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
			Non-shaded			
0	0.50	1.00	2.17	0.80	1064	19.3
50	0.51	0.70	3.31	1.25	3239	26.5
100	0.50	0.67	3.45	1.38	3284	27.4
150	0.51	0.74	3.57	1.27	3354	28.6
			Shaded			
0	0.42	0.62	2.43	0.94	777	15.9
50	0.45	0.61	2.80	1.29	3009	20.0
100	0.45	0.67	3.06	1.13	3569	21.6
150	0.44	0.61	3.01	1.13	3585	21.5
l.s.d. (P=0.05)	0.04	0.21	0.17	0.35	216	2.5

6.3.1.2 Nitrate concentration in the shoot

Statistical analysis showed that there was interaction between N and shade treatment with respect to shoot NO_3-N concentration. With the non-shaded treatment, the NO_3-N concentration in the tops of the plants treated with 0 and 50 mg N per pot was more than in the plants under shaded treatment while in the plants that received 100 and 150 mg N per pot under the shaded treatment the concentration of NO_3-N was higher than that in the plant under non-shaded treatment (Table 6.2). The effect of shade on the concentration of NO_3-N in shoots was more pronounced at higher levels of N (100 and 150 mg N per pot). This may be attributed to the effect of shade which does not allow NO_3-N to change to organic form of N.

6.3.1.3 Total nitrogen concentration in the plant

Two days after adding N, root and shoot N concentration were significantly increased (Table 6.1). Total N concentration in the shoots increased with increasing N

application (Table 6.2). Shading reduced the N concentration in the shoot at all N rates (except no N), but only at 100 and 150 mg N in the roots. There was an interaction between shade and N treatment with respect to the shoot total N concentration. Shading decreased the total N content of all plants and the total N concentration in plants that were treated with 50, 100 or 150 mg N compared with non-shaded plants (Table 6.2).

6.3.1.4 Root activity

The two plant densities resulted in different root length densities. There was a reduction in root length between harvest 1 and 2, except at 9 plants per pot (Table 6.3). Root activity was estimated from the rate of N uptake during the 2 days after N application and the mean root length or root dry weight. The uptake rate by roots at the 3 plants were greater than that of the roots at the 9 plants, indicating considerable compensation between root length and rate of uptake. There was a substantial increase in the N uptake by the 3 plants under non-shaded treatment. In the non-shaded treatment the N uptake per gram or per cm of root in the pot with low or high plant density increased a little by increasing N application, while with the shaded treatment N uptake at the 9 plants remained steady (Fig 6.1, 6. 2).

Table 6.3. Gravimetric water content, root length density and total root length in the soil with different plant density (Experiment 5).

Plant density (plants per pot)	Water content (kg kg^{-1})	Root length density (cm cm^{-3})	Total root length (m)
	Before nitrogen and light treatment (Harvest 1)		
3	0.038	3.70	70.5
9	0.040	6.53	124.0
	After nitrogen and light treatment (Harvest 2)		
3	0.044	3.29	62.5
9	0.039	5.11	97.2
l.s.d.(P=0.05)	0.003	0.38	7.23

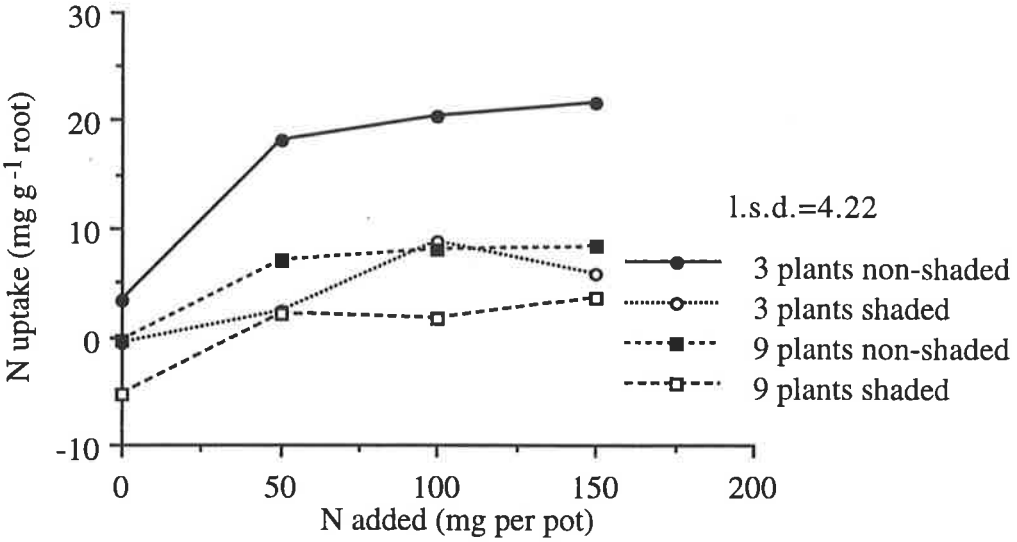

Fig 6.1. Short term (48 hour) N uptake by wheat at different plant densities under different shade treatments (Experiment 5)

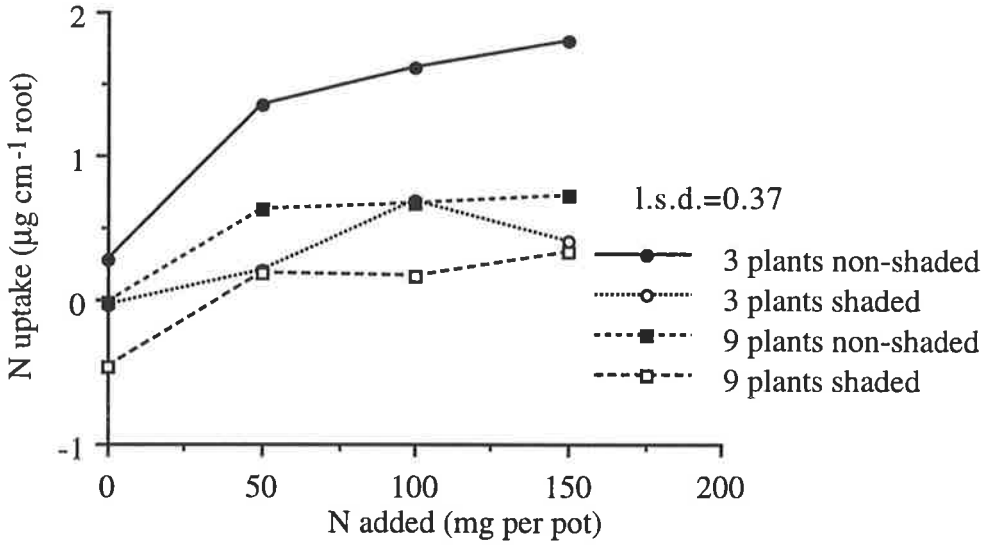

Fig 6.2. Short term (48 hour) N uptake by wheat at different plant densities under different shade treatments (Experiment 5)

6.3.1.5 Nitrogen uptake

Total uptake of N per pot, calculated as the difference in plant total N content between the first and second harvests, was substantially smaller at 9 than at 3 plants density (Table 6.1). The rate of N uptake was calculated by dividing the total N uptake by the mean root dry weight or mean root length between the first and second harvest. The rate of N uptake per unit root dry weight or root length was greater at 3 plants (Table 6.4).

Table 6. 4 Mean root dry weight, root length and rate of nitrogen uptake by plant between the first and second harvests (Experiment 5).

Plant density Plants per pot	Mean root dry weight (g)	Mean root length (cm)	Nitrogen uptake (mg)	Nitrogen uptake (mg g^{-1})[A]	Nitrogen uptake (µg cm^{-1})[B]
3	0.51	6650	5.32	10.43	0.80
9	0.98	11060	3.10	3.16	0.28
l.s.d.(P=0.05)	0.05	361	1.27	1.44	0.13

[A] mg uptake per gram of roots (dry weight basis). [B] µg uptake per cm of root length.

The rate of N uptake per unit root growth by 3 plants under shade treatment increased with increasing N application until 100 mg per pot (Fig 6.1, 6.2). In the pot with high plant density the rate of N uptake increased when 50 mg N was applied but was not further increased with higher rates of N application (Fig 6.1, 6.2). The rate of N uptake per unit root growth in the pot with low plant density under non shaded treatment was more than all other treatments (Fig 6.1, 6.2). Increasing the N application from 50 to 150 mg per pot did not increase the rate of N uptake in the plant under non-shaded treatment. The N uptake rate in 3 plants or 9 plants was reduced by shading (Fig 6.1). There was similar response per cm of root length (Fig 6.2). There was an apparent loss of N from the plant (shoot+root) at N0 in the 9 plants shaded treatment (Fig 6.1, 6.2). A possible cause of this was loss of some of the root through washing.

6.3.1.6 Soil water and water use

Water use per pot at the 9 plants density was greater than that at the 3 plants density (Table 6.5). Short term water use by the non-shaded plants was more than the water use by the shaded plants. The short term water use by non-shaded plants decreased after applying more than 50 mg N per pot with 3 plants density (Fig 6. 3).

Table 6.5. Effect of the plant density on the soil water content at the end of the experiment, total water used and water use after applying nitrogen (Experiment 5).

Plant density (Plants per pot)	Soil water content (mL per pot)	Total water use (mL per pot)	Water use efficiency ($g\ L^{-1}$)[A]	Water use after 2 day (mL per pot)	Water use by root ($\mu L\ cm^{-1}$)	Water use by root ($mL\ g^{-1}$)
3	102.2	278.0	1.35	25.67	3.7	49.1
9	92.4	393.4	1.45	28.37	2.6	28.7
l.s.d(P=0.05)	6.39	8.47	0.05	1.48	0.29	2.8

[A] Total water use efficiency was calculated for the shoot by weight.

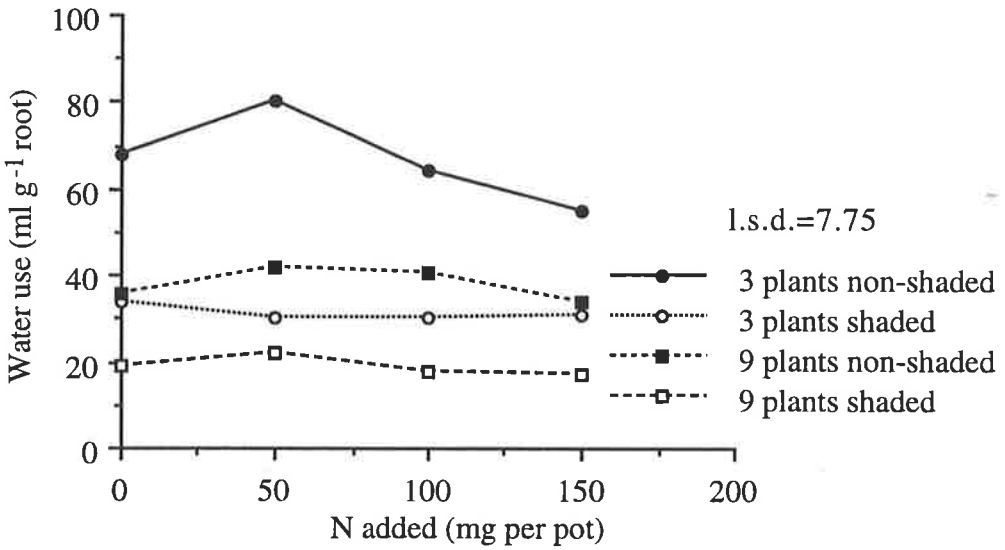

Fig. 6.3. Short term (48 hour) water use by wheat at different plant densities under different shade treatments (Experiment 5)

6.3.2 Experiment 6

6.3.2.1 Dry matter yield

Compared with Experiment 5, the plants grew more vigorously. There was no significant change in the dry weight of shoot and root over the 48 hours, which was an aim of the experiment. Statistical analysis showed that there was significant difference in shoot dry weight in pots with different plant densities (Table 6.6). Shading restricted the plant growth and the shoot dry weight compared with the non-shaded plants. The shoot dry weights of the plants with leaves half covered were similar to those of non-shaded plants (Table 6.7). Plants at low density under the non-shaded had higher shoot and root growth than under the shaded treatment.

Table 6. 6. Dry weight, concentration of nitrate and total nitrogen in the plants with different plant density (Experiment 6)

Plant density (Plants per pot)	Dry weight (g per pot)		Nitrogen (%)		Nitrate (mg kg^{-1})	Total N (mg per pot)
	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
	Before nitrogen and shade treatment (Harvest 1)					
3	0.49	0.88	2.05	0.84	863	17.8
9	0.67	1.47	1.72	0.65	1036	21.0
	After nitrogen and shade treatment (Harvest 2)					
3	0.49	0.91	2.55	0.94	2951	22.4
9	0.67	1.50	2.24	0.79	2575	28.5
l.s.d. (P=0.05)	0.02	0.09	0.05	0.04	99.6	0.81

6.3.2.2 Nitrate concentration in the shoot

The NO_3-N concentrations in the tops of the shaded plants was more than that in the non-shaded plants (Table 6.7). Statistical analysis showed that there was an interaction between the N and shade treatments with respect to shoot NO_3-N concentration. In the non-shaded treatment application of N up to 100 mg N per pot increased the concentration of NO_3-N. Under the shaded treatment and with leaves half covered the concentration of NO_3-N was increased by increasing the N fertiliser rate.

Table 6. 7. Dry weight, concentration of nitrate and total nitrogen in the plant under different shade treatments at harvest 2 (Experiment 6)

Plant density	Dry weight (g per pot)		Nitrogen (%)		Nitrate (mg kg^{-1})	Total N (mg per pot)
Plants per pot	Shoot	Root	Shoot	Root	Shoot	Shoot+Root
			Non-shaded			
3	0.51	0.94	2.68	1.02	2885	24.6
9	0.71	1.45	2.23	0.85	2358	29.7
			Shaded			
3	0.47	0.84	2.47	0.89	3056	20.5
9	0.62	1.55	2.29	0.74	2951	27.2
			Leaves half covered			
3	0.49	0.93	2.50	0.92	2911	22.2
9	0.69	1.50	2.21	0.78	2415	28.6
l.s.d. (P=0.05)	0.02	0.16	0.11	0.06	234	1.5

l.s.d. applies to interaction between shade treatment and plant density.

6.3.2.3 Total nitrogen concentration in the plant

Concentration of total N in the shoots increased at harvest 2 with N application (Table 6.6). There was an interaction between plant density and shade treatment with respect to the total N concentration of the shoot. Shade treatments (shaded and leaves half covered) decreased the total N concentration in the 3 plants. Nitrogen application increased the content of total N in the plant at harvest 2 compared with harvest 1. The content of total N in the 9 plants under non-shade treatment was high compared with the other treatments (Table 6.7).

6.3.2.4 Root growth and root activity

Shade treatment decreased the root length density and total root length in the pot with high plant density (Table 6.8). There was a substantial increase in the N uptake by the plant under non-shade treatment. The N uptake per gram or per cm of root was increased sharply by applying 50 mg N per pot. In the non-shade treatment, N uptake

continued to increase with increasing amount of N fertiliser. In the plant with half leaves covered, N uptake remained steady. With the shaded treatment, N uptake decreased (Fig 6.4, 6.5).

Table 6.8. Gravimetric water content, root length density and total root length in the soil with different plant densities (Experiment 6).

Plant density Plants per pot	Water content (kg kg^{-1})	Root length density (cm cm^{-3})	Total root length (m)
	Before nitrogen and shade treatment		
3	0.041	5.25	100
9	0.041	9.35	178
	After nitrogen and shade treatment		
3	0.051	5.84	111
9	0.044	8.21	156
l.s.d.(P=0.05)	0.005	0.56	10.7

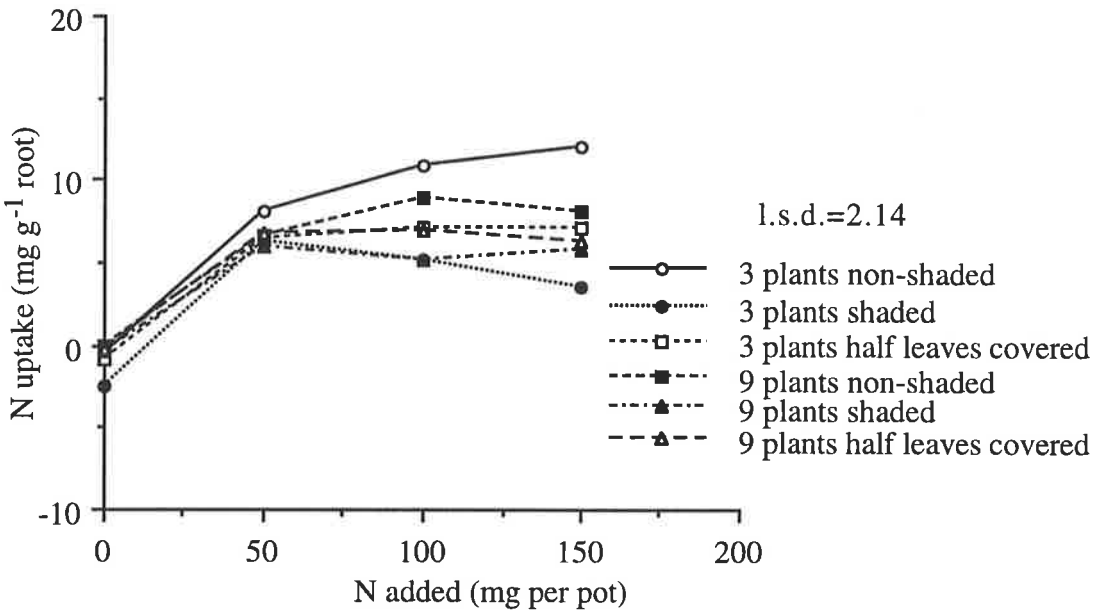

Fig 6.4. Short term (48 hour) N uptake by wheat at different plant densities under different shade treatments (Experiment 6)

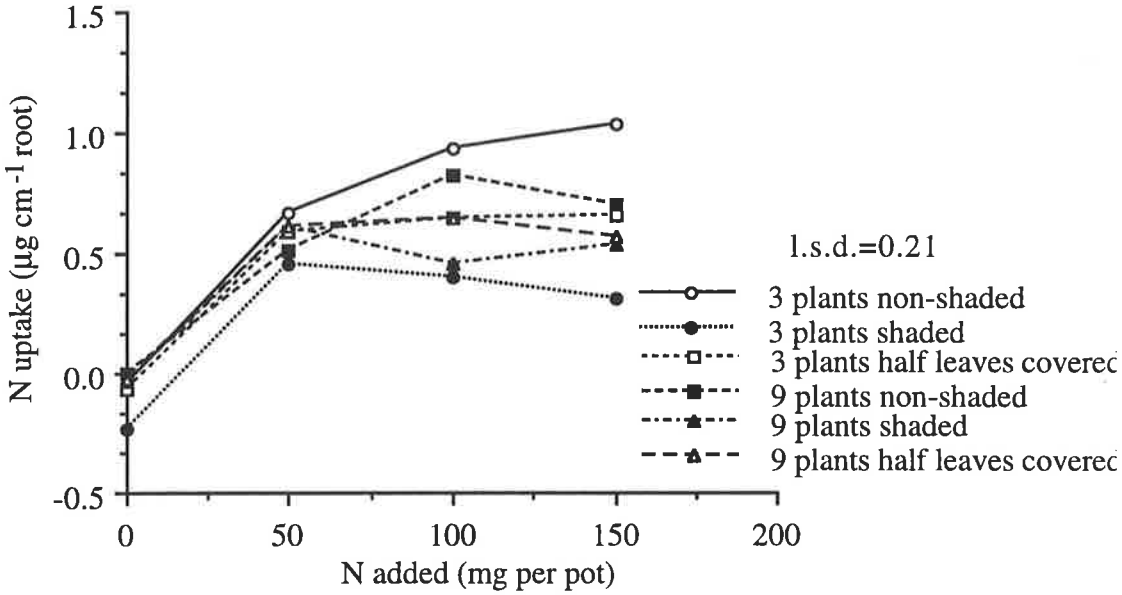

Fig 6.5. Short term (48 hour) N uptake by wheat at different plant densities under different shade treatments (Experiment 6)

6.3.2.5 Nitrogen uptake

The N uptake in the 9 plants was more than that in the 3 plants (Table 6.9). Increasing the N application from 50 to 150 mg per pot did not increase the N uptake in the 3 plants (Fig 6.6). The N uptake in plants decreased under the shade treatment after applying more than 50 mg N while the N uptake by the plant with half leaves covered did not change any more (Fig 6.4, 6.5). The rate of N uptake per g of root dry weight increased when 50 mg N was applied but was not further increased by higher rates of application. This response to N was independent of plant density (Fig 6.7). There was similar response per cm of root length (Fig 6.8).

Table 6.9. Mean root dry weight, root length density and rate of nitrogen uptake by plant between the first and second harvests (Experiment 6).

Plant density Plants per pot	Mean root dry weight (g)	Mean root length (cm)	Nitrogen uptake (mg)	Nitrogen uptake (mg g^{-1})[A]	Nitrogen uptake (µg cm^{-1})[B]
3	0.90	10500	4.64	5.23	0.45
9	1.49	16700	7.47	4.99	0.45
l.s.d.(P=0.05)	0.05	535	0.81	n.s.	n.s.

[A] mg uptake per gram of roots (dry weight basis). [B] µg uptake per cm of root length.

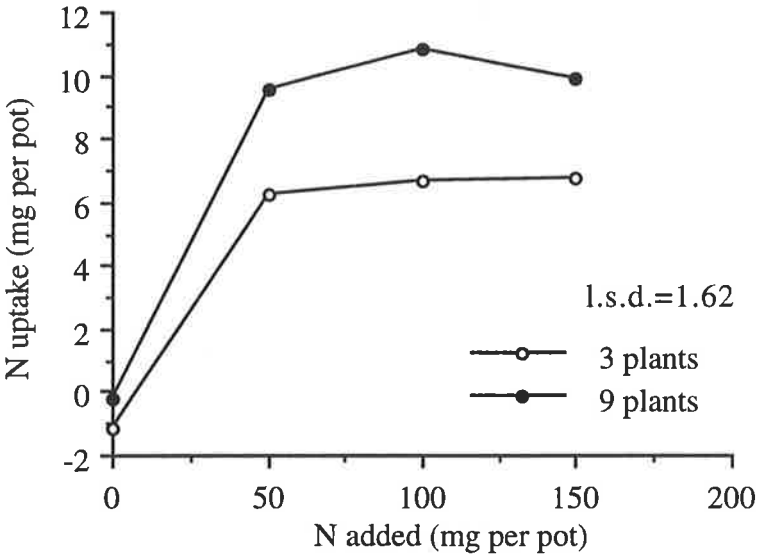

Fig. 6.6. Short term (48 hour) N uptake by wheat at different plant densities (Experiment 6)

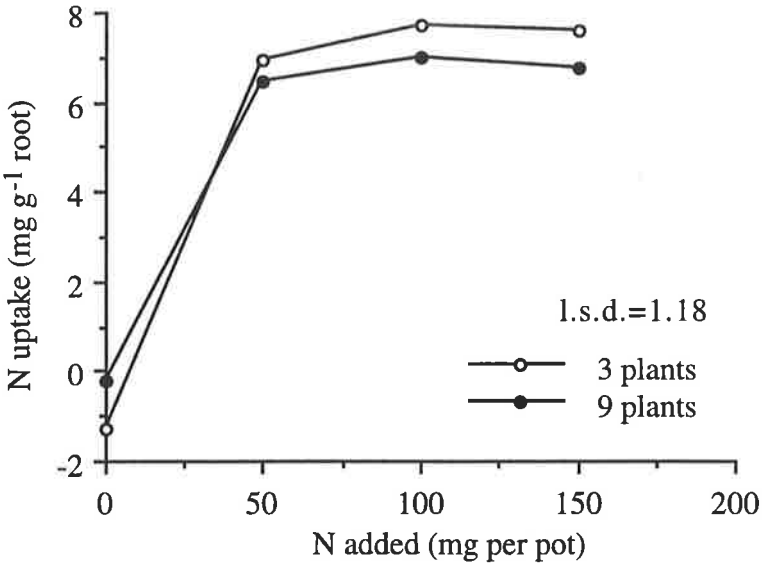

Fig.6.7. Short term (48 hour) N uptake by wheat at different plant densities (Experiment 6).

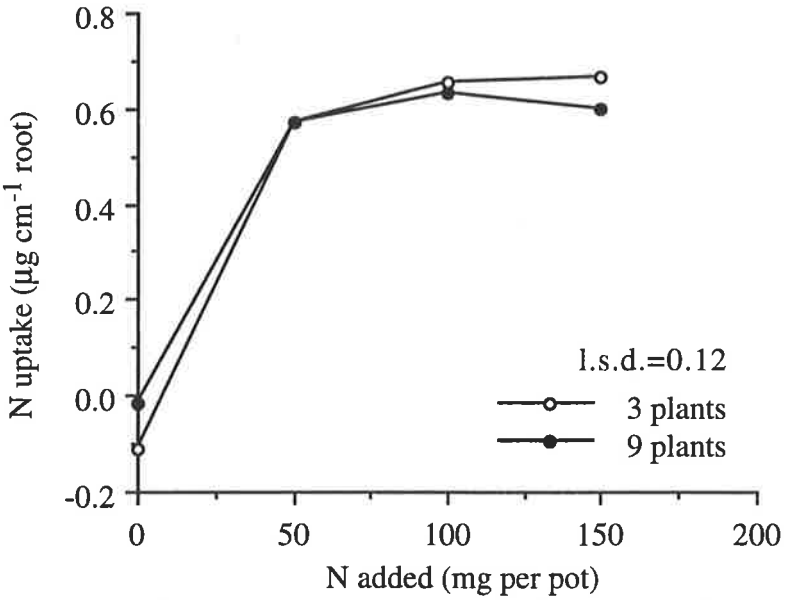

Fig.6.8. Short term (48 hour) N uptake by wheat at different plant densities (Experiment 6).

6.3.2.6 Soil water and water use

Water use per pot at the higher plant density was greater than that at the lower density. Short term water use by the plants under non-shade treatment was more than the

water use by the plants under shade treatment (Table 6.10). The water used per gram or per cm of the root was greater under non-shaded treatment and lower under shaded treatment (Table 6.11).

Table 6. 10 Effect of the plant density on the soil water content at the end of the experiment, total water used and water use after applying nitrogen (Experiment 6).

Plant density Plants per pot	Soil water content (mL per pot)	Total water use (mL per pot)	Water use efficiency (g L^{-1})[A]	Water use after 2 day (mL per pot)	Water use by root (µL cm^{-1})	Water use by root (mL g^{-1})
3	119.2	436	1.13	43.9	4.1	48.9
9	104.4	577	1.16	45.3	2.7	30.8
l.s.d. (P=0.05)	12	14	n.s.	1.02	0.2	1.27

[A] Total water use efficiency was calculated for the shoot by weight.

Table 6. 11. Effect of the plant densities and light treatments on the water use and nitrogen uptake by plant between the first and second harvests (Experiment 6).

Plant density Plants per pot	Water use by root (µLcm^{-1})	Water use by root (mL g^{-1})	Nitrogen uptake (mg g^{-1})[A]	Nitrogen uptake (µg cm^{-1})[B]	Rooting density (cm cm^{-3})	Nitrogen uptake (µg $µL^{-1}$)
			Non-shaded			
3	5.5	63.7	7.63	0.65	6.04	0.118
9	3.3	39.9	5.87	0.50	8.53	0.152
			Shaded			
3	2.6	32.2	3.10	0.23	5.87	0.088
9	2.0	19.5	4.15	0.39	7.40	0.195
			Leaves half covered			
3	4.3	50.8	4.97	0.45	5.59	0.105
9	2.8	32.9	4.95	0.45	8.70	0.161
l.s.d. (P = 0.05)	0.4	2.57	1.2	0.10	1.5	0.25

[A] mg uptake per gram of roots (dry weight basis). [B] µg uptake per cm of root length.

6.4 Discussion

The two experiments, especially Experiment 5 showed that shading restricted both shoot and root growth. Robinson (1986) predicted that self-shading by leaves and the production of non-photosynthetic tissues would decrease the theoretical rate of C assimilation in the shoot system, and shading would also decrease the plant demand for N and water use.

The light would also influence the plant NO_3-N concentration. The NO_3-N concentration increased in the plant with applying more than 50 mg per pot in Experiment 5 and also in Experiment 6 under shaded treatment. The effect of light on conversion of NO_3-N is more than the effect on the uptake (Kessler 1964, Beevers and Hageman 1969). Nitrate reductase activity is affected by light intensity and conversion of NO_3-N to organic forms will be slow under low light (Hageman and Flesher 1960, Ziersel *et al.* 1963). The activity of nitrate reductase in the top leaves is higher than the bottom ones (Zierseral *et al.* 1963) due to shading and the senescence of the bottom leaves (Egmond and Breteler 1972, Darwinkel 1975). Hageman *et al.* (1961) reported that NO_3-N concentration and nitrate reductase activity appear to be inversely related but Breniman *et al.* (1961) reported high concentration of NO_3-N on a sunny day. The major reason for diurnal variation in NO_3-N concentration is changing light intensity because light intensity will affect conversation of NO_3-N to organic form. There is agreement of such variation during the day (Hageman *et al.* 1961) and it was specifically shown by Minotti and Stankey (1973) and Papastylianou (1987).

Total uptake of N was greater at the high than at low plant density in both experiments. The rate of N uptake per unit length of root increased when 50 mg N was applied but was not further increased by higher rates of application. This response to N was independent of plant density in Experiment 5. The rate of N uptake was different in Experiment 5 compared with Experiment 6 (Fig 6.1 and 6.2 compared with Fig 6.4 and 6.5). In Experiment 5 the rate of N uptake increased sharply by increasing 50 mg per pot but was not further increased by higher rates of application. The lower root length density and also lower dry weight of shoots and roots are possible reasons for these

differences. The maximum light intensity in Experiment 5 was higher than that in Experiment 6. Restricting the growth of the plants by shading or reducing the area of leaf exposed to light in Experiment 6 had no effect on the response of the plants to 50 mg N, but it decreased the ability of the plant to respond to higher levels of N. With the non-shade treatment, N uptake continued to increase with increasing amounts of N fertiliser but no such increase was observed with the shaded treatments. The response was not significantly affected by plant density. These results show that the total uptake was greater by the 9 plants. The rate of uptake was sensitive to shoot growth.

One of the most important factors governing uptake of nutrients of low mobility in the soil such as phosphate is the root length per unit volume of soil but relatively sparse root systems may be sufficient to remove almost quantitatively from the soil highly mobile nutrients such as NO_3-N (Bray 1954). Various pot experiments (Wiersum 1962, Cornforth 1968, Andrews and Newman 1970) have shown that P uptake to be correlated with root density but N uptake not so. Several authors (*e.g.* Daigger and Sander 1976, Matzel *et al.* 1984) in field studies found that deep placement of N or leaching of NO_3-N into deeper soil layers where root length densities usually are small (Herbst and Stumpe 1984) hardly affected N uptake of winter wheat. When the N supply in the subsoil at the beginning of the growing season was relatively low, the soil profile was nearly fully depleted during the growing season by sugarbeet, barley and wheat (Strebel and Duynisveld 1989, Kuhlmann *et al.* 1989). Similarly to these experimental approaches, several model calculations (*e.g.* DeWilligen and Van Noordwijk 1987, Robinson and Rorison 1983) showed that N uptake from wet soils would be hardly affected by root length density. Barraclough (1986, 1989) concluded that NO_3-N transport through the soil will be a rate-limiting factor in N uptake since large root growth of wheat and rape permitted slow inflow rates (*i.e.* low diffusive demand) per unit root length. Wheat crops with average rooting densities of about 0.04 cm cm^{-3} at depth of 120-150 cm between tillering and anthesis extracted 5 kg ha^{-1} N while the amount of N extracted between tillering and anthesis from these depths with average rooting densities of 0.13 cm cm^{-3} was 10 kg ha^{-1} (Kuhlmann *et al.* 1989). This showed

that a small amount of root was able to extract N effectively. Kuhlmann and Barraclough (1987) reported that the maximum uptake rate of N was 1 pmol cm^{-1} root sec^{-1} for winter wheat, suggesting that the small amounts of root mentioned above have the potential to absorb a considerable amount of N. The rate of NO_3-N transport to the root has an essential role for uptake. Brady *et al.* (1993) reported that there was small differences in NO_3-N uptake activity of different root sections with 14-day-old wheat plant. Uptake of ^{15}N over 24 hour was 5, 5 and 7 μg cm^{-1} total root length in basal, middle and apical segments respectively. There were no significant differences in uptake rate along young, rapidly growing roots. The N uptake during 48 hours was 0.80 and 0.30 μg cm^{-1} with low and high plant density in Experiment 5 and 0.45 μg cm^{-1} with low or high plant density in Experiment 6 which was much smaller than the values reported by Brady *et al.* (1993).

A number of factors have to be taken into account in considering the amount of nutrients that crop can taken up from the soil. The most important are rooting density, the nutrient status of the soil and its water content (Wiersum 1967). In most soil types, rooting in the deeper layers is sparse and the fertility of the subsoil is usually poorer than that of the topsoil. The potential uptake ability of roots in the deeper soil layers becomes important in dry periods when roots in the upper soil layer may become inactive. The root length density in these two experiment was more than the 1 cm cm^{-3} reported by Walter and Barley (1974) for root length density at a depth of 100 cm in a field experiment in Southern Australia. Wind (1961) reported that root density has to be about 1 to 2 cm cm^{-3} to fully extract the water from subsoil. Therefore the plant can takeup N and water from subsoil even with low root density.

Shading treatment decreased the plant demand on N and water use and for this reason the plant under shaded treatment used less water compared with the plants under non-shaded treatment.Water use was decreased by shading in both experiment. Over the 48 hour period. Water use varied little with planting density.

6.5 Conclusion

The experiments showed that the rate of uptake of N per unit root length by wheat with different supplies of N was dependent on shoot growth. The effect of shading related to N uptake was greater than the effect of plant density. The plant can take up a significant amount of N during the short term period (48 hour) and the rate of uptake was affected by the shoot growth. Therefore a transient increase in subsoil mineral N due, for example, to mineralisation and leaching, can improve the N uptake by plant. The ability of the plant to respond to this N will depend on its growth rate. The N fertiliser to increase the rate of N uptake must be balanced with the amount of soil water available. Roots in the subsoil are certainly of potential value in feeding the plant particularly if the water content and nutrient availability of the topsoil is low. Their contribution to plant nutrition will further depend on the fertility of the subsoil layers, the water content of these layers and the amount of roots that have been able to develop in these regions. In the field, the subsoil water content in the late season is often more than the water content in the topsoil. If the N is available in the subsoil the plant can absorb water and N even with low density of root.

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

This general discussion seeks to interpret the main findings of the experiments and identify gaps in knowledge for future research and will build on the discussions of individual experiments in previous chapters. The study examined the importance of subsoil NO_3-N availability on GPC of wheat and the factors that contribute to the effective utilisation of this N. The issue of low GPC in parts of southern Australia has arisen because of the decline in wheat GPC during the 1980s and early 1990s (Palmer 1990, Dyson and Fewings 1990). There was also widespread concern that N deficiency was becoming a major factor limiting grain yield of winter cereals as well as GPC in South Australia as the intensity of cropping increased, pastures were replaced with grain legume or oilseed crops and the use of N fertiliser increased (McDonald 1989, Reuter 1989, Xu *et al.* 1991 and 1992, Xu and Elliott 1993). Most of the fertiliser is applied prior to or at sowing, and there is potential for significant leaching to occur over winter. Subsoil nitrate (NO_3-N) accumulation often occurs in southern Australia and because rainfall is generally insufficient to cause leaching beyond the roots, NO_3-N usually remains within the root zone of crops (Storrier 1965, Greenland 1971). Late in the season the NO_3-N can be taken up by the wheat and thus it constitutes a potential source of N for enhancement of GPC. However, the magnitude of the effect and its interaction with post-anthesis water deficit have not been studied extensively. The series of experiments reported in this thesis was therefore conducted to examine the ability of wheat to utilise N from deep (60 cm) in the profile, the effects of different rates and placement of N fertiliser and the effect of post-anthesis topsoil drying on utilisation of N from the subsoil. The experiments were conducted in long pots under controlled conditions in a glasshouse in which leaching beyond the root-zone did not occur, in an attempt to overcome the inherent variability associated with measuring root growth and soil NO_3-N in the field. The results therefore represent an ideal situation in which recovery of N was most likely to be high. Nevertheless, the results are generally

consistent with observations from field experiments, and the more detailed measurements, compared with those possible in the field, provide a good picture of post-anthesis NO_3-N uptake. Where major differences with field observation occur, these will be discussed.

7.2 Leaching and the use of subsoil N

In Experiments 1-4 (Chapter 4 and 5) post anthesis N uptake from below 60 cm came from 2 main sources, the NO_3-N that was leached from the surface soil during the experiment and the NO_3-N that was placed in the subsoil as a N treatment. In all the experiments, leaching occurred and NO_3-N accumulated below 60 cm. The reason for this was probably related to the frequency of watering (2-3 times per week) as well as the high rates of N used. However, even when no N was applied at sowing (Experiment 4, Chapter 5) a considerable amount of NO_3-N was found below 60 cm at tillering, suggesting that under the conditions of the experiments some of the NO_3-N in the subsoil was derived from mineralisation of organic N.

In Experiments 1-4 (Chapters 4 and 5) significant amounts of NO_3-N accumulated below 60 cm by tillering, which ranged from 35 days after sowing (DAS) (Experiment 3, Chapter 4) to 45 DAS (Experiment 1, Chapter 4). The amounts of water applied until tillering ranged from 700 ml (Experiment 4 in Chapter 5) to 1210 ml (Experiment 3, Chapter 4), equivalent to 74-127 mm of rainfall. In the medium to high rainfall areas of the cereal zone in South Australia (400-550 mm per annum), rainfall during June and July frequently equals or exceeds these amounts, so the leaching observed in the present experiments is not dissimilar to that which may occur in the field. These results highlight the potential for significant amounts of leaching up to tillering in many parts of the cereal belt. Factors that may mitigate against this are soil texture and soil structure, which will affect infiltration of water through the profile, and the lower frequency and amount of rainfall in the field (*e.g* Storrier 1965, Greenland 1971). The experiments used a uniform profile in which the soil columns were packed to a bulk density of 1.3 g cm^3, which contrasts with many of the soils of the cereal belt

with duplex profiles and bulk densities up to 1.8 g cm^3 in the subsoil. Therefore, the depth of leaching in the field may be less than that observed in the present experiments. For example, preliminary measurements in the field at the Waite Institute showed a loss of mineral N from the surface horizon of the soil to below 30 cm, part of which was attributed to leaching. Prescott and Piper (1930) measured leaching of NO_3-N in the field in South Australia and reported that the NO_3-N was washed down (leached) by the rain and redistributed in the soil profile (0 to 100 cm depth) but most of it remained in the top 45 cm. However, movement of NO_3-N from the surface layers to deeper in the subsoil in the winter has been reported (Waring and Teakle 1960, Storrier 1965), so examining uptake from 60 cm depth was valid.

The other important aspect of leaching in these experiments was its occurrence in relation to root development. In all experiments, leaching to below 60 cm had occurred by tillering, at which time there was little or no root growth below 60 cm. It was only when the NO_3-N in the topsoil had been depleted and when there was significant amount of root growth below 60 cm that the plants used the subsoil NO_3-N. The results confirm the results of experiments by Kuhlmann *et al.* (1989), who found that N uptake during the vegetative growth of winter wheat occurred mainly from the upper soil layers, whereas N uptake after anthesis was restricted to the subsoil. Therefore, subsoil accumulation of NO_3-N due to leaching early in the season may be common with average winter rainfall totals experienced in parts of southern Australia and it is a potential source of N for plant in the late season. The value of this N reserve will depend in part on the ability of the crop to exploit it.

7.3 Root development

Rooting density in the subsoil is one of the important factors in determining nutrient uptake by plants (Wiersum 1967). Generally, the fertility of the subsoil is poorer and the density of the root in the subsoil is lower than that of the topsoil. In the Mediterranean-type climate of southern Australia rainfall declines during spring and early summer, resulting in topsoil drying. When this occurs, root length in the topsoil

declines (Experiment 4, Chapter 5) and the root growth in the moist subsoil becomes important for sustaining water and nutrient uptake during grain filling. The results from Experiments 1 and 2 in Chapter 4 show an increase in the root length in the subsoil (60-80 cm depth) after anthesis and a co-incident decrease in measured root length in the topsoil. This agrees with the observation by Campbell *et al.* (1977), who reported that there was a decrease in root length and root weight in the topsoil sometime after anthesis and before the dough stage. An important issue therefore, is whether the uptake of NO_3-N from the subsoil will be limited by root length density.

The results of Experiment 1 (Chapter 4) imply that growth and distribution of roots in the subsoil have major roles in the post-anthesis N economy of plants because substantial uptake of NO_3-N did not occur until there was an increase in root length density. Although there was an increase in uptake per cm of root, total uptake was small immediately after the addition of N to the subsoil . Recovery of N occurred gradually over a 6 weeks period. This period probably corresponded to the time when the plant was increasing lateral root development in response to the localised increase in soil N (Passioura and Wetselaar 1972, Drew *et al.* 1973, Drew and Saker 1975). This suggests that although uptake rates per cm of root can increase substantially with high concentration of subsoil NO_3-N (Table 4.7), the development of an extensive root system in the subsoil is more important to the effective use of subsoil NO_3-N.

The rates of N used in the experiments were equivalent to field rates of approximately 75 and 150 kg N ha^{-1} and the root length densities below 60 cm in Experiments 1 and 2 (2-2.5 cm cm^{-3}) were higher than those often found in the field. For example, subsoil NO_3-N concentrations after anthesis are often less than 10-20 mg kg^{-1} (Prescott and Piper 1930, Storrier 1965, Greenland 1971, Strebel and Duynisveld 1989), and root length densities of 0.5-1.0 cm cm^{-3} are common (Schultz 1971, Walter and Barley 1974, Hamblin and Hamblin 1989). Do these low root length densities limit the use of subsoil N? Kuhlmann *et al.* (1989) examined NO_3-N uptake by winter wheat that was irrigated. They measured post anthesis uptake of N from the 120-150 cm layer to be 10 kg N ha^{-1}, and the root length density to be 0.13 cm cm^{-3}. Calculations based

on mineral N uptake rates of winter wheat showed potential uptake of 24 kg N ha^{-1} after anthesis at this root length density. Therefore, it appeared from this work that even at small root densities wheat can take up significant amounts of N. This conclusion is supported by the results of Burns (1980), Kuhlmann and Barraclough (1987) and Robinson (1991). This amount of N (24 kg ha^{-1}) taken up after anthesis could make a significant contribution to grain protein. However, the N was taken up over an 8 week period, which is much longer than the grain filling period (4-5 weeks) of field-grown wheat in southern Australia. Hence N uptake in southern Australia, with its relatively short grain filling period may be less than that reported by Kuhlmann *et al.* (1989) and root length density may become important.

Some compensation for low root length densities can be made by greater rates of uptake (Experiment 1), although the degree to which this compensation can occur is affected by the photosynthetic production of the shoot (Chapter 6). The production of new roots and the increase in NO_3-N uptake across the root both require the diversion of photo-assimilate to the root system, and a reduction in this supply will limit the ability of the plant to exploit subsoil N. Normally, the crop starts to senesce soon after anthesis and the rate of photosynthesis falls (Puckridge 1968). Consequently, the ability of the crop to allocate photosynthate to the roots and support continued uptake of N will decline. As well, the demand for N may fall with increased senescence. The exploitation of subsoil NO_3-N after anthesis may therefore be limited by the reduction in growth and photosynthesis after anthesis, which are characteristic features of wheat growth in the southern Australian environment.

The importance of root length density can be examined by using some simple calculations based on the data from the present experiments. The average grain yield in the South Australia is about 1.8 t ha^{-1}. Assuming that all of the N taken up after anthesis goes to the grain, a small amount of N taken up after anthesis (for example 5 kg N ha^{-1}) can increase in GPC by about 1.5%. In Experiment 3 (Chapter 4) the rate of uptake of N after anthesis was about 0.1 μg cm^{-1} d^{-1} (Table 4.17) most of which came from the subsoil (60-100 cm) because the concentration of NO_3-N in the topsoil was negligible

(Table 4.18). The root length density in these layers was about 2 cm cm^{-3}. Over a 30 d period, the amount of N taken up will be about 24 kg ha^{-1}. At root length densities more commonly found in the subsoil of Australian soils (0.5-1.0 cm cm^{-3}), the amount taken up is of the order of 6-12 kg N ha^{-1}, enough to increase GPC significantly. Therefore it is possible for the plant to takeup about 5 kg ha^{-1} under southern Australian conditions even with low concentration of NO_3-N and low root density.

7.4 Effect of available water after anthesis on GPC.

The water content of the soil is very important to N uptake by wheat because if the topsoil or subsoil contains enough mineral N but water is not available, the plant cannot take up the N. Low soil water in the root zone may also change the accessibility of NO_3-N in the soil profile.

A potential problem in post-anthesis uptake is that roots die after anthesis, particularly in the field when the topsoil dries late in the season. Drying of the topsoil after anthesis decreased the root length in the topsoil at maturity (Chapter 4, 5). Therefore, the roots in the subsoil play an important role in post-anthesis water and N uptake. Hamblin and Tennant (1987) suggested that water use was closely related to rooting depth rather than root length. The depth of rooting is generally limited to the depth of profile wetting, and about 50% of the rainfall is lost by evaporation from the soil surface in Mediterranean environments. Walter and Barley (1974) found in a field experiment in South Australia that root length density at a depth of 100 cm was 1 cm cm^{-3}, which is much lower than in our experiments. Such low root length densities may explain why significant amounts of soil water sometimes remain unexploited at crop maturity (Schultz 1971, Ridge 1986). Brown *et al.* (1987) reported that as root length increased to 1 cm cm^{-3} the rate of water uptake from each soil layer increased. The water uptake rate from a soil volume depends on evaporative demand, on rooting density, on water potential difference between soil and xylem, and on radial resistance to flow of water from bulk soil to the root xylem (Taylor and Klepper 1978). Wheat root systems vary from one cultivar to another due to genetic potential, but environment

interacts strongly with genetic potential to control root growth and effectiveness. There is an opportunity to select cultivars that reduce the effect of each adverse soil condition, at least within certain ranges. Sowing a variety of wheat with a long root system may improve subsoil water and mineral N uptake late in the season. Therefore improving the rooting depth of wheat has the potential not only to increase crop water use and production, and also offers the possibility of increasing N content of the grain. Whether it will increase GPC will depend on the relative increases in grain weight and grain N that result from this better utilisation of subsoil water. The result of the Experiment 2 (Chapter 4) showed some evidence of increased root length in the subsoil at maturity (Table 4.12). Nitrogen fertiliser increased the water use and efficiency of water use compared with the control.

Allowing the topsoil to dry and reducing the supply of water decreased the grain yield but increased the GPC (Table 7.1). When grain yield is limited by factors other than N (*e.g.* water) or at high rates of N, the yield response is low or sometimes negative, while GPC continues to increase. There may be a negative correlation between grain yield and GPC in this case (Perry and Hillman 1991). Campbell *et al.* (1981) reported that the effect of water stress on GPC was mainly through its influence on grain yield (dilution effect). Subsoil N had more effect on GPC when post-anthesis water stress was imposed.

Table 7.1. The effect of water treatments on grain yield, GPC, apparent N recovery and the contribution of remobilised N to wheat grain N in Experiments 2 and 4.

Water treatment	Grain yield (g per pot)	Grain protein (%)	Apparent N recovery (%)	N remobilised N in grain (%)
	Experiment 2 (Chapter 4)			
W1[A]	13.81	12.26	84	39.9
W2	12.87	13.08	73	37.3
l.s.d (P = 0.05)	n.s	0.44	n.s	n.s
	Experiment 4 (Chapter 5)			
W1	13.65	13.78	58.5	17.7
W2	11.36	15.01	61.7	13.8
l.s.d (P = 0.05)	1.35	0.62	n.s	n.s

[A]W1= surface irrigation, W2 = subsoil irrigation

Table 7.2. The effect of nitrogen treatments on grain yield, GPC, apparent N recovery, and the contribution of remobilised N to wheat grain N in Experiments 1, 2 and 4.

Nitrogen treatment mg N per pot	Grain yield (g per pot)	Grain protein (%)	Apparent N recovery (%)	N remobilised N in grain (%)
	Experiment 1 (Chapter 4)			
0	6.64	7.8	-	81
150 subsoil after anthesis	8.57	12.4	72	27
l.s.d (P = 0.05)	1.37	0.6	-	30
	Experiment 2 (Chapter 4)			
0	11.7	11.8	-	-
150 topsoil at sowing	13.7	12.7	77	35.7
75 topsoil+75 sub at sowing	13.9	12.9	80	40.5
150 subsoil at sowing	14.0	13.0	85	43.6
75 top at sowing+75 sub late	13.5	13.0	73	34.8
l.s.d (P = 0.05)	1.7	0.70	n.s	n.s
	Experiment 4 (Chapter 5)			
0	11.98	13.7	-	35.5
150 top at sowing	13.27	14.3	64	24.5
75 at tillering+75 at booting	13.32	14.6	73	-0.5
150 sub after anthesis	11.45	15.0	42	4.2
l.s.d (P = 0.05)	n.s	0.88	n.s	28

7.5 Recovery of N by wheat

The apparent recovery of N by plant tops from the subsoil was high (>70%) in Experiments 1 and 2, Chapter 4 (Table 7.2). By maturity in Experiment 1, 121 mg of extra N was measured in plants given subsoil N, equivalent to an apparent recovery in the roots and shoots of 80%. Most of the N had been translocated to the grain. This suggests that the ability of the plant to recover N from the subsoil after anthesis was high. In Experiment 2, the apparent recoveries tended to be slightly lower with post-anthesis water stress for all of the N treatments except for the plants that received all of the N subsoil at sowing (Table 7.3). The apparent recoveries of N by plant tops in Experiment 4 (Chapter 5) were lower than those in Experiments 1 and 2 (Chapter 4), especially by the plants that received 150 mg N subsoil after anthesis (Table 7.2).

In the field, recovery of autumn-applied fertiliser N is low (11-40%), with between 40 and 80% of the fertiliser N lost mainly (if not entirely) by leaching (Powlson *et al.* 1986b). Cooper and Blakeney (1990) reported that the effective recoveries of N in the plant tops were 78.5% and 48.9 % after applying 40 and 80 kg N ha^{-1} respectively, which are similar to values reported by Whitfield *et al.* (1989) and Smith *et al.* (1989) in Victoria, but much higher than the values of Strong (1982) in Queensland. Such high recovery (78.5% and 48.9%) of N only 3 weeks after it was applied indicates rapid N uptake, which is supported by the data of Recous *et al.* (1988) and Smith *et al.* (1989) who reported the mean recovery of applied N (50 kg N ha^{-1}) was 84%. Total recoveries of ^{15}N in the above-ground portion of the plants were 41, 45, 55 and 61% of the amounts applied at depths of 45, 75, 105 and 135 cm, respectively (Gass *et al.* 1971). The apparent recovery of N by plant tops was high in Experiments 1 and 2 (Chapter 4) compared with the some of the values reported above perhaps because leaching was prevented in the pot experiments and other losses were low.

The apparent recovery of N in the grain can vary greatly (13%, Strong 1982; 56% Smith *et al.* 1989). The values obtained by Cooper and Blakeney (1990), 30.8% and 36.4 % with applying 40 and 80 kg N ha^{-1} respectively, were similar to values reported by other workers (Sadaphal and Das 1966, Hamid and Sarwar 1976). Doyle

and Shapland (1991) reported that the apparent recovery in the grain was 48-56% of N applied at sowing, but only 25-48 % of N applied post-sowing.

Table 7.3. The effect of nitrogen treatments on grain yield, GPC, apparent N recovery and the contribution of remobilised N to wheat grain N in Experiments 2 and 4.

Nitrogen treatment mg N per pot	Grain yield (g per pot)	Grain protein (%)	Apparent N recovery (%)	N remobilised N in grain (%)
		Experiment 2 (Chapter 4)		
		Surface irrigation (W1)		
0	11.69	11.46	-	-
150 topsoil at sowing	14.47	12.46	84	38.0
75 topsoil+75 sub at sowing	14.34	12.14	85	36.2
150 subsoil at sowing	14.50	12.45	82	50.3
75 top at sowing+75 sub late	14.03	12.77	86	35.1
		Subsoil irrigation (W2)		
0	11.64	12.12	-	-
150 topsoil at sowing	12.88	12.98	70	33.3
75 topsoil+75 sub at sowing	13.50	13.61	74	44.7
150 subsoil at sowing	13.44	13.51	88	36.8
75 top at sowing+75 sub late	12.87	13.19	60	34.5
l.s.d (P = 0.05)	n.s	n.s	n.s	n.s
		Experiment 4 (Chapter 5)		
		Surface irrigation (W1)		
0	13.16	12.77	-	33
150 topsoil at sowing	14.86	13.85	65.9	25
75 at tillering+75 at booting	13.50	14.25	56.2	5
150 subsoil after anthesis	13.19	14.25	53.3	8
		Subsoil irrigation (W2)		
0	10.91	14.75	-	38
150 topsoil at sowing	11.68	14.69	61.2	24
75 at tillering+75 at booting	13.13	14.90	92.2	-6
150 subsoil after anthesis	9.70	15.86	31.6	0.3
l.s.d (P = 0.05)	n.s	n.s	n.s	n.s

l.s.d applies to interaction between nitrogen and water treatments.

7.6 Remobilisation of N

The mobility of N within the plant is an important characteristic of N metabolism. During grain development, grain yield and GPC are affected by translocation of N (Pearson and Miurhead 1984, Anderson *et al.* 1991). For example, at anthesis wheat can contain about 80% of the N that is present in the plant at maturity (Dalling *et al.* 1976, Austin *et al.* 1977). The general trend was for late applications of N to reduce the contribution of remobilised N to grain N. In Experiment 1, the amount of N remobilised after anthesis from the root and shoot to the grain in the control plants was high, equivalent to 81% of the N in the grain, while it was 27% in the N treated plants (Table 7.2). When the N was available in the subsoil after anthesis, total N remobilised from leaf and stem decreased. The apparent contribution of remobilised N to grain in Experiment 2 was not significantly different between treatments but in Experiment 4, N applied at booting or after anthesis did reduce remobilisation of N significantly. Decreasing the N supply to the topsoil may have increased N uptake from the subsoil. Total N remobilised to the grain in Experiment 4 (Chapter 5) was low compared with Experiment 2 (Table 7.2). Drying of the topsoil after anthesis slightly, but not significantly, decreased the apparent contribution of remobilised N to the grain in both Experiments 2 and 4 (Table 7.1). Therefore, plants may preferentially utilise soil N available in the post anthesis period, rather than remobilise N, although the effect of this on grain yield and GPC may vary.

Given the capacity of plants to remobilise N to the grain, is there any benefit from post-anthesis uptake of N from subsoil? Under N-deficient conditions, post anthesis uptake can improve both yield and GPC (Experiment 1), probably by maintaining green leaf area. Under more favourable conditions, post anthesis uptake may benefit GPC in years of favourable grain filling conditions. As mentioned before, the mean grain yield in South Australia is about 1.8 t ha^{-1}. The amount of dry matter at anthesis for this yield is about 4 t ha^{-1} (McDonald 1992). If the concentration of total N at anthesis is 1.2%, then N uptake will be 48 kg ha^{-1}. If 80% of this N remobilised to the grain, this will result in a GPC of 12%. If grain set and filling is enhanced by favourable

conditions, which increases yield to 2.0 t ha^{-1}, GPC will decline from 12% to 10.9% assuming 80% remobilised to the grain and another extra 3.7 kg N ha^{-1} is needed for the plant. It is feasible that this could be satisfied by post-anthesis uptake (see section 7.3). Alternatively, increasing remobilisation from 80% to 90% results in an extra 4 kg N ha^{-1} remobilised giving a GPC of 12.4%. Therefore, uptake of subsoil N can be useful when high yields are favoured at the expense of GPC. Where subsoil N is not available, greater remobilisation may occur.

7.7 Yield and protein response to N

The Experiments 1, 2 and 4 examined the effect of subsoil mineral N on GPC of wheat. Adding N to the subsoil after anthesis increased both yield and GPC in Experiment 1 (Table 7.2). Yield and GPC were also increased by N application in Experiment 2, although the yield response was not as great as that in Experiment 1. In Experiment 2 application of N significantly increased the yield and GPC irrespective of the depth or the time of application. This shows that placement of N had no effect on N uptake by plants. All apparent recoveries were similar for all N treatments (Table 7.2). This suggests that as long as the N is not leached from the root zone application of N to the topsoil and subsoil has an equivalent effect. Nitrogen supply during the growth of the plant and a late supply of N to the plant could be important in determining GPC.

In all experiments, the general trend was for late applications of N to increase GPC, but the effect on yield was less consistent. In Experiment 4 (Chapter 5) the GPC of wheat significantly increased by placement of 150 mg N at 60 cm depth after anthesis or 75 mg N topsoil at tillering and 75 mg N topsoil at booting. In Experiment 1 (Chapter 4), adding N to the subsoil after anthesis increased both yield and GPC (Table 7.2). Overall, GPC with all N treatments Experiment 4 (Chapter 5) was higher than that in Experiment 2 (Chapter 4) possibly because there was more NO_3-N in the subsoil at anthesis than in Experiment 2 (Chapter 4).

The low level of N in the soil in Experiment 1 was one possible reason for increase both GPC and grain yield after N application. When the N status of soil is low

the application of N after anthesis generally affects both GPC and grain yield, but only GPC is likely to be affected when soil N status is high. The effect of a split N application on GPC was comparable to the subsoil N application after anthesis due to the leaching of the N into the subsoil from where it was taken up after anthesis. Doyle and Shapland (1991) reported that GPC was increased by application of N after sowing and the effect of N application at booting on GPC was more than that at tillering. Subsoil N application increased the GPC. This result is in agreement with the result of Smika and Grabouski (1976) who reported that in dryland farming, enhanced GPC and yield of winter wheat were associated with NO_3-N in the deeper portions of the rooting zone after water had been depleted from upper zone prior to the grain filling stage. Smika and Greb (1973) reported that when high NO_3-N concentration were found in the soil below the 60 cm depth, the GPC was higher. The results of these experiments support the suggestion of Ladd (1990) that subsoil mineral N may be taken up by wheat late in the season and increase the GPC.

7.8 General conclusions

The findings from this work showed that NO_3-N which accumulates in the subsoil from leaching early in the season has the potential to be a good N reserve for the wheat late in the season when there is no water available in the topsoil for the plant. The ability of plant roots to recover N from the subsoil is high and N uptake from subsoil after anthesis can increase the GPC of wheat. Where N was available in the subsoil and taken up after anthesis the amounts of N remobilised from the vegetative parts of the plant to the grain were decreased (Experiments 1 and 4). The results also showed that as long as the N is not leached from the root zone topsoil N application at sowing or split N application at tillering and booting has an equivalent effect as application to the subsoil. Water use and efficiency of water use were increased by N application. The rate of uptake per unit root length was not affected by the density of roots but was sensitive to shoot growth. Therefore a transient increase in subsoil mineral N due, for example, to mineralisation and leaching, can improve the N uptake by plant. The ability of the plant

to respond to this N will depend on its growth rate. Further research is needed to investigate the effect of subsoil mineral N on GPC of wheat under field conditions. For example, different methods of cultivation (direct drill or conventional cultivation) can have different effects on subsoil mineral N and will probably affect the GPC of wheat. Growing of cultivars selected for high root length densities, long root systems and long grain filling period may improve the subsoil mineral N uptake and increase GPC.

REFERENCES

Addiscott T M, Whitemore A P and Powlson D S 1991 Measuring nitrate losses from soil. *In* Farming, Fertiliser and the Nitrate Problem. pp 55-72. Redwood Press: Melksham, UK.

Alessi J, Power J F and Sibbit L P 1979 Yield, quality and nitrogen fertiliser recovery of standard and semi-dwarf spring wheat as effected by sowing date and fertiliser rate. J. Agric. Sci. Camb. 93, 87-93.

Allison F E 1973 Soil Organic Matter and its Role in Crop Production. Elsevier Scientific: New York.

Alston A M 1976 Effect of depth of fertiliser placement on wheat grown under three water regimes. Aust. J. Agric. Res. 27, 1-10.

Alston A M 1979 Effect of soil water content and foliar fertilization with nitrogen and phosphorus in late season on the yield and composition of wheat. Aust. J. Agric. Res. 30, 577-585.

Alston A M 1980 Response of wheat to deep placement of nitrogen and phosphorus fertiliser on a soil high in phosphorus fertilisers in the surface layer. Aust. J. Agric. Res. 31, 13-24.

Amir J , Mufradi I , Klitman S and Asido S 1994 Long-term comparative study of soil nitrate test, Gilat plant indicator method and wheat nitrogen uptake. Plant and Soil 158, 223-231.

Anderson W K, Belford R K, Crosbie G B, Loss S P, Mason M G and Perry M W 1991 Crop management. *In* The Wheat Book. A Technical Manual for Wheat Producers. Eds M Perry and B Hillman. pp 87-115. Department of Agriculture, Western Australia. Bull. No. 4196.

Andrew W D 1965 The effect of some pasture species on soil structure. Aust. J. Exp. Agric. Anim. Husb. 5, 133-136.

Andrews R E and Newman E I 1970 Root density and competition for nutrients. Oecol. Plant 5, 319-334.

Anonymous 1972 Remember the plant food content of your crops. *In* Better Crops with Plant Food. 56(1), 1-2. Potash Institute of North America.

Arnon I 1979 Optimising yields and water use in Mediterranean agriculture in soil. *In* Mediterranean Type Climates and Their Yield Potential. pp 311-348. Proc 14th Coll. Int. Potash Institute, Sevilla, Spain.

Austin R B, Ford M A, Edrich J A and Blackwell R D 1977. The nitrogen economy of winter wheat. J. Agric. Sci. Camb. 88, 159-167.

Baldwin I P, Ney P H and Tinker P B 1973 Uptake of solutes by multiple root systems from soil. III. A model for calculating the solute uptake by a randomly dispersed root system developing in a finite volume of soil. Plant and Soil 38, 621-635.

Ball B C 1981 Pore characteristics of soils from two cultivation experiments as shown by gas diffusivities and permeabilities and air-filled porosities. J. Soil Sci. 32, 483-498.

Ball R P and Ryden J C 1984 Nitrogen relationship in intensively managed temperate grasslands. Plant and Soil 76, 23-33.

Barber S A 1962 A diffusion and mass-flow concept of soil nutrient availability. Soil Sci. 93, 39-49.

Bargh B J 1978 Output of water, suspended sediment , Phosphorus and nitrogen forms from a small agricultural catchment. N. Z. J. Agric. Res. 21, 29-38.

Barker A V and Maynard D N 1971 Nutritional factors affecting nitrate accumulation in spinach. Commun. Soil Sci. Plant Anal. 2, 471-478.

Barley K P and Naidu N A 1964 The performance of three Australian wheat varieties at high levels of nitrogen supply. Aust. J. Exp. Agric. Anim. Husb. 4, 39-48.

Barnes B T and Ellis F B 1979 Effects of different methods of cultivation and direct drilling and diaposal of straw residues on populations of earthworms. J. Soil Sci. 30, 669-679.

Barraclough P B 1986 The growth and activity of winter wheat roots in the field: Nutrient inflows of high yielding crops. J. Agric. Sci. Camb. 106, 53-59.

Barraclough P B 1989 Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. Plant and Soil 119, 59-70.

Barrow N J 1960 Stimulated decomposition of organic matter during the decomposition of added organic materials. Aust. J. Agric. Res. 11, 331-338.

Bauder J W and Montgomery B R 1979 Overwinter redistribution and leaching of fall-applied nitrogen. Soil Sci. Soc. Am. J. 43, 744-747.

Beachell H M, Khush R S and Juliano B O 1972 Breeding for high protein content in rice *In* Rice Breeding. pp 419-428. Int. Rice Res. Inst: Los Banos, Philippines.

Bechtel D B, Gains R L and Pomeranz Y 1982 Protein secretion in wheat endosperm formation of the matrix protein. Cereal Chem. 59, 336-343.

Beevers L and Hageman R H 1969 Nitrate reduction in higher plants. Ann. Rev. Plant Physiol. 20, 495-522.

Benbi D K 1990 Efficiency of nitrogen use by dryland wheat in a subhumid region in relation to optimising the amount of available water. J. Agric. Sci. Camb. 115, 7-10.

Best, E K 1976 An automated method for determining nitrate-nitrogen in soil extracts. Qld. J. Agric. Anim. Sci. 33, 161-166.

Bhatia C R and Rabson R 1976 Bioenergetic considerations in cereal breeding for protein improvement. Sci. 194, 1418-1421.

Bhullar S S and Jenner C F 1986 Effect of temperature on the conversion of sucrose to starch in the developing wheat endosperm. Aust. J. Plant Physiol. 13, 605-615.

Biggar J W 1978 Spatial variability of nitrogen in soils. *In* Nitrogen in the Environment: Vol. 1, Nitrogen Behaviour in Field Soil. Eds. D R Nielsen and J G MacDonald. pp 201-222. Academic Press: New York.

Blum A and Pnuel Y 1990 Physiological attributes associated with drought resistance of wheat cultivars in a Mediterranean environment. Aust. J. Agric. Res. 41, 799-810.

Boatwright C O and Haas H J 1961 Development and composition of spring wheat as influenced by nitrogen and phosphorus fertilisation. Agron. J. 53, 33-36.

Bolin B and Arrhenius E 1977 Nitrogen an essential life factor and a growing environmental hazard. Ambio 6, 96-105.

Brady D J, Gregory P J and Fillery I R P 1993 The contribution of different regions of the seminal roots of wheat to uptake of nitrate from soil. Plant and Soil 155/156, 155-158.

Bray R H 1954 A nutrient mobility concept of soil-plant relationships. Soil Sci. 78, 9-22.

Bremner, J. M. 1965 Nitrogen availability indices. *In* Methods of Soil Analysis, part 2 Ed. C A Black. pp 1324-1345. Am. Soc. Agron : Madison.

Breniman G W, Neuman A L, Smith G S and Zimmerman J E 1961 Factors affecting the nitrate content of forages. J. Anim Sci. 20, 926.

Brooks A, Jenner C F and Aspinall D 1982 Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. Aust. J. Plant. Physiol. 9, 423-436.

Brown J R and Smith G E 1966 Soil fertilisation and nitrate accumulation in vegetables. Agron. J. 58, 209-212.

Brown J R and Smith G E 1967 Nitrate accumulation in vegetable crops as influenced by soil fertility practices. Univ Miss Coll Agric Expt Sta Res Bull No. 920.

Brown S C, Gregory P J and Wahbi 1987 Root characteristic and water use in Mediterranean environments. *In* Drought Tolerance in Winter Cereals. Eds. J P Srivastava, E Proceddu, E Acevedo and S Varm. pp 275-283. Wiley: New York.

Brown T H 1977 Rate of loss of dry matter and change in chemical composition of nine pasture species over summer. Aust. J. Exp. Agric. Anim. Husb. 17, 75-79.

Buddenhagen I W 1990 Legumes in farmer systems in Mediterranean climates. *In* The Role of Legumes in the Farming Systems of the Mediterranean areas. Eds. A E Osman, M H I Brabim and M A Jones. pp 3-29. Kluwer: Amsterdam.

Burden R J 1982 Nitrate contamination of New Zealand aquifers: a review. N. Z. J. Sci. 25, 205-220.

Burns I G 1980 Influence of the spatial distribution of nitrate on the uptake of N by plants: A review and a model for rooting depth. J. Soil Sci. 31, 155-173.

Cameron I H 1966 Dry matter and quality change during and after making pasture hay. Aust. J. Exp. Agric. Anim. Husb. 6, 394-401.

Cameron K C and Scotter D R 1986 Nitrate leaching losses from temperate agricultural systems *In* Nitrogen Cycling in Temperate Agricultural System. Eds. P E Bacon, J Bvans, R R Storrier and A C Taylor. pp 251-281.

Cameron K C and Wild A 1984 Potential aquifer pollution from nitrate leaching following the plowing of temporary grassland. J . Environ Qual. 13, 274-278.

Campbell C A and E A Paul 1978 Effect of fertiliser N and soil moisture on mineralisation, N recovery, A-values under spring wheat grown in small lysimeters. Can. J. Soil Sci. 58, 39-51.

Campbell C A, Davidson H R and Warder F G 1977 Effects of fertiliser N and soil moisture on yield, yield components, protein content and N accumulation in the aboveground parts of spring wheat. Can. J. Soil. Sci. 57, 311-327.

Campbell C A, Davidson H R and Winkelman G E 1981 Effect of nitrogen, temperature, growth stage and duration of moisture stress on yield components and protein content of Manitou spring wheat. Can. J. Plant Sci. 61, 549-563.

Cassman K G and Munns D N 1980 Nitrogen mineralization as affected by soil moisture, temperature and depth. Soil Sci. Soc. Am. J. 44, 1233-1237.

Cataldo D A, Haroon M, Schrader L E and Youngs V L 1975 Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6 , 71-80.

Ceccareli S, Acevedo E and Grando S 1991 Breeding for field stability in unpredictable environments: Single traits, interaction between traits, and architecture of genotypes. Euphytica 56, 169-185.

Chancellor W J and Roos J R 1976 Balancing energy and food production: 1975-2000. Sci. 192, 213-218.

Clarke A L and Russell J S 1977 Crop sequential practices. *In* Soil Factor in Crop Production in a Semi-arid Environmen. Eds. E L Greacen and J S Russell. pp 279-300. Univ. Queensland Press: St. Lucia.

Clay D E, Clapp C E, Dowdy R H and Molina J A E 1993 Mineralization of nitrogen in fertilizer-acidified lime-amended soils. Biolo. Fertil. Soils. 15, 249-252.

Colwell J D 1963 The estimation of phosphorus fertiliser requirements of wheat in southern New South Wales by soil analysis. Aust. J. Exp. Agric. Anim. Husb. 3, 190-197.

Cooper J L and Blakeney A B 1990 The effect of two forms of nitrogen fertiliser applied near anthesis on the grain quality of irrigated wheat. Aust. J. Exp. Agric. 30, 615-619.

Cooper P J M 1983 Crop management in rainfed agriculture with special reference to water use efficiency. *In*: Nutrient Balances and the Need for Fertilisers in Semi-arid and Arid Regions. pp 63-79. Proc. 17th Coll. Int. Potash Institute, Rabat and Marrakech, Morocco, Bern.

Cornforth I S 1968 Relationships between soil volume used by roots and nutrient accessibility. J. Soil Sci. 19, 291-301.

Cox M C, Qualset C O and Rains D W 1985 Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein. Crop Sci. 25, 435-440.

Craswell E T and Godwin D C 1984 The efficiency of nitrogen fertiliser applied to cereals in different climates. Adv. Plant. Nut. 1, 1-55.

Craswell E T and Strong W M 1976 Isotopic studies of the nitrogen balance in a cracking clay. Nitrogen recovery in plant and soil in relation to the depth of fertiliser addition and rainfall. Aust. J. Soil Res. 14, 75-83.

Crawford R F Kennedy W K and Jonson W C 1961 Some factors that affect nitrate accumulation in forages. Agron. J. 53, 159-162.

Crisp D T 1966 Input and output of minerals for an area of pennine moorland: the importance of precipitation, drainage, peat erosion and animals. J. Appl Ecol. 3, 327-348.

Cuttle S P and Bournce P C 1993 Uptake and leaching of nitrogen from artificial urine applied to grass land on different dates during the growing season. Plant and Soil 150, 77-86.

Dahmane A B K 1978 Nitrogen accretion by annual medicago species in a semi-arid environment. Ph.D. thesis University of Adelaide, South Australia.

Daigger L A and Sander D H 1976 Nitrogen availability to wheat as affected by depth of nitrogen placement. Agron. J. 68, 524-526.

Dalling M J, Boland G and Wilson J H 1976 Relation between acid proteinase activity and redistribution of N during grain development in wheat. Aust. J. Plant Physiol. 3, 721-730.

Dann P R 1969 Response by wheat to phosphorus and nitrogen with particular reference to " haying off". Aust. J. Exp. Agric. Anim. Husb. 9, 625-629.

Darwinkel A 1975 Aspects of assimilation and accumulation of nitrate in some cultivated plants. Ph.D. thesis, University of Wageningen.

Darwinkel A 1983 Ear formation and grain yield of winter wheat as affected by time of nitrogen supply. Neth. J. Agric. Sci. 31, 211-25.

Dassk, Subba Reddy G, Sharma K L, Vittal K P R, Venkates warlu B, Narayana Reddy M and Reddy Y V R 1993 Prediction of nitrogen availability in soil after crop residue incorporation. Fert. Res. 34, 209-215.

De Willigen P and Van Noordwijk M 1987 Roots, plant production and nutrient use efficiency. Ph.D. thesis, University of Wageningen, The Netherlands. 282p.

Deckard E L, Tsai C Y and Tucker T C 1986 Effect of nitrogen nutrition on quality of agronomic crops. *In*: Nitrogen in Crop Production. Ed. R D Hauck. pp 601-615. Amer.Soc. Agron: Madison

Dedatta S K, Obcemea W N and Jana R K 1972 Protein content of rice grain as affected by nitrogen fertiliser and some triazines and substituted ureas. Agron. J. 64, 785-788.

Department of Agriculture South Australia 1985 Termination Report (Project 14) for the Wheat Industry Research Committee of South Australia.

Department of Agriculture South Australia 1990 Beating the wheat protein crisis. Bulletin 4/90.

Diggle A J, Bowden J W and D'Antuono M F 1990 A comparison of the effects of mineral and organic nitrogen sources on the distribution of wheat roots in a leaching environment. Aust. J. Soil Res. 28, 963-971.

Donald C M 1963 Grass or crop in the land use of tomorrow. Aust. J. Sci. 25, 386-395.

Donald C M 1964 The progress of Australian agriculture and the role of pastures in environmental change. Aust. J. Sci. 27, 187-198.

Donovan G R and Lee J W and Hill R D 1977 Composition changes in the developing grain of high and low protein wheats. I. Chemical composition. Cereal Chem. 54, 638-645.

Dowdell R J and Cannell R Q 1975 Effect of ploughing and direct drilling on soil nitrate content. J. Soil Sci. 26, 53-61.

Doyle A D and Shapland R A 1991 Effect of split nitrogen applications on the yield and protein content of dryland wheat in northern New South Wales. Aust. J. Exp. Agric. 31, 85-92.

Drew M C and Saker L R 1975 Nutrient supply and the growth of the seminal root system in barley. II. Localised, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. J. Exp. Bot. 26,70-90.

Drew M C, Saker L R and Ashley T W 1973 I. The effect of nitrate concentration on the growth of axes and laterals. J. Exp. Bot. 24, 1189-1202.

Dyson C and Fewings P 1990 Trends in protein levels of A.S.W. wheat grown in South Australia. Dept. Agric. South. Aust. Technical Report No.158.

Eck H V 1988 Winter wheat response to nitrogen and irrigation. Agron. J. 80, 902-908.

Egmond F V and Breteler H 1972 Nitrate reduction activity and oxalate content of sugarbeet leaves. Neth. J. Agric. Sci. 20, 193-198.

Ehlers W 1976 Rapid determination of unsaturated hydraulic conductivity in tilled and untilled loess soil. Soil Sci. Soc. Am. J. 40, 837-840.

Ehlers W 1977 Measurement and calculation of hydraulic conductivity in horizons of tilled and untilled loess-derived soil, Germany. Geoderma 19, 293-306.

El-Sheikhler A M and Broyer T C 1970 Concentration of total nitrogen in squash cucumber and melon in relation to growth and to a Piper-Steenbjerg effect. Commun. Soil Sci. Plant Anal. 1, 213-219.

Elliott B R and Jardine R 1972 The influence of rotation system on long-term trends in wheat yield. Aust. J. Agric. Res. 23, 935-944.

Elliott D E and Clarke A L 1975 Seasonal response of pasture to nitrogen fertiliser in the Mt. lofty Ranges, South Australia. Aust. J. Exp. Agric. Anim Husb. 15, 231-238.

Elliott D E, Growden B and Reuter D J 1985 Nitrogen deficiency and wheat, its prediction and correction. Termination report (Project 14) for the Wheat Industry Research Committee of South Australia. Dept. Agric. South. Aust. Adelaide.

Elrick D E and French L K 1966 Miscible displacement patterns on disturbed and undisturbed soil cores. Soil Sci. Soc. Am. Proc. 30, 153-156.

Engel R E 1991 Simulated growing season precipitation and nitrogen effects on winter wheat. Agron. J. 83, 180-185.

Farquhar G B, Firth P M, Wetselaar R and Weir B 1980 On the gaseous exchange of ammonia between leaves and the environment. Determination of the ammonia compensation point. Plant. Physiol. 6, 710-714.

Fenn L B and Hossner L R 1985 Ammonium volatilization from ammonia or ammonium-containing fertiliser. Adv. Soil Sci. 1, 123-169.

Feyter C and Cossens G G 1977 Effect of rates and methods of nitrogen application on grain yields and yield components of spring-sown wheats in South Otago N. Z. J. Exp. Agric. 5, 371-376.

Fillery I R P 1983 Biological denitrification. *In* Gaseous Losses of Nitrogen from Plant-Soil Systems. Eds. J R Freney and J R Simpson. pp 33-64. Nijhoff: The Hague.

Finney K F, Meyer J W, Smith F W and Fryer H C 1957 Effect of foliar spraying of Pawnee wheat with urea solutions on yield, protein content and protein quality. Agron. J. 49, 341-347.

Ford G W 1968 Partly humified organic matter in soils, its contribution to mineralisable nitrogen. Ph.D. Thesis, University of Adelaide, South. Australia.

Fox R L and Lipps R C 1960 Distribution and activity of roots in relation to soil properties. Trans.7th Int. Congr. Soil Sci, Madison 3, 260-267.

French R J 1978a The effect of fallowing on the yield of wheat. I. The effect on soil water storage and nitrate supply. Aust. J. Agric. Res. 29, 653-668.

French R J 1978b The effect of fallowing on the yield of wheat. II. The effect of grain yield. Aust. J. Agric. Res. 29, 669-684.

French R J and Schultz J E 1984 Water use efficiency of wheat production in a Mediterranean-type environment. I. The relationship between yield, water use and climate. Aust. J. Agric. Res. 35, 743-764.

French R J, Matheson W E and Clarke A L 1968 Soils and Agriculture of the Northern and York Peninsula Region of South Australia. Dept. Agric. South. Aust. Adelaide.

Freney J R and Simpson J R 1983 Preface. *In* Gaseous Loss of Nitrogen from Plant-Soil Systems Eds. J R Freney and J R Simpson. p 7. Martinus Nijhoff/Dr W. Junk Publishers: The Hague, Netherlands.

Gardner B R and Jackson E B 1976 Fertilisation nutrient composition and yield relationship in irrigated spring wheat. Agron. J. 68, 75-78.

Gass W B, Peterson G A, Hauck R D and Olson R A 1971 Recovery of residual nitrogen by corn (Zea mays L.) from various soil depths as measured by ^{15}N tracer techniques. Soil Sci. Soci. Am. Proc. 35, 290-294.

Gasser J K R 1964 Some factors affecting losses of ammonium from urea and ammonium sulfate applied to soils. J. Soil Sci. 15, 258-272.

Gates C T, Jones D B, Muller W J and Hicks J S 1981 The interaction of nutrients and tillage methods on wheat and weed development. Aust. J. Agric. Res. 32, 227-241.

Gentilli J (Editor) 1971 Climates of Australia and New Zealand. World Survey of Climatology. Vol. 13 p 405. Elsevier: Amsterdam.

George J R, Rhykerd C L and Noller C H 1971 Effect of light intensity temperature nitrogen and stage of growth on nitrate accumulation and dry matter production of a sorghum-sudangrass hybrid. Agron. J. 63, 413-415.

George T, Ladha J K, Buresh R J and Garrity D P 1993 Nitrate dynamics during the aerobic soil phase in lowland Rice-Based Cropping systems. Soil Sci. Soc. Am. J. 57, 1526-1532.

Gilbert C S, Eppson H F, Bradley W B and Beath O A 1946 Nitrate accumulation in cultivated plants and weeds. Univ. Wyom. Agric. Expt. Sta. Bul. 277.

Giles L C, Hancock T W, Eckert S R and Pattison S 1994 An introduction to GENSTAT 5. Faculty of Agricultural and Natural Resource Science, University of Adelaide.

Goulding K W T, Webster C P, Powlson D S and poultion P R 1993 Denitrification losses of nitrogen fertiliser applied to winter wheat following ley and arable rotation as estimated by acetylene inhibition and ^{15}N balance. J. Soil Sci. 44, 63-72.

Greenland D J 1971 Changes in the nitrogen status and physical condition of soils under pasture, with special reference to the maintenance of the fertility of Australian soils used for growing wheat. Soils Fert. 34, 237-251.

Gregory P J , Shepherd K D and Cooper P J M 1984 Effects of fertiliser on root growth and water use of barley in northern Syria. J. Agric. Sci. Camb. 103, 429-438.

Gregory P J, McGowan M, Biscoe P V and Hunter B 1978 Water relations of winter wheat. I. Growth of the root system. J. Agric. Sci. Camb. 91, 91-102.

Guettinger D L and Koehler F E 1963 Nitrogen relationship in wheat plant Agron. J. 55, 409-410.

Gupta S K, Chaudhary T N and Pandey R N 1982 Nitrate movement in sandy soil under varying water and fertilizer management. Aust. J. Soil Res. 20, 225-232.

Hadas A, Feigenbaum S and Portnoy R 1989 Nitrogen mineralisation in the field at various soil depths. J. Soil Sci. 40, 131-137.

Hageman R H and Flesher D 1960 Nitrate reductase activity in corn seedling as affected by light and nitrate content of nutrient media. Plant Physiol. 35, 700-708.

Hageman R H, Flesher D and Gitter A 1961 Diurnal variation and other light effect influences the activity of NO_3 reductase and nitrogen metabolism in corn. Crop Sci. 1, 201-204.

Halloran G M 1981 Grain yield and protein relationships in a wheat crops. Crop Sci. 21, 699-701.

Halse N J, Greenwood E A N, Lapins P and Boundy C A P 1969 An analysis of the effect of nitrogen deficiency on the growth and yield of a Western Australian wheat crop. Aust. J. Agric. Res. 20, 987-988.

Hamblin A and Tennant D 1987 Root length density and water uptake in cereals and grain legumes: How well are they correlated? Aust. J. Agric. Res. 38, 513-27.

Hamblin A P and Hamblin J 1985 Root characteristics of some temperate legume species and varieties on deep, free-draining cntisols. Aust. J. Agric. Res. 36, 63-72.

Hamblin A, Tennant D and Perry M W 1987 Management of soil water for wheat production in western Australia. Soil Use Manag. 3, 63-69.

Hamid A and Sarwar G 1976 Effect of split application on N uptake by wheat from N labelled ammonium nitrate and urea. Exp. Agric. 12, 189-193.

Hanway J J 1962 Corn growth and composition in relation to soil fertility: II. Uptake of N, P and K and their distribution in different plant parts during the growing season. Agron. J. 54, 217-227.

Hanway J J and Englehorn A J 1958 Nitrate accumulation in some Iowa crop plants Agron. J. 50, 331-334.

Hanway J J and weber C R 1971 Accumulation of N, P and K by soybean (Glycine max. (L.) Merrill) plants. Agron. J. 63, 406-408.

Harms C L and Tucker B B 1973 Influence of nitrogen fertilisation and other factors on yield, prussic acid, nitrate, and total nitrogen concentration of sudangrass cultivars Agron. J. 65, 21-26.

Harmsen G W 1961 The effect of weather conditions fertilizing and vegetation on the nitrogen content of soil. Landw Forsch Sonderh. 15, 61-74.

Harmsen G W and Van Schreven D A 1955 Mineralisation of organic nitrogen in soil. Adv. Agron. 7, 299-398.

Harmsen K 1984 Nitrogen fertiliser use in rainfed agriculture. Fert. Res. 5, 371-382.

Harmsen K, Shepherd K D and Allan A Y 1983 Crop response to nitrogen and phosphorus in rainfed agriculture. *In*: Nutrient Balances and the Need for Fertilisers in Semi-arid and Arid Regions. pp 223-257. Proc. 17th Coll. Int. Potash Institute, Rabat and Marrakech, Morocco, Bern.

Hart P B S, Goh K M and Ludecke T E 1979 Nitrogen mineralisation in Fallow and wheat soils under field and laboratory conditions. N. Z. J. Agric. Res. 22, 115-125.

Hauck R D and Bremner J M 1976 Use of tracers for soil nitrogen research. Adv. Agron. 28, 219-266.

Haynes R J 1986 Retention and movement of nitrogen in soil. *In* Mineral Nitrogen in the Plant-Soil System. Ed. R J Haynes. pp 166-240. Academic Press: New York.

Henin, S. and Gras, R. 1962 Vigueur des arbers fruitiers et epaisseur de sol exploitee par les racines. Compt Rend. Acad. Sci. Paris. 255, 1775-1777.

Herbst F and Stumpe H 1984 Stickstoffverlust und Stickstoffwirkung bei Dungerausbringung im Herbst. Arch. Acker- Pflanzenbau Bodenk. 28, 603-609.

Hewitt E J and Smith T A 1975 Plant Mineral Nutrition. Wiley: New York

Holford I C R 1981 Changes in nitrogen and organic carbon of wheat-growing soil after various periods of grazed lucerne extended fallowing and continuous wheat. Aust. J. Soil Res. 19, 239-249.

Holford I C R and Doyle A D 1978 Effect of grazed lucerne on the moisture status of wheat-growing soils. Aust. J. Exp. Agric. Anim Husb. 18, 112-117.

Holford I C R and Doyle A D 1992 Yield responses and nitrogen fertilizer requirements of wheat in relation to soil nitrate levels at various depths. Aust. J. Soil Res. 30, 683-694.

Holland P T and During C 1977 Movement of nitrate-N and transformation of urea-N under field conditions. N. Z. J. Agric Res. 20, 479-488.

Holt E C and F L Fisher 1960 Root development of coastal bermuda grass with high nitrogen fertilisation. Agron. J. 52, 593-596.

Hunter A A and Stanford G 1973 Protein content of winter wheat in relation to rate and time of nitrogen fertiliser application . Agron. J. 65, 772-774.

Huntjens J L M 1971a Influence of living plants on immobilisation of nitrogen in permanent pastures. Plant and Soil 34, 393-404.

Huntjens J L M 1971b The influence of living plants on mineralisation and immobilisation on nitrogen. Plant and Soil 35, 77-94.

Hurd E A and Spratt E D 1975 Root patterns in crops as related to water and nutrient uptake. *In* Physiological Aspects of Dryland Farming. Ed. U S Gupta. pp 167-235 Oxford IBH: New Dehli, India.

Hylton D L, Williams D E, Ulrich A and Cornelius D R 1964 Critical nitrate levels for growth of Italian ryegrass. Crop Sci. 4, 16-19.

Hylton L O, Cornelius D R and Ulrich A 1970 Nitrogen nutrition and growth relations of tall and intermediate wheat grasses. Agron. J. 62, 353-356.

Jarvis S C and Pain B F 1990 Ammonia volatilization from agricultural land. Proc. Fert. Soc. 298, 1-35.

Jenkinson D S 1990a An introduction to the global nitrogen cycle. Soil Use Manag. 6, 56-61.

Jenner C F, Ugalde T D and Aspinall D 1991 The physiology of starch and protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. 18, 211-226.

Jones J B, Jr 1967 Interpretation of plant analysis for several agronomic crops. *In* Soil Testing and Plant Analysis. pp 49-58. Special Publ Ser No 2. Soil Sci. Soc. Am: Madison, Wisc.

Kanemasu E T, Stainer J L, Biere A W, Worman E D and Stone J F 1983 Irrigation in great plains. Agric. Water Manag. 7, 157-178.

Kaul A K 1977 Protein resources and production. Ambio 6, 141-145.

Keeney D R 1982 Nitrogen management for maximum efficiency and minimum pollution. *In* Nitrogen in Agricultural Soils. Ed F J Stevenson. pp 605-650. Am Soc Agron: Madison Wisc.

Keller W D and Smith G E 1967 Ground water contamination by dissolved nitrate. Geol. Soc. Am. Spec. Pap. 90, 48-59.

Kessler E 1964 Nitrate assimilation by plants. Ann. Rev. Plant Physiol. 15, 57-72.

Kinjo T and Pratt P F 1971 Nitrate adsorption: I. In Some Acid Soil of Mexico and South America. Soil Sci. Soc. Am. Proc. 35, 722-732.

Kissel D F, Ritchie J T and Burnett E 1974 Nitrate and chloride leaching in a swelling clay soil. J. Environ. Qual. 3, 401-404.

Kmoch H G, Raming R E, Fox R L and Koehler F E 1957 Root development of winter wheat as influenced by soil moisture and nitrogen fertilisation. Agron. J. 49, 20-25.

Kohn G D , Osborne G J, Batten G D, Smith A N and Lill W J 1977 The effect of top dressed superphosphate on changes in nitrogen, carbon: Sulphur, phosphorus and pH on a red earth soil during a long term grazing experiment. Aust. J. Soil Res. 15, 147-158.

Kramer T 1978 Environmental and genetic variation for protein content in the winter wheat (*Triticum aestivum L.*). Euphytica 28, 209-218.

Kretschmer A E 1958 Nitrate accumulation in everglades forages. Agron. J. 50, 314-316.

Kuhlmann H and Barraclough P B 1987 Comparison between the seminal and nodal systems of winter wheat in their activity for N and K uptake. Z Pflanzenernahr. Bodenkd 150, 24-30.

Kuhlmann H, Barraclough P B and Weir A H 1989 Utilisation of mineral nitrogen in the sub soil by winter wheat. Z Pflanzenernaehr. Bodenkd. 152, 291-295.

Ladd J N 1990 Soil nitrogen dynamics and nitrogen uptake by wheat crops. Dept. Agric. South. Aust. Technical Report No. 159.

Ladd J N and Amato M 1986 The fate of nitrogen from legume and fertilizer sources in soils successively cropped with wheat under field conditions. Soil Biol. Biochem. 18, 417-425.

Ladd J N, Oades J M and Amato M 1981 Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biol. Biochem. 13, 251-256.

Ladd J N, Oades J M and Amato M 1981a Microbial biomass formed from ^{14}C, ^{15}N-labelled plant material decomposing in soils in the field . Soil Biol. Biochem. 13.119-126.

Langer R H M and Liew F K Y 1973 Effect of varying nitrogen supply at different stages of the reproductive phase on spikelet and grain production and on grain nitrogen in wheat. Aust. J. Agric. Res. 24, 647-665.

Large E C 1954 Growth stages in cereals. Plant Pathol. 3, 128.

Leeper G W 1970 The Australian Environment. 4th Edition p 163.CSIRO/MUP: Melbourne.

Lieshout J W V 1960 Invloed van het bodemmilieu op ontwikkeling en activiteit van het wortelstelsel. Diss. Wageningen (Pudoc) p 91.

Littler J W 1963 Effect of time and rate of application of urea to wheat. Qld. J. Agric. Sci. 20, 323-344.

Loffler G M and Busch R H 1982 Selection for grain protein yield and nitrogen partitioning efficiency in hard red spring wheat. Crop Sci. 22, 591-595.

Loffler G M, Rauch T L and Busch R H 1985 Grain and plant protein relationships in hard red spring wheat. Crop Sci. 25, 521-524.

Lovelace D A, Holt E C and Anderson W B 1968 Nitrate and nutrient accumulation in two varieties of bermuda grass (Cynodon dactylon (L.) pers.) as influenced by soil-applied fertiliser nutrients. Agron. J. 60, 551-554.

MacGregor J M, Blake G R and Evans S D 1974 Mineral nitrogen movement into subsoils following continued annual fertilisation for corn. Soil Sci. Soc. Am. Proc. 38, 110-113.

MacKenzie A J Spenser W F Stockinger K R and Krantz B A 1963 Seasonal nitrate-nitrogen content of cotton petioles as affected by nitrogen application and its relationship to yield. Agron. J. 55, 55-59.

MacKown C T and van Sanford D A 1986 Post anthesis nitrate assimilation in winter wheat. Plant Physiol. 81,17-21.

Martin W E and Matocha J W 1973 Plant analysis as an aid in the fertilisation of forage crops. *In* Soil Testing and Plant Analysis. Eds. L M Walsh and J D Beatona pp 393-426 Soil Sci Soc Am: Madison.

Mason M G 1986b Late nitrogen for wheat crops. Western Australian Department of Agriculture, Division of Plant Research Technical Report No. 5.

Mason M G 1991 Timing of nitrogen fertiliser application for cereals. Division of plant Industries Western Australian Department of Agriculture, Division of Plant Industries Technical Report No. 36.

Mason M G, Rowley A M and Quayle D J 1972 The fate of urea applied at various intervals after sowing of a wheat crop on a sandy soil in Western Australia. Aust. J. Exp. Agric. Anim. Husb. 12, 171-175.

Matzel W, Teske W and Lippold H 1984 Nitrataufnahme aus unterschiedlichen Bodentiefen durch Wintergetreide. Arch. Ackerpflanzenbau Bodenkd. 28, 393-401.

Maynard D N, Barker A V, Minotti P L and Peck N H 1976 Nitrate accumulation in vegetables. Adv. Agron. 28, 71-117.

McDonald G K 1989 The contribution of nitrogen fertilizer to the nitrogen nutrition of rainfed wheat crops in Australia. a review. Aust. J. Exp. Agric. 29, 455-481.

McDonald G K 1992 Effects of nitrogenous fertiliser on the growth, grain yield and grain protein concentration of wheat. Aust. J. Agric. 43, 949-967.

McGarity J W and Myers R J K 1973 Seasonal trends in the content of mineral nitrogen in solodised solonets soil. Aust. J. Exp. Agric. Anim Husb. 13, 423-429.

McKenzie, H A and Wallace H S 1954 The Kjeldahl determination of nitrogen: A critical study of digestion condition-temperature, catalyst and oxidizing agent. Aust. J. Chem. 7, 55-70.

McTaggart I P and Smith K A 1993 Estimation of potentially mineralisable nitrogen in soil by KCl extraction. Plant and Soil 157, 175-184.

Meagher J W and Rooney D R 1966 The effect of crop rotation in the Victorian Wimmera on the cereal cyst nematode (Heterodera avenae) nitrogen fertility and wheat yield. Aust. J. Exp. Agric. Anim. Husb. 6, 425-431.

Meek B D, Grass L B and MacKenzine A J 1969 Applied nitrogen loss in relation to oxygen status of soils. Soil Sci. Soc. Am. Proc. 33, 575-578.

Mengel D B and Barber S A 1974 Rate of nutrient uptake per unit of corn root under field conditions. Agron. J. 66, 399-402.

Miller R D and Johnstone D D 1964 The effect of soil moisture tension on carbon dioxide evolution nitrification and nitrogen mineralization. Soil Sci. Soc. Am. Proc. 28, 644-647.

Minotti P L and Stankey D L 1973 Durnal variation in the nitrate concentration of beets. Hort. Sci. 8, 33-34.

Mohammed I H, Scotter D R and Gregg P E H 1984 The short-term fate of urea applied to barley in a humid climate. I Experiments. Aust. J. Soil Res. 22, 173-180.

Muirhead W A, Melhuish F M, White R J G and Higgins M L 1985 Comparison of several nitrogen fertiliser applied in surface irrigation systems. II Nitrogen transformations. Fert. Res. 8, 49-65.

Murphy L S and Smith G E 1967 Nitrate accumulation in forage crops. Agron. J. 59, 171-174.

Myers R J K 1978 Nitrogen and phosphorus nutrition of dryland grain sorghum at Katherine Northern Terriotory. Aust. J. Exp. Agric. Anim. Husb. 18, 834-843.

Myers R J K 1983 The effect of plant residues on plant uptake and leaching of soil and fertilizer nitrogen in a tropical red earth soil. Fert. Res. 4, 249-260.

National Research Council 1978 Nitrates: an environmental assessment. Nat. Acad. Sci: Washington, D.C.

Nicolas M E, Gleadow R M and Dalling M J 1985a Effect of post-anthesis drought on cell division and starch accumulation in developing wheat grains. Ann. Bot. 54, 433-444.

Nicolas M E, Simpson R J, Lambers H and Dalling M 1985 Effects of drought on partitioning of nitrogen in two wheat varieties differing in drought-tolerance. Ann. Bot. 55, 743-754.

Nix E L 1975 The Australian climate and its effects on grain yield and quality. In: Australian Field Crops Vol. 1: Wheat and other temperate cereals. Eds. A. Lazenly and E.M. Matheson. pp 183-226. Angus and Robertson: Sydney.

Nommik Hans 1973 Assessment of volatilisation loss of ammonium from surface-applied urea on forest soil by N recovery. Plant and Soil 38, 589-603.

Novoa R and Loomis R S 1981 Nitrogen and plant production. Plant and Soil. 58, 177-204.

Nowakowski T Z 1961 The effect of different nitrogenous fertilisers applied as solids or solutions on the yield and nitrate-N content of established grass and newly sown ryegrass. J. Agric. Sci. Camb. 56, 287-292.

Noy-Meir I and Harpaz Y 1977 Agroecosystem in Israel. Agroecosystems. 4, 143-167.

Nye P H and Tinker P B 1977 Solute Movement in the Soil Root System. p 212. Blackwell: Oxford.

Olson R A and Kurtz L T 1982 Crop nitrogen requirements, utilization, and fertilization. *In* Nitrogen in Agricultural Soils. Ed. F G Stevenson. pp 567-604. Madison: Wisc.

Olson R A, Dreier A F, Thompson C, Frank K and Grabouski P H 1964a Using fertiliser nitrogen effectively on grain crops. Nebr. Agric. Exp. Stn. Bull 479. pp 10-14.

Osborne G J 1984 Tillage practices and soil properties. Proc. Nat. Soils Conf. pp 104-126. Aust. Soc. Soil Science: Brisbane.

Owens L B 1981 Effects of nitrapyrin on nitrate movement in soil columns. J. Environ Qual. 10, 308-310.

Palmer G A 1990 Wheat protein and end product quality. Dept Agric South. Aust. Report No. 161.

Papastylianou I 1980 Nitrate concentration in cereal stems and its use in evaluation rotations and predicting nitrogen fertiliser requirement. Ph.D. Thesis, University of Adelaide.

Papastylianou I 1987 Effect of wheat and barley varietal differences in nitrate concentration on plant analysis interpretation. Fert. Res. 12, 157-163.

Papastylianou I and Puckridge D W 1981 Nitrogen nutrition of cereal in a short-term rotation. II. Stem nitrate as an indication of nitrogen availability. Aust. J. Agric. Res. 32, 713-23.

Papastylianou I and Puckridge D W 1983 Stem nitrate nitrogen and yield of wheat in a permanent rotation experiment. Aust. J. Agric. Res. 34, 599-606.

Parrott R T and Donald C M 1970 Growth and ignitability on annual pastures in a Mediterranean environment. I. Effect of length of season and the defoliation on the growth water content and desiccation of annual pastures. Aust. J. Exp. Agric. Anim Husb. 10, 67-75.

Parton W J, Mogan J A, Altenhofen J M and Harper L A 1988 Ammonia volatilization from spring wheat plants. Agron. J. 80, 1423-1436.

Passioura J B and Wetselaar R 1972 Consequence of banding fertiliser N in soil. II Effect on the growth of wheat roots. Plant and Soil. 36, 461-473.

Pate J S 1971 Movement of nitrogenous solutes in plants. *In* Nitrogen-15 in Soil Plant Studies. pp 165-187. Atomic Energy Agency: Vienna.

Pate J S and Layzell D B 1981 Carbon and nitrogen partitioning in the whole plant- A thesis based on empirical modelling. *In* Nitrogen and Carbon Metabolism. Ed. J D Bewley. pp 94-134. Nijhoff: the Hague, Netherlands.

Pate J S, Layzell D B and McNeil D L 1979 Modelling the transport and utilisation of carbon and nitrogen in a nodulated legume. Plant Physiol. 63, 730-737.

Patrick Wm H, J R. and Wyatt Ronald 1964 Soil nitrogen loss as a result of alternate submergence and drying. Soil Sci. Soc. Am. 28, 647-653.

Pearson C J and Muirhead W A 1984 Nitrogen uptake. *In* Control of Crop Productivity. Ed C J Pearson. pp 73-88. Academic Press: New York.

Penning de Vries F W T, Brunsting A H M and Van laar H H 1974 Products requirements and efficiency of biosynthesis: a quantitative approach. J. Theor. Biol. 45, 339-337.

Perry M and Hillman B (eds) 1991 The Wheat Book. A Technical Manual for Wheat Producers. Department of Agriculture, Western Australia. Bull No. 4196. p 109.

Pimentel D, Dritschilo W, Krummel J and Kutzman J 1975 Energy and land constraints in food protein production. Sci. 190, 754-761.

Powlson D S, Hart P B S, Pruden G and Jenkinson D S 1986b Recovery of N-labelled fertiliser applied in autumn to winter wheat at four sites in eastern England. J. Agric. Sci. Camb. 107, 611-620.

Pratt P F , Jones W W and Hunsaker V E 1972 Nitrate in deep soil profile in relation to fertiliser rates and leaching volume J. Environ Qual. 1, 97-102.

Pratt P F, Stertling D, Sharpless R G, Paugh W J and Bishop S E 1976 Nitrate content of sudangrass and barley grown in plots treated with animal manures. Agron. J. 68, 311-314.

Prescott J A and Piper G R 1930 Nitrate fluctuations in a South Australian soil. J. Agric. Sci. Camb. 20, 517-531.

Prescott J A and Thomas J A 1949 The length of the growing season in Australia as determined by the effectiveness of the rainfall. Proc. Roy. Geog. Soc. South Aust. 50, 42-46.

Puckridge D W 1968 Photosynthesis of wheat under field conditions I. The interaction of photosynthetic organs. Aust. J. Agric. Res. 19,711-19.

Randall P J, Freney J R, Smith C J, Moss H J, Wrigley C W and Galbally I E 1990 Effect of additions of nitrogen and sulphur to irrigated wheat at heading on grain yield composition and milling and baking quality. Aust. J. Exp. Agric. 30, 95-101.

Recous S, Machet J M and Mary B 1988 The fate of Labelled N urea and ammonium nitrate applied to a winter wheat crop. II. Plant uptake and N efficiency. Plant and Soil 112, 215-224.

Reuter D J 1989 Philosophical priorities for future nutrition research strategies for South Australian field crops. Discussion paper prepared for the Wheat and Barley Research Committees for S.A.

Reuter D J and Dyson C B 1990 Farming practices and protein levels in ASW wheat. Dept. Agric. South. Aust. Report No.162.

Ridge P E 1986 A review of long fallows for dry land wheat production in southern Australia. J. Aust. Inst. Agric. Sci. 52, 37-44.

Ritter W F, Scarborough R W and Chirnside A E M 1993 Nitrate leaching under irrigation corn. J. Irrig. Drain. Eng.119, 544-553.

Rixon A J 1969 The influence of annual and perennial irrigated pastures on soil fertility as shown by the yield and quality of a subsequent wheat crop. Aust. J. Agric. Res. 20, 243-255.

Rixon A J 1972 Yield and quality of four wheat crops subsequent to annual and perennial irrigated pastures. Aust. J. Agric. Res. 23, 561-571.

Robertson F, Myers R and Saffigna P 1993 Distribution of carbon and nitrogen in a long-term cropping system and permanent pasture system. Aust. J. Agric. Res. 44, 1323-1336.

Robinson D 1986 Compensatory changes in the partitioning of dry matter in relation to nitrogen uptake and optimal variation in growth. Ann Bot. 58, 841-848.

Robinson D and Rorison I H 1983 Relationships between root morphology and nitrogen availability in a recent theoretical model for describing nitrogen uptake from soil. Plant, Cell Environ 6, 641-647.

Robinson D, Linehan D J and Caul S 1991 What limits nitrate uptake from soil? Plant, Cell Environ. 14, 77-85.

Robinson J B D 1957-58 The critical relationship between soil moisture content in the region of wilting point and the mineralisation of natural soil nitrogen. J. Agric. Sci. 49-50, 100-105.

Russell E W 1973 Soil Conditions and Plant Growth. 10th ed. Longman: London.

Russell J S 1963 Nitrogen content of wheat grain as an indicator of potential yield response to nitrogen fertilizer.Aust. J. Exp. Agric. Anim. Husb. 3, 319-323.

Russell J S 1968a Nitrogen fertiliser and wheat in a semi-arid environment: 2. climatic factors affecting response. Aust. J. Exp. Agric. Anim. Husb. 8, 223-231.

Russell J S 1968b Nitrogen fertiliser and wheat in a semi-arid environment: 3. Soil and cultural factors affecting response. Aust. J. Exp. Agric. Anim. Husb 8, 340-348.

Russell J S 1968c Nitrogen fertiliser and wheat in a semi-arid environment: 4. Empirical yield response models and economic factors. Aust. J. Exp. Agric. Anim. Husb 8, 736.

Russell J S 1980 Nitrogen in dry land agriculture. Proc. Inter. Congr. on Dry land Farming. pp 202-207. Dept. Agric. South. Aust. Adelaide.

Russell R S 1977 Plant Root Systems: Their Function and Interaction with the Soil. McGraw-Hill: Maidenhead, England. 298 p.

Sadaphal M N and Das N P 1966 Effect of spraying urea on winter wheat (Triticum aestivum). Agron. J. 58, 137-141.

Sahrawat K L and Keeney D R 1986 Nitrous oxide emissions from soils. Advances in Soil Sci. 4, 103-148.

Salter P J and Goode J E 1967 Crop responses to water at different stages of growth. Commonw. Bur. Hort. Plant. Crops Res. Rev. No.2.

Sander D H, Allaway W H and Olson R A 1987 Modification of nutritional quality by environment and production practices. *In* Nutritional Quality of Cereal Grains Genetic and Agronomic Improvement. Eds. R A Olson and K J Frey. pp 45-82. Am. Soc. Agron: Madison, Wisc.

Scarsbrook C E 1965 Nitrogen availability. *In* Soil Nitrogen. Eds. W V Bartholomew and F E Clarc. pp 451-502. Am. Soc. Agron: Madison, Wisc.

Schorring J K, Nielsen N E, Jensen H E and Gottschau A 1989 Nitrogen losses from field-grown spring barley plants as affected by rate of nitrogen application. Plant and Soil 116, 167-175.

Schultz E J 1972 The effect of surface treatments on soil water storage and yield of wheat. Aust. J. Exp. Agric. Anim. Husb. 12, 299-305.

Schultz J E 1971 Soil water changes under fallow-crop treatments in relation to soil type, rainfall and yield of wheat. Aust. J. Exp. Agric. Anim. Husb. 11, 236-242.

Schultz J E 1974 Root development of wheat at the flowering stage under different cultural practices. Agric. Record. 1, 12-17.

Schumann G E , McCalla T M , Sacton K E and Knox H T 1975 Nitrate movement and its distribution in the soil profile of differentially fertilised watersheds. Soil Sci. Soc. Am. Proc. 39, 1192-1197.

Scott W R 1981 Improving wheat quality by agronomic management. Proc. Agron. Soc. N. Z. 11, 91-97.

Scotter D R 1978 Preferential solute movement through large soil voids. I Some computation using simple theory. Aust. J. Soil Res. 16, 257-267.

Sharma B R and Chaudhary T N 1984 Nitrogen uptake by wheat in relation to water regime and depth of N placement in a loamy sand soil measurement and model simulation. J. Soil Sci. 35, 141-148.

Shaw K 1962 Loss of mineral nitrogen from soil. J Agric Sci. 58, 145-151.

Shuford J W, Fritton D R and Baker D E 1977 Nitrate nitrogen and chloride movement through undisturbed field soil. J. Environ. Qual. 6, 255-259.

Simpson J R 1962 Mineral nitrogen fluctuation in soils under improved pasture in southern New South Wales. Aust. J. Agric. Res. 13, 1059-1072.

Simpson J R and Freney J R 1974 The fate of fertiliser nitrogen under different cropping systems. *In* Fertilisers and the Environment. Ed. D R Leece pp 27-33. AIAS: Sydney.

Simpson R J, Lambers H and Dalling M J 1983 Nitrogen redistribution during grain growth in wheat (*Triticum aestivum L.*). IV. Development of a quantitative model of the translocation of nitrogen to the grain. Plant Physiol. 71, 7-14.

Singh R and Prihar S S 1978 Fertiliser use in rainfed areas. Proc. India/FAO/Norway Seminar on the development of the complementary use of mineral fertilisers and organic materials in India. pp 63-55.

Singh R, Singh Y, Prihar S S and Singh P 1975 Effect of N fertilisation on yield water use efficiency of dry land wheat as affected by stored water and rainfall. Agron. J. 67, 599-603.

Smika D E and Greb B W 1973 Protein content of winter wheat grain as related to soil and climate factor in the semiarid central great plains. Agron. J. 65, 433-436.

Smika D E and P H Grabouski 1976 Anhydrous ammonia applications during fallow for winter wheat production. Agron. J. 68, 919-922.

Smith C J , Whitfield D M, Gyles O A and Wright G C 1989b Nitrogen fertiliser balance of irrigated wheat grown on a red-brown earth in south-eastern Australia. Field Crops Res. 21, 265-275.

Smith C J, Freney J R, Chapman S L and Galbally I E 1989a Fate of urea nitrogen applied to irrigated wheat at heading. Aust. J. Agric. Res 40, 951-963.

Smith R C G and Harris H C 1981 Environmental resources and restraints to agricultural production in a Mediterranean-type environment. *In* Soil Water and Nitrogen in Mediterranean-type Environments. Eds. J Monteith and C Webb. pp 31-57. Martinus Nijhoff/Dr W Junk Publishers: The Hague.

Sommerfeldt T G, Chang C and Carefoot J M 1982 A laboratory study on the effects of soil moisture content, texture and timing of leaching on N loss from two southern Alberta soils. Can. J. Soil Sci. 62, 407-413.

Spiertz J H J and De Vos N M 1983 Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. Plant and Soil. 75, 379-391.

Spiertz J H J and Ellen J 1978 Effect of nitrogen on crop development and grain growth of winter wheat in relation to the assimilation and utilization of assimilates and nutrients. Neth J Agric Sci 26, 210-231.

Stanford G 1982 Assessment of soil nitrogen availability. *In* Nitrogen in Agricultural Soils. p 653. Am. Soc. Agron. Madison, Wisc.

Stanford G and Epstein E 1974 Nitrogen mineralisation-Water relation in soil. Soil Sci. Soc. Am. Proc. 38, 103-107.

Stanford G, Frere M H and Schwaninger D H 1973 Temperature coefficient of soil nitrogen mineralization. Soil Sci. 115, 321-323.

Steel R G D and Torrie J H 1960 Principles and procedures of statistics. p 813. MacGraw-Hill: New York.

Steele K W, Judd M J and Shannon P W 1984 Leaching of nitrate and other nutrients from a grazed pasture. N. Z. J. Agric Res. 27, 5-11.

Stevenson F J 1982 Nitrogen in Agricultural Soils. Am. Soc. Agron: Madison, Wisc. 940p.

Stewart B A, Viets F G, Jr , Hutchinson G L and Kemper W D 1967 Nitrate and other water pollutants under fields and feedlots. Environ. Sci. Technol. 1, 736-739.

Stewart B A, Viets F G, Jr and Hutchinson G L 1968 J Soil Water Conserv 23, 13-15.

Stoddard F L and Marshall D R 1990 Variability in grain protein in Australia hexaploid wheats. Aust. J. Agric. Res. 41, 277-288.

Storrier R R 1962 The availability of mineral nitrogen in a wheat soil from southern New South Wales . Aust. J. Exp. Agric. Anim. Husb. 2, 185-192.

Storrier R R 1965 The leaching of nitrogen and its uptake by wheat in a soil from southern New South Wales . Aust. J. Exp. Agric. Anim. Husb. 5, 323-328.

Storrier R R 1975 Wheat nutrition. *In* : Australian Field Crops volume 1: Wheat and other Temperate cereals. Eds A Lazenby and E M Matheson pp 256-287. Angus and Robertson: Sydney.

Stoteman T C 1973 Soil structure changes under wheat belt farming systems. J. Dept. Agric. West. Aust. 14, 209-214.

Strebel O and Duynisveld W H M. 1989 Nitrogen supply to cereals and sugar beet by mass flow and diffusion on a silty loam soil. Z. Pflanzenernahr, Bodenkd. 152, 135-141.

Strong W M 1981 Nitrogen requirement of irrigated wheat on the Darling Downs. Aust. J. Exp. Agric. Anim. Husb. 21, 424-431.

Strong W M 1982 Effect of late application of nitrogen on the yield and protein content of wheat. Aust. J. Exp. Agric. Anim. Husb. 22, 54-61.

Strong W M 1986 Effect of Nitrogen applications before sowing compared with the effects of split applications before and after sowing for irrigated wheat on the Darling Downs. Aust. J. Exp. Agric. 26, 201-207.

Strong W M and Clarke A L 1977 The accumulation of soil mineral nitrogen in fallow following various legume and non-legume crops. *In* Proc. Australian Cereal Agronomy Conference, Perth W. Aust. Section 7, pp 36-37.

Strong W M and Cooper J E 1980 Recovery of nitrogen by wheat from various depth in a cracking clay soil . Aust. J. Exp. Agric. Anim Husb. 20, 82-87.

Sukhatme P V 1977 Nitrogen in malnutrition. Ambio 6, 137-140.

Taylor A C, Lill W J and McNeill A A 1988 Importance of mineral nitrogen in the subsoil to yield and uptake of nitrogen by wheat in Southern New South Wales. Aust. J. Exp. Agric. 28, 215-22.

Taylor H M and Klepper Betty 1978 The role of rooting characteristics in the supply of water to plants. Adv. Agron. 30, 99-128.

Tennant D 1975 A test of a modified line intersect method of estimating root length. J. Appl. Ecol. 63, 995-1001.

Terman G L and Allen S E 1978 Crop yield Nitrate-N, total N and total K relationship: leafy vegetables. Commun. Soil Sci. Plant Anal. 9, 813-825.

Terman G L, Noggle J C and Hunt C M 1976 Nitrate-N and total N concentration relationships in several plant species. Agron. J. 68, 556-560.

Thomas G W and Swoboda A R 1970 Anion exclusion effects on chloride movement in soils. Soil Sci 110, 163-166.

Tinker P B and Widdowson F V 1982 Maximising wheat yields and some causes of yield variation. Proc. Fert. Soc. 211, 149-184.

Tinker P B H 1978 Uptake and consumption of soil nitrogen in relation to agronomic practice. *In* Nitrogen Assimilation of Plants. Eds. E J Hewitt and C V Cutting. pp 101-122. Academic Press: New York.

Tuohey C L 1973 The effect of increased soil nitrogen associated with medic ley on the performance of a standard and a semi-dward wheat cultivar. Aust. J. Exp. Agric. Anim. Husb. 13, 63-68.

Tyler D D and Thomas G W 1977 Lysimeter measurements of nitrate and chloride losses from soil under conventional and no-tillage corn. J. Environ Qual. 6, 63-66.

Ulrich A and Hills F J 1973 Plant analysis as an aid in fertilizing sugar crops: 1. Sugar beets. *In* Soil Testing and Plant Analysis. Eds. L M Walsh and J D Beaton. pp 271-288. Soil Sci Soc Amer: Madison, Wisc.

Vallis I 1990 Nitrogen: The most limiting element in agriculture. Agric. Sci. 3 (3), 19-23.

Van Oosterom E J , Ceccarelli S and Peacock J M 1993 Yield response of barley to rainfall and temperature in Mediterranean environments. J. Agric Sci. Camb 121, 307-313.

Van Veen J A and Frissel M J 1981 Simulation model of the behaviour of N in soil. *In* Simulation of Nitrogen Behaviour in Soil-Plant Systems. Eds M J Frissel and J A vanVeen. pp. 126-144. Pudoc: Wageningen.

Varshney P, Kanwar R S, Baker J L and Anderson C E 1993 Tillage and nitrogen management effects on nitrate-nitrogen in the soil profile Amer. Soc. Agric. Eng. 36, 783-789.

Viets F G J 1965 The plant's need for and use of nitrogen. *In* Soil Nitrogen. Eds. W V Bartholomew and F E Clark. pp 503-549. Am. Soc. Agron: Madison.

Walter C J and Barley K P 1974 The depletion of soil water by wheat at low, intermediate and high rates of seeding. Trans. 10th Int. Congr. Soil Sci, Moscow. 1, 150-158.

Ward R C, Whitney D A and Westfall D G 1973 Plant analysis as an aid in fertilising small grains. *In* Soil Testing and Plant Analysis (Revised ed.). Eds. L M Walsh and J. D. Beaton. pp 329-348. Soil Sci. Soc. Am: Madison, Wisc.

Waring S A and Teakle L J H 1960 Fertility investigation on the black earth wheat lands of the Darling Downs, Queensland. III. Mineral nitrogen in the soil and its relation to the wheat crop. Aust. J. Agric. Res. 11, 27-41.

Watson E R 1963 The influence of subterranean clover pastures on soil fertility. I. Short-term effects. Aust. J. Agric. Res. 14, 796-807.

Watson E R and Lapins P 1972 Loss of nitrogen from plant residues on the soil surface. Field Station Records Division of Plant Industry CSIRO Aust. 11, 5-10.

Weier K L and Macrae I C 1993 Net mineralisation, net nitrification and potentially available nitrogen in the subsoil beneath a cultivated crop and a permanent pasture. J. Soil Sci. 44, 451-458.

Wetselaar R 1962 Nitrate distribution in tropical soils. Plant and Soil 16, 19-31.

Wetselaar R and Farquhar G D 1980 Nitrogen losses from tops of plants. Adv. Agron. 33, 263-302.

White R E 1988 Advance in Nitrogen Cycling in Agricultural Ecosystems. Ed. J R Wilson. pp 193-211. CAB International: Wallingford U K.

Whitfield D M, Smith C J, Gyles O A and Wright G C 1989 Effects of irrigation, nitrogen and gypsum on yield, nitrogen accumulation and water use by wheat. Field Crops Res. 20, 261-77.

Wiersum L K 1962 Uptake of nitrogen and phosphorus in relation to soil structure and nutrient mobility. Plant and Soil 16, 62-70.

Wiersum L K 1967 Potential subsoil utilisation by roots . Plant and Soil 27, 383-400.

Wiesler F and Horts W J 1993 Differences among maize cultivars in the utilization of soil nitrate and the related losses of nitrate through leaching. Plant and Soil 151, 193- 203.

Wild A 1972 Nitrate leaching under Bare fallow at a site in northern Nigeria. J. Soil Sci. 23, 315-324.

Wild A 1981 Mass flow and diffusion. *In* The Chemistry of Soil Processes. Eds. D J Greenland and M H B Hayes. pp 37-80. Wiley: New York.

Wind G P 1961 Capillary rise and some applications of the theory of moisture movement in unsaturated soils. Institute for Land and Water Management Research, Tech. Bull. No.22.

Wright M J and Davidson K L 1964 Nitrate accumulation in crops and nitrate poisoning in animals. Adv. Agron. 16, 197-247.

Wright N and Trautman R J 1962 Influence of management on nitrate accumulation in blue panicgrass. Agron. J. 54, 363-364.

XU ZH and Elliott D E 1993 Tillage system, Rotation, Nutrition and associated root diseases. Workshops held at Waite Campus University of Adelaide, 25 and 26 March 1993.

Xu Z H, Elliott D E and Winn D 1992 Influence of rainfall and previous land use on optimum rate of N fertiliser applied on wheat and barley in South Australia. Proceedings of Workshop on Tillage Systems, Rotations, Nutrition and Associated Root Diseases. Ed. R G Fawett. pp 63-64. Waite Agricultural Research Institute, University of Adelaide, 26-27 March 1992.

Xu Z H, Elliott D E, Hannam R J and Winn D 1991 Yield and quality responses of wheat and barley to application of nitrogen fertiliser in South Australia. Proceedings of Workshop on Tillage Systems, Rotations, Nutrition and Associated Root Diseases. Ed. R G Fawett. pp. 68-69. Waite Agricultural Research Institute, University of Adelaide, 11-12 April 1991.

Young R A, Ozbun J L, Bauer A and vasey E H 1967 Yield response of spring wheat and barely to nitrogen fertiliser in relation to soil and climatic factors. Soil Sci. Soc Am Proc. 31, 407-410.

Zierserl J F Rivenbark W L and Hageman R H 1963 Nitrate reductase activity protein content and yield of four maize hybrids at varying plant populations. Crop Sci. 3, 27-32.

Aust. J. Agric. Res., 1997, 48, 1–0

Effect of nitrogen fertiliser placement on grain protein concentration of wheat under different water regimes

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Abstract. Two experiments were conducted in pots 105 cm deep and 11 cm in diameter to determine the effects of subsoil nitrogen (N) on grain yield and grain protein concentration (GPC) of wheat (*Triticum aestivum* L. cv. Molineux). In both experiments, KNO_3 was applied in solution at different times and depths in the profile. In the first experiment, in which a sandy soil low in available N was used, application of 150 mg N at 60 cm, 2 weeks after anthesis, significantly increased grain yield and GPC. The N was taken up gradually by the plant after N was applied. Adding N to the subsoil increased root growth and this resulted in increased water use and water use efficiency. Although there was an increase in the rate of N uptake by the roots, the main factor that influenced the utilisation of subsoil N was the root length density. In the second experiment, the effects of depth and time of N application, and of a reduction in post-anthesis water supply, were determined. A more fertile soil than the one used in the first experiment was used. There were 5 KNO_3 treatments: nil N; 150 mg N applied to the topsoil at sowing; 75 mg N to the topsoil and 75 mg N to the subsoil (60 cm depth) at sowing; 150 mg N to the subsoil at sowing; 75 mg N to the topsoil at sowing and 75 mg N to the subsoil 1 week after anthesis. The effect of post-anthesis water stress was assessed by allowing the topsoil to dry and then supplying half the amount of water used by the well-watered control treatment at 60 cm in half of the pots. Adding N increased yield and GPC but there was no significant difference in yield and GPC between the different N treatments. When N was applied to the topsoil only, most of it was used by the wheat plants or leached to the subsoil by anthesis; post-anthesis uptake of N depended on the amount of N in the subsoil. Adding N, irrespective of the depth of placement or time of application, increased water use and water use efficiency. In both experiments, increasing the availability of N in the soil after anthesis reduced the amount of N that was remobilised from the roots and stem to the grain. The recovery of applied N in both experiments was high (about 80%). These experiments have shown that N available in the subsoil after anthesis can be used very efficiently and can contribute to both grain yield and GPC. A critical factor in the efficient use of this N appears to be root length density in the subsoil.

Additional keywords: mineral nitrogen.

Introduction

Maintaining an adequate concentration of grain protein is of economic importance to wheat producers. As farmers strive for higher yields, the nitrogen (N) available to the crop needs to be increased to maintain grain quality standards. To achieve this, wheat crops must exploit the available soil N effectively to maintain an adequate supply of N throughout the year. Nitrogen supplied as fertiliser during autumn and winter, or the flush of N from mineralisation of organic matter early in the growing season may benefit yield, but unless the supply of N to the grain is sustained during grain filling, the grain protein concentration (GPC) of the crop may be low.

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In the cereal-growing areas of southern Australia, the cultivated and fertilised topsoil contains most of the labile forms of N. When the topsoil dries in spring and summer and the N become less available and roots less active, plants may suffer from a shortage of N which may adversely affect yield and GPC. Experiments have been conducted which showed that when fertiliser was added to the subsoil the nutrient shortage caused by topsoil drying was overcome (Alston 1980; Strong and Cooper 1980), but commercial application of this technique is limited. Subsoil mineral N may be a useful N source for crops when N in the surface soil is unavailable or is not accessible because the topsoil is dry (Gass *et al.* 1971).

Leaching of NO_3-N from the topsoil is an important factor which increases the concentration of NO_3-N in the subsoil (Kissel *et al.* 1974; Ladd 1990; Weier and MacRae 1993). In southern Australia, subsoil accumulation of NO_3-N is common, and the NO_3-N usually remains within the root zone of crops (Storrier 1965; Greenland 1971) because rainfall is generally insufficient to cause leaching beyond the roots. The NO_3-N can be taken up by the crop late in the season and Ladd (1990) suggests that late-season uptake of N from the subsoil can play an important role in determining GPC of wheat.

There is considerable evidence that uptake of N late in the season (at or after anthesis) can increase GPC (e.g. Strong 1982, 1986), and that in some environments substantial uptake from the subsoil does occur at this time. The main factors which appear to determine the amount of subsoil N taken up are the amount of mineral N, the soil water content, and the amount of root growth in the subsoil. Storrier (1962) in southern NSW, found that N uptake stopped after anthesis and little subsoil N was used; however, Strong and Copper (1980) found that post-anthesis uptake of N during a dry year was increased when the N uptake was increased by deep placement of fertiliser. Sharma and Chaudhary (1984) reported that deep placement of fertiliser N in a coarse textured soil resulted in its more uniform distribution in the root zone, more extensive root proliferation, and enhanced subsoil water utilisation. Kuhlmann *et al.* (1989) found that N taken up after flowering of winter wheat in northern Germany and Rothamsted (UK) mainly occurred from the subsoil layers, whereas N uptake during the vegetative growth was restricted to the topsoil. Smika and Greb (1973) in an experiment on the Central Great Plains of the USA, showed that GPC was higher when higher concentrations of NO_3-N were found in the subsoil. These studies, though demonstrating the importance of the availability of subsoil N to GPC, provide relatively little information on the efficiency with which wheat plants can utilise N from the subsoil, or the rate at which this N can be extracted from the soil. Nitrogen in the grain comes from 2 sources: post-anthesis uptake and remobilisation and transport of N from the vegetative parts of the plant to the grain. Many studies on GPC have been conducted under conditions when post-anthesis uptake is low and remobilisation accounts for most of the N in the grain at maturity. Fewer studies have examined the importance of subsoil N availability on GPC. Therefore, 2 greenhouse experiments were conducted to determine the effects of the availability of N, the time of increased N availability, and the soil water content, on GPC in wheat.

Materials and methods

Plants were grown in PVC pots 105 cm deep and 11 cm in diameter, sealed at the base. The soil was packed to a bulk density of 1·3 g/cm^3 to a depth of 100 cm. A polyethylene tube, 70 cm long and 2·5 cm diameter, was placed down the centre of each pot to a depth of 60 cm to allow fertiliser and water to be added to the subsoil.

Experiment 1

The first experiment examined the ability of wheat plants to take up subsoil mineral N and its subsequent effect on GPC. A 'pulse' of N was applied to the subsoil and its fate was examined over the subsequent period. Wheat (*Triticum aestivum* L. cv. Molineux) was grown in a sandy soil (sand 90%, silt 6%, clay 4%) of pH(1:5 soil:water) 6·8. The soil contained 200 mg/kg of total N, 3 mg/kg of NO_3-N, and 10 mg/kg of $NaHCO_3$-extractable phosphorus (Colwell 1963). The experiment had a randomised complete block design with 3 replications. The factors tested were 2 N treatments and 8 harvest dates, arranged in a factorial combination. Six wheat seeds were sown per pot on 1 April 1994 at a depth of 1·5 cm, and a basal fertiliser solution containing 20 mg N as KNO_3, 100 mg phosphorus as $CaH_4(PO_4)_2.H_2O$, 10 mg copper as $CuSO_4 \cdot 5H_2O$, 10 mg zinc as $ZnSO_4 \cdot 7H_2O$, and 20 mg magnesium as $MgSO_4 \cdot 7H_2O$ was added to the surface of the soil 3 weeks later. Three days after emergence, the plants were thinned to 3 seedlings per pot. Shadecloth was placed around the experiment at a height of two-thirds that of the plants to reduce edge effects and prevent the excessive tillering and growth that commonly occur with plants grown in spaced pots. The mean daily minimum and maximum air temperatures in the greenhouse ranged from 18° and 25°C, respectively, in April, to 17° and 20°C in September. The pots were weighed once per week. A measured amount of water was added to the surface of the soil 2 or 3 times weekly to maintain the soil water near field capacity. In this soil, water held at field capacity was 0·05 kg/kg. Signs of N deficiency appeared during the experiment; thus on 8 May, 24 May, 17 June, 5 July, and 19 July a solution of KNO_3 containing 20, 20, 10, 10, and 5 mg N, respectively, was added to the surface soil in all pots. The post-anthesis N treatments (0 and 150 mg N) were applied to the subsoil on 23 July, 2 weeks after anthesis, by adding 40 mL of 0·27 M KNO_3 to the treated pots and an equivalent amount of water to each control pot.

Plants were harvested at tillering (15 May), stem elongation (1 June), anthesis (9 July), 2 weeks after anthesis (23 July),

2 days (25 July) and 7 days (30 July) after applying N, at the dough stage (22 August), and at maturity (14 September). The plants were cut at soil level, dried at 80°C, weighed, and ground. At the last 2 harvests, the plant material was separated into grain and straw. Total N was determined in a sulfuric acid digest of the plant material by a micro-Kjeldahl method (Bremner 1965), and the NO_3-N concentration in the plant shoots was assessed by the salicylic acid method (Cataldo *et al.* 1975). The soil from each pot was separated into depths of 0–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm. A subsample of soil was taken from each layer to determine water content, and NO_3-N concentration by the method of Best (1976). Roots were separated from the remaining soil samples by flotation and root lengths measured by the modified line intercept method of Tennant (1975).

Experiment 2

In the second pot experiment, we determined the effects of depth, time, and amount of N application, and soil water regime on N uptake and GPC. In this experiment, a post-anthesis N application was compared to a treatment in which the N was placed either in the topsoil or in the subsoil during the early part of the season. This experiment was designed to simulate possible conditions in the field, i.e. subsoil N derived from decomposition of organic material or from fertiliser application in the early season (leaching). Because the sandy soil in Expt 1 was very low in N and tended to foster N deficiency, we decided to use a loamy soil which had a higher supply of available N in this second experiment. The soil was a red-brown earth with a pH(1:5 soil:water) of 6·0. It contained 800 mg/kg of total N, 20 mg/kg of NO_3-N, and 56 mg/kg of $NaHCO_3$-extractable phosphorus (Colwell 1963). The experiment had a randomised complete block design with 3 replications. Factors tested were 5 N treatments, 3 harvest dates, and 2 water regimes arranged in factorial combination. The N treatments (KNO_3) were nil N (N0); 150 mg N/pot placed in the topsoil at sowing (N1); 75 mg N placed in the topsoil and 75 mg N in the subsoil at sowing (N2); 150 mg N placed in the subsoil at sowing (N3); and 75 mg N placed in the topsoil at sowing and 75 mg N placed in the subsoil 1 week after anthesis (N4). The topsoil applications were applied in solution to the surface of the soil which was then lightly cultivated. Plants were harvested at tillering, anthesis, and maturity. The plants that did not receive N (N0) were harvested only at maturity. The 2 water treatments, introduced when the wheat was at anthesis, were surface irrigation sufficient to keep water stress low in the plants (W1), and no surface water but subsoil irrigation at 60 cm depth (W2). The pots were weighed 3 times per week and water added to maintain the soil water content near field capacity. Water held at field capacity in this soil was 0·12 kg/kg. The pots with the subsoil irrigation treatment (W2) received half the quantity of water that would have been needed to bring all the soil to field capacity. Six wheat seeds (cv. Molineux) were sown per pot on 15 November 1994 at a depth of 1·5 cm, and a basal fertiliser solution containing 50 mg phosphorus as $CaH_4(PO_4)_2 \cdot H_2O$, 10 mg copper as $CuSO_4 \cdot 5H_2O$, 10 mg zinc as $ZnSO_4 \cdot 7H_2O$, and 20 mg magnesium as $MgSO_4 \cdot 7H_2O$ was added to the surface of the soil 1 week later. The mean daily minimum and maximum air temperatures in the greenhouse ranged from 20° and 23°C respectively for November to 25° and 27°C for February. The measurements on growth and plant and soil N in this experiment were similar to those described for the first experiment.

To follow the leaching of N from the topsoil, an additional series of pots in which 150 mg N was applied at sowing (N1) was established and watered as for the well-watered treatment (i.e. W1). Between sowing and maturity, 9 harvests were made and the distribution of soil NO_3-N, amount of soil water, and root growth were measured.

Results

Experiment 1

Dry matter yield

Nitrogen increased the dry matter production of both roots and shoots (Table 1). The effect was particularly evident in root growth where without additional N, root weight decreased during the post-anthesis period, whereas the additional N maintained root growth.

Table 1. Experiment 1. Dry weight and concentration of nitrate and total nitrogen in the plant

Harvest[A]	Dry weight (g/pot)		Nitrogen (%)		NO_3-N (mg/kg)	Total N (mg/pot)
	Shoot	Root	Shoot	Root	Shoot	Shoot +root
			Before adding nitrogen			
15 May	1·12	1·33	3·06	1·31	1325	53
1 June	2·27	1·95	2·63	1·19	1064	85
9 July	7·98	3·42	1·29	0·93	554	139
23 July	9·73	3·00	1·08	0·93	589	138
l.s.d. ($P = 0·05$)	0·79	1·24	0·15	0·24	276	21
			After adding nitrogen			
			Control (nil N)			
25 July	9·81	4·05	0·93	0·86	793	133
30 July	10·44	4·05	0·92	0·80	740	139
22 Aug	14·81	3·86	0·83	0·88	925	167
14 Sept	16·05	3·59	0·78	0·76	380	155
			Nitrogen (150 mg/pot)			
25 July	9·71	3·25	1·06	0·98	1130	145
30 July	10·74	4·34	1·04	0·97	825	162
22 Aug	15·59	5·51	1·22	0·86	1141	251
14 Sept	18·72	6·18	1·23	0·64	429	276
l.s.d. ($P = 0·05$)	1·57	0·99	0·05	0·11	147	19

[A] Growth stage at date of harvest was tillering (15 May), stem elongation (1 June), flowering (9 July), 2 weeks after flowering (23 July), 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.), and maturity (14 Sept.).

Table 2. Experiment 1. Effect of nitrogen treatments on the dry matter yield, grain protein concentration, and apparent N recovery

Nitrogen (mg/pot)	DM yield (g/pot)		Nitrogen (%)	Protein (%)	Apparent N recovery (%)
	Grain	Straw	Straw	Grain	Shoots
			22 August		
0	3·95	10·86	0·60	8·4	–
150	4·36	11·24	0·91	11·1	45
			14 September		
0	6·64	9·41	0·36	7·8	–
150	8·57	10·16	0·45	12·4	72
l.s.d. ($P = 0·05$)	1·37	1·04	0·12	0·6	18

Table 3. Experiment 1. Root length density (cm/cm^3 in the soil profile

The growth stage at the date of harvest was tillering (15 May), stem elongation (1 June), flowering (9 July), 2 weeks after flowering (23 July), 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.), and maturity (14 Sept.); l.s.d. applies to interaction between depth and harvest.

Depth (cm)	Before adding N				After adding N							
					Control (nil N)				N(150 mg/pot)			
	15 May	1 June	9 July	23 July	25 July	30 July	22 Aug.	14 Sept.	25 July	30 July	27 Aug.	14 Sept.
0–10	5·04	9·94	75·5	18·36	16·40	19·79	17·66	11·87	17·55	19·29	16·15	12·08
10–20	2·67	3·69	4·71	4·35	4·73	6·45	5·15	4·68	5·42	4·56	6·86	5·46
20–40	1·47	2·03	2·22	2·73	4·04	2·28	3·51	3·38	3·63	3·20	3·80	3·32
40–60	1·62	1·85	2·01	2·51	2·91	2·23	2·80	2·88	2·58	2·30	3·56	3·70
60–80	0·63	1·38	1·70	1·88	0·98	1·85	1·53	1·44	1·73	1·88	2·47	2·97
80–100	0·00	0·25	1·26	0·96	0·16	0·34	0·92	0·07	0·38	0·30	0·27	0·79
l.s.d. ($P = 0·05$)			1·68									

Table 4. Experiment 1. Root dry weight (g) in the soil profile

The growth stage at the date of harvest was tillering (15 May), stem elongation (1 June), flowering (9 July), 2 weeks after flowering (23 July), 2 days after adding N (25 July), 7 days after adding N (30 July), dough stage (22 Aug.), and maturity (14 Sept.); l.s.d. applies to interaction between depth and harvest

Depth (cm)	Before adding N				After adding N							
					Control (nil N)				N(150 mg/pot)			
	15 May	1 June	9 July	23 July	25 July	30 July	22 Aug.	14 Sept.	25 July	30 July	27 Aug.	14 Sept.
0–10	0·52	0·84	1·49	1·27	1·44	1·85	1·46	1·22	1·04	1·52	1·39	1·34
10–20	0·13	0·19	0·29	0·27	0·34	0·42	0·35	0·41	0·30	0·29	0·59	0·44
20–40	0·19	0·23	0·28	0·40	0·62	0·52	0·52	0·52	0·46	0·56	0·52	0·56
40–60	0·28	0·25	0·32	0·45	0·93	0·60	0·53	0·64	0·50	0·59	1·11	1·41
60–80	0·21	0·36	0·45	0·66	0·58	0·79	0·56	0·72	0·71	1·12	1·76	2·01
80–100	0·00	0·14	0·58	0·56	0·14	0·29	0·44	0·07	0·25	0·26	0·15	0·43
l.s.d. ($P = 0·05$)			0·27									

Adding N to the subsoil 2 weeks after anthesis significantly increased dry weight of grain and straw (Table 2).

Root length density increased rapidly after 1 June (stem elongation) until 23 July (2 weeks after anthesis), especially in the surface layers (Table 3). The roots did not extend beyond 80 cm until stem elongation (1 June). Adding N to the subsoil just after anthesis resulted in an increase in root length in the subsoil that was apparent by the dough stage (22 August). The root length density in the topsoil decreased 3 weeks after anthesis (30 July). Root dry weight followed a similar trend to root length during the growing season, but dry weight was more affected than length by the addition of N (Table 4).

Nitrate concentration in the shoot

The NO_3-N concentration in the shoots generally decreased with plant growth (Table 1). The decrease was most pronounced between dough stage (22 August) and maturity (14 September). Concentrations were higher in the system that received additional N after anthesis.

Total N and grain protein concentration in the plant

The N concentration of the shoots declined with plant maturation (Table 1). During the week after adding N to the subsoil, the concentration of total N in the shoots stopped decreasing and had increased again by the dough stage (22 August). From 30 July onwards, the system receiving subsoil N reflected this treatment in the total plant N. The GPC was increased significantly ($P < 0·05$) by applying N to the subsoil after anthesis (Table 2).

Recovery of N and remobilisation to the grain

Two days after applying the N, 90% of the N could be accounted for in the plant and soil, but over the next month, recovery diminished to 60–80%. Initially, little of the added N was taken up by the plants; for example, of the 150 mg added to the subsoil, only an extra 12 mg was found in the plants after 2 days (Table 1) and uptake occurred slowly over the next 6 weeks. By maturity, 121 mg of extra N was measured in plants to which the subsoil N was applied. This was equivalent to an apparent recovery in the root and shoots of 80%. By maturity, most of the N had been translocated to the grain. The addition of N after anthesis reduced the amount of N remobilised from the straw from 73 to 51 mg per pot ($P < 0·05$) and it reduced the apparent contribution of remobilised N to grain from 81 to 27% ($P < 0·05$).

Soil water and water use

Applying N increased water use (5342 *v.* 4896 mL/pot) due to increase in plant growth, but the efficiency of water use (based on grain yield) was also increased (1·36 to 1·60 g/L). There was greater use of water from the subsoil when N was added.

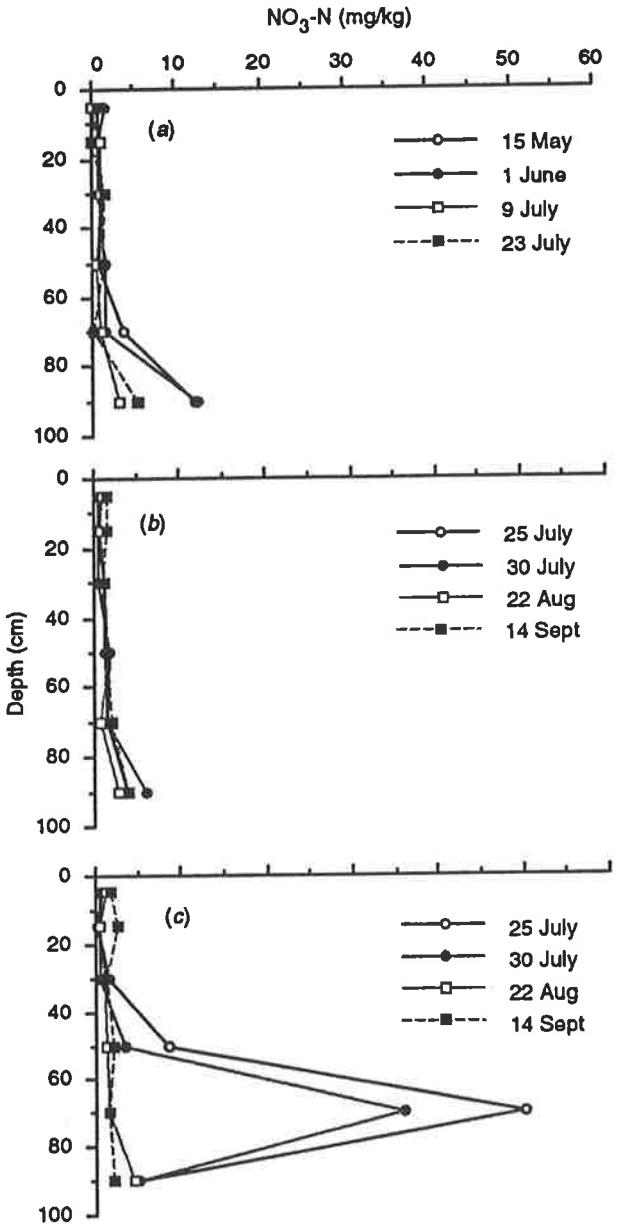

Fig. 1. Experiment 1. Concentration of NO_3-N in the soil profile (*a*) before adding N; after adding N (*b*) control, (*c*) N treatment.

Soil nitrate

The accumulation of NO_3-N at the bottom of the pot prior to 15 May was attributed to leaching (Fig. 1). Between stem elongation (1 June) and anthesis (9 July), the concentration of NO_3-N at 80–100 cm decreased as the root growth in these layers increased. As expected, there was a large increase in the concentration of NO_3-N when the nitrate solution was added to the subsoil 2 weeks after anthesis. The concentration of NO_3-N declined within 1 week (30 July) of adding the N. The N uptake by plant from soil during this period was high (Fig. 1) and by the last 2 harvests (22 August and 14 September) the subsoil contained only trace amounts of NO_3-N.

Experiment 2

In most cases, N added to wheat is applied as a single application before or near to sowing. Therefore, in this experiment, N1 rather than N0 can be regarded as the appropriate control. The inclusion of N0 was to allow the N responsiveness of the different water treatments to be assessed.

Table 5. Experiment 2. Dry weight and concentrations of nitrate nitrogen and total nitrogen in the plant

N0, nil N; N1, 150 mg N topsoil at sowing; N2, 75 mg N topsoil and 75 mg N subsoil at sowing; N3, 150 mg N subsoil at sowing; N4, 75 mg N topsoil at sowing and 75 mg N subsoil after flowering

Nitrogen treatment	Dry weight (g/pot)		Nitrogen (%)		NO_3-N (mg/kg)	Total N (mg/pot)
	Shoot	Root	Shoot	Root	Shoot	Shoot +root
			Tillering			
N1	3·2	2·20	3·75	1·64	9167	184
N2	2·8	1·80	3·62	1·60	8708	152
N3	2·7	2·20	3·32	1·40	7208	142
N4	2·8	1·80	3·36	1·60	8750	147
			Anthesis			
N1	13·6	5·20	1·94	1·16	2847	358
N2	13·0	5·40	2·07	1·12	2582	362
N3	14·5	6·30	1·90	1·06	2277	373
N4	13·4	6·40	1·85	0·95	2115	336
l.s.d.[A] ($P = 0·05$)	n.s.	0·86	0·20	0·11	921	n.s.
			Maturity			
			Surface irrigation (W1)			
N0	27·2	5·24	1·22	1·04	529	400
N1	33·8	5·55	1·35	1·14	988	554
N2	35·0	6·40	1·31	1·10	833	557
N3	33·8	4·84	1·36	1·04	750	529
N4	33·5	5·35	1·37	1·04	817	541
			Subsoil irrigation (W2)			
N0	25·6	4·04	1·32	1·14	792	402
N1	32·3	5·69	1·37	1·02	1688	554
N2	32·5	5·22	1·38	1·02	1313	543
N3	32·3	5·56	1·45	1·00	1604	574
N4	30·5	5·67	1·40	0·98	1583	530
l.s.d.[B] ($P = 0·05$)	n.s.	1·04	n.s.	0·09	308	n.s.

n.s., not significant.
[A] Applies to N×harvest interaction.
[B] Applies to N×water treatment interaction.

Dry matter yield

Nitrogen increased shoot dry matter at maturity, but there was no significant difference ($P < 0.05$) in effect between N treatments (Table 5). The interaction between N treatment and water was not significant. Similarly, the grain yield of plants treated with N was higher than for the N0 treatment, but neither the time nor method of N placement affected the grain yield significantly (Table 6). Reducing the supply of water after anthesis restricted grain yield compared with surface irrigation (12·9 *v.* 13·8 g per pot), but the difference was not significant. The post-anthesis water regime had no effect on the response to N.

Table 6. Experiment 2. Effect of nitrogen treatments on the dry matter yield, grain protein concentration and apparent N recovery at maturity

Values have been averaged over water treatments. N0, nil N; N1, 150 mg N topsoil at sowing; N2, 75 mg N topsoil and 75 mg N subsoil at sowing; N3, 150 mg N subsoil at sowing; N4, 75 mg N topsoil at sowing and 75 mg N subsoil after flowering

Nitrogen treatment	DM yield (g/pot)		Nitrogen (%)	Protein (%)	Apparent N recovery (%)
	Grain	Straw	Straw	Grain	Shoots
N0	11·7	14·7	0·64	11·8	–
N1	13·7	19·4	0·75	12·7	77
N2	13·9	19·8	0·70	12·9	80
N3	14·0	19·1	0·77	13·0	85
N4	13·5	18·6	0·75	13·0	73
l.s.d. ($P = 0.05$)	1·7	3·0	n.s.	0·70	n.s.

Table 7. Experiment 2. Root length density and root dry weight in the soil profile

Values have been averaged over all N treatments because the effect of N was not significant; l.s.d. applies to the interaction between depth and harvest

Depth (cm)	Harvest		
	Tillering	Anthesis	Maturity
	Root length density (cm/cm^3)		
0–10	7·10	14·49	9·46
10–20	4·50	12·43	8·55
20–40	3·37	6·85	6·34
40–60	1·98	4·65	3·92
60–80	0·21	1·52	2·31
80–100	0·05	1·16	2·30
l.s.d. ($P = 0.05$)		1·36	
	Root dry weight (g)		
0–10	0·63	1·75	1·38
10–20	0·36	0·92	0·88
20–40	0·51	1·41	1·20
40–60	0·39	0·99	0·89
60–80	0·08	0·42	0·57
80–100	0·01	0·30	0·67
l.s.d. ($P = 0.05$)		0·15	

Root growth

Roots grew slowly until tillering, then root length density increased sharply from tillering to anthesis, especially in the surface layers (Table 7). Neither the addition of N, nor its placement, affected root length density. Root dry weight increased until anthesis, then decreased sharply in the top 10 cm of soil (Table 7). However, root dry weight in the 60 to 100 cm depth increased up to maturity.

Nitrate concentration in the shoot

The NO_3-N concentration in the tops of the plants decreased during the season (Table 5). At the tillering stage, the concentration of NO_3-N in the plant that received the entire 150 mg N in subsoil at sowing (N3) was low compared with the other N treatments but differences between N treatments were small at other times. Subsoil irrigation resulted in a significant increase in the concentration of NO_3-N in the plant shoots at maturity.

Total N and grain protein concentration in the plant

Total N concentration in the shoots at tillering was high, but N concentration decreased as the plants matured (Table 5). At tillering, N concentration in shoots was greater for N1 and N2 than for N3 and N4 and at anthesis it was greater for N2. At tillering, the N concentration of the root where 150 mg was placed in the subsoil at sowing (N3) was low compared with other N treatments. However, from anthesis on, N4 tended to result in the lowest root N concentration, and where subsoil irrigation was used, N0 had the highest root N concentration. Total N in the plant (shoot and root) increased during the growing season (Table 5). At maturity, all N treatments increased plant N compared with N0, but there was no difference due to N placement or water treatment. The content of the N in the plants that received 150 mg N in subsoil at sowing (N3) under subsoil irrigation was higher than with the other treatments. All N treatments significantly increased the GPC compared with N0 (Table 6). The GPC was significantly higher (13·1%) with the limited subsoil irrigation than with surface irrigation (12·3%).

Soil water and water use

The N-treated plants used similar amounts of water, and more than the unfertilised control plants. However, water use efficiency, though tending to be higher where N was applied, was not significantly ($P > 0.05$) affected (Table 8). Total water used by wheat was greater, whereas water use efficiency was poorer, with surface irrigation than with subsoil irrigation.

Table 8. Experiment 2. Effect of the nitrogen and water treatments on the soil water content at the end of the experiment, amount of water used, and efficiency of water use

	Soil water content (mL/pot)	Total water use (mL/pot)	water use efficiency (g/L)[A]
	N treatment		
N0	480	7489	1·57
N1	404	7855	1·74
N2	384	7912	1·77
N3	426	7741	1·81
N4	342	7844	1·72
l.s.d. ($P = 0·05$)	62	215	n.s.
	Water treatment		
Surface irrigation	422	8389	1·65
Subsoil irrigation	392	7148	1·80
l.s.d. ($P = 0·05$)	n.s.	136	0·13

n.s., not significant.
[A] Water use efficiency was calculated for the weight of grain.

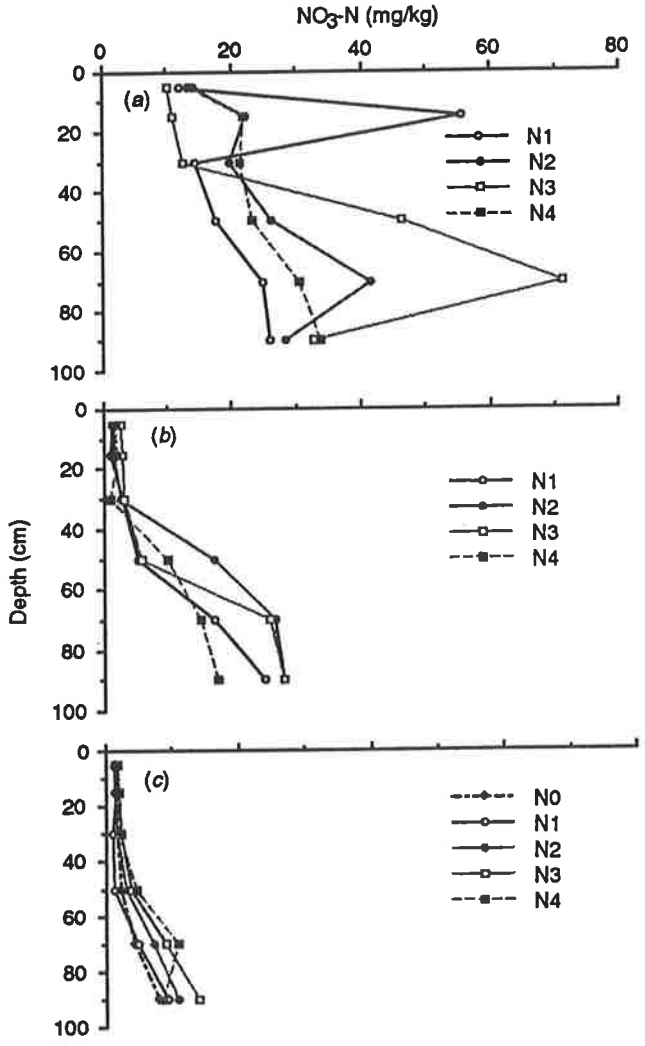

Fig. 2. Experiment 2. Concentration of NO_3-N in the soil profile at (*a*) tillering, (*b*) flowering and (*c*) maturity. N0, no N; N1, 150 mg N topsoil at sowing; N2, 75 mg N topsoil and 75 mg N subsoil at sowing; N3, 150 mg N subsoil at sowing; N4, 75 mg N topsoil at sowing and 75 mg N subsoil after flowering.

Soil nitrate

As expected, there was a large amount of NO_3-N in the topsoil at tillering for treatments with 150 mg N added to the topsoil at sowing, and in the subsoil for pots that received 150 mg N in the subsoil at sowing (Fig. 2). By anthesis, the concentration of NO_3-N had decreased in the topsoil but was still appreciable in the subsoil. At anthesis, the concentration of NO_3-N in the 60–100 cm depth for the pots that received 75 mg N in the topsoil and 75 mg N in the subsoil (N2), and those receiving 150 mg N in the subsoil at sowing (N3), were similar (Fig. 2). Measurements of NO_3-N over the growing season in soil with the N1 treatment indicated that there was an accumulation of NO_3-N in the subsoil prior to anthesis (Fig. 3). There was little NO_3-N present in the topsoil at stem elongation (31 December) and the plants relied on NO_3-N in the subsoil thereafter.

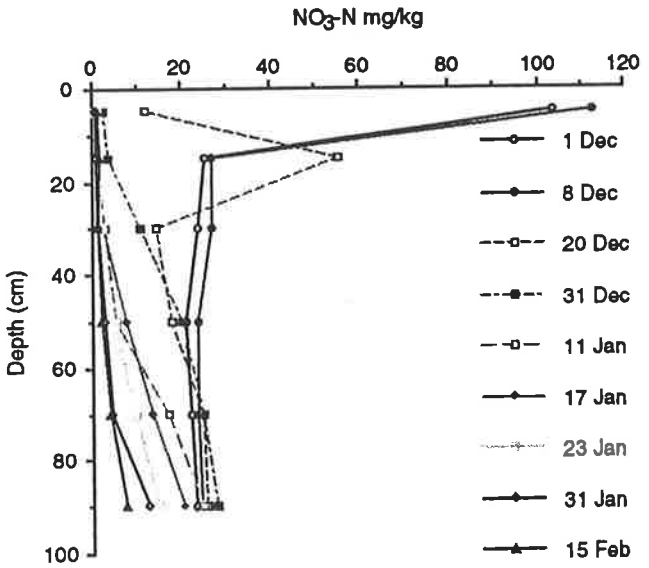

Fig. 3. Experiment 2 (additional pots). Concentration of NO_3-N in the soil for pots receiving 150 mg N topsoil at sowing.

Discussion

The 2 experiments examined N responses by wheat in soils with quite different N status. Measurement of plant NO_3-N concentration in Expt 1 showed that the values were low enough to restrict plant growth. Papastylianou (1980) reported that plant growth in pot experiments was restricted when the stem NO_3-N concentrations at tillering, jointing, and anthesis fell below 5500, 1200, and 500 mg/kg, respectively. Consequently, there was a large increase in grain yield and a significant increase in GPC following the addition of N. The use of a loamy soil with greater N availability in Expt 2 improved growth and yield compared with

Expt 1 and the plant NO_3-N concentrations at tillering (7000–9000 mg/ kg) were well above the critical levels. Despite this, yield and GPC were again significantly increased by addition of N, although the yield response was not as great as obtained in Expt 1. The timing and the initial placement of N in Expt 2 had no effect on yield nor on the GPC response. Furthermore, responses to the different N treatments were not affected by restricting the supply of water during the post-anthesis period. The only N treatment in which no N was applied to the subsoil was N1. However, when the fate of this N was followed during the pre-anthesis period, it was found that a considerable proportion of it was leached into the subsoil. We also observed that very little soil NO_3-N located below 60 cm was used prior to anthesis, except when all the N was applied in subsoil at sowing. However, following the depletion of soil NO_3-N in the surface layers, plants relied on subsoil NO_3-N to sustain growth (Fig. 2). When all the N was supplied to the subsoil at sowing (N3), the plant exploited this reserve without any detrimental effect on grain yield or GPC. When applied N was split between the topsoil and subsoil at sowing (N2), there was greater use of topsoil N prior to anthesis, but a greater reliance on subsoil N reserves after anthesis when the topsoil concentration was very low (Fig. 2). This pattern of uptake has been reported elsewhere under field conditions (e.g. Kuhlmann *et al.* 1989). The preferential use of topsoil N initially is probably associated with the greater root length densities in the topsoil layers. The results from the 2 experiments support the suggestion by Ladd (1990) that some subsoil mineral N may be taken up late in the season and thereby contribute to GPC.

The other source of N used for grain filling is that remobilised from the non-grain parts of the plant. Under dryland conditions, when the availability of post-anthesis soil N is low, remobilisation is the major source of N for grain filling. Increasing applied N during the post-anthesis period substantially reduced the amount of N remobilised in the plant and its contribution to grain N yield. In Expt 2, the effect of applying all the N to the topsoil at sowing (N1) was comparable either with applying the N to the topsoil and subsoil at sowing (N2), or with applying the N only to the subsoil at sowing (N3), or with delaying the application of some of the N until after anthesis (N4). This suggests that as long as the N is not leached from the root-zone, application of N to the topsoil and subsoil has an equivalent effect.

An important factor that influences the ability of plants to exploit N from the subsoil is the root length density (Wiersum 1967). Root length density declines with depth and often rooting in the subsoil is sparse. The ability of plants to take up N from the subsoil may be limited if the root length density is very low. However, the importance of this factor will depend on how transient is the increased availability of N. Localised, high concentrations of N in the soil can increase proliferation and branching of wheat roots, which in turn can increase N uptake from the soil (Passioura and Wetselaar 1972). Immediately after applying N to the subsoil in Expt 1, the recovery of N was low; 2 days after applying N only 12 mg N per pot was recovered in the roots and shoots, and 7 days after application 23 mg N was recovered per pot (calculated from Table 1). However, over the time to maturity there was a steady increase in the amount of N recovered so that by maturity the recovery was 120 mg per pot.

When N was added to the subsoil, root growth below 60 cm depth continued to increase up to maturity and paralleled the continued uptake of N from below this depth (Table 9), suggesting that the exploitation of subsoil N depended on active root growth. However, the rate of NO_3-N uptake per g root dry matter (or per cm root) was also greater than the control treatment, so the initial increase in the root length density alone did not account for the increase in soil NO_3-N uptake. It appears that the initial uptake of NO_3-N from the soil was limited by the amount of root growth in the zone of N application and, although

Table 9. Experiment 1. Estimation of root activity in the subsoil (depth 60–100 cm) 2 days after adding nitrogen

Harvest	Average root dry weight (g)	Average root length (cm)	NO_3-N uptake (mg)	NO_3-N uptake (mg/g.day)	NO_3-N uptake (μg/cm.day)
		Control (nil N)			
25 July–30 July	0·90	3164	−5·2	–	–
30 July–22 Aug.	1·04	4408	12·3	0·5	0·1
22 Aug–14 Sept.	0·90	3762	−5·1	–	–
		Nitrogen (150 mg/pot)			
25 July–30 July	1·17	4075	33·9	4·1	1·1
30 July–22 Aug.	1·65	4674	83·2	2·2	0·8
22 Aug–14 Sept.	2·17	6175	6·4	0·1	0·04

there was an increase in the rate of uptake per unit root growth, complete exploitation of the NO_3-N depended on the stimulation of root growth in the subsoil. The root length densities measured in the experiment were generally greater than those measured in the field, and the use of a non-draining pot ensured that NO_3-N was not leached from the pot. This resulted in a steady uptake of N by the wheat, whereas in the field uptake generally slows down after heading (Campbell *et al.* 1977). These results probably represent an ideal situation of relatively high root length density and no movement of NO_3-N beyond the roots. If the ability of roots to respond to N is limited by soil chemical or physical properties, or if the soil texture allows the NO_3-N to be leached beyond the root zone, the ability of the plant to exploit the subsoil N may be poor. Our results imply that growth and distribution of roots in the soil play major roles in the post-anthesis N economy of plants.

The increased root growth in response to N was also associated with greater water use. A number of studies in southern Australia have shown the existence of significant amounts of subsoil water under cereal crops at maturity, even though roots are present in the subsoil (Schultz 1971; Walter and Barley 1974). Moreover, the water use efficiency of the plants in Expt 1 was improved by addition of N. Thus it appears that the presence of substantial reserves of N in the subsoil and its subsequent effect on root growth is not only beneficial to GPC but may lead to more effective exploitation of soil water reserves and thus improve water use efficiency.

Conclusion

We concluded that the ability of plant roots to recover N from the subsoil is high. The results indicated that a transient increase in subsoil N increased root growth and N uptake per unit root length and thereby increased shoot N and grain protein concentration (GPC). Nitrogen placement increased grain yield and GPC of wheat compared with the control (zero N treatment). Water use and water use efficiency were increased by N application. Further research is needed to quantify the amount of N taken up by roots as a function of soil depth and to assess other factors which may affect N uptake from the subsoil by wheat.

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References

Alston, A. M. (1980). Response of wheat to deep placement of nitrogen and phosphorus fertiliser on a soil high in phosphorus fertilisers in the surface layer. *Australian Journal of Agricultural Research* **31**, 13–24.

Best, E. K. (1976). An automated method for determining nitrate–nitrogen in soil extracts. *Queensland Journal of Agriculture and Animal Sciences* **33**, 161–6.

Bremner, J. M. (1965). Nitrogen availability indices. *In* 'Methods of Soil Analysis, Part 2'. (Ed. C. A. Black.) pp. 1324–45. (American Society of Agronomy: Madison, WI.)

Campbell, C. A., Davidson, H. R., and Warder, F. G. (1977). Effects of fertiliser N and soil moisture on yield, yield components, protein content and N accumulation in the aboveground parts of spring wheat. *Canadian Journal of Soil Science* **57**, 311–27.

Cataldo, D. A., Haroon, M., Schrader, L. E., and Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* **6**, 71–80.

Colwell, J. D. (1963). The estimation of phosphorus fertiliser requirements of wheat in southern New South Wales by soil analysis. *Australian Journal of Experimental Agriculture and Animal Husbandry* **3**, 190–7.

Gass, W. B., Peterson, G. A., Hauck, R. D., and Olson, R. A. (1971). Recovery of residual nitrogen by corn (*Zea mays* L.) from various soil depths as measured by ^{15}N tracer techniques. *Soil Science Society of America Proceedings* **35**, 290–4.

Greenland, D. J. (1971). Changes in the nitrogen status and physical condition of soils under pasture, with special reference to the maintenance of the fertility of Australian soils used for growing wheat. *Soils and Fertilisers* **34**, 237–51.

Kissel, D. F., Ritchie, J. T., and Burnett, E. (1974). Nitrate and chloride leaching in a swelling clay soil. *Journal of Environmental Quality* **3**, 401–4.

Kuhlmann, H., Barraclough, P. B., and Weir, A. H. (1989). Utilisation of mineral nitrogen in the subsoil by winter wheat. *Zeitschrift fuer Pflanzenernaehrung und Bodenkunde* **152**, 291–5.

Ladd, J. N. (1990). Soil nitrogen dynamics and nitrogen uptake by wheat crops. Department of Agriculture, South Australia. Technical Report No. 159.

Papastylianou, I. (1980). Nitrate concentration in cereal stems and its use in evaluation rotations and predicting nitrogen fertiliser requirement. PhD Thesis, University of Adelaide.

Passioura, J. B., and Wetselaar, R. (1972). Consequence of banding fertiliser nitrogen in soil. II. Effects on the growth of wheat roots. *Plant and Soil* **36**, 461–73.

Schultz, J. E. (1971). Soil water changes under fallow-crop treatments in relation to soil type, rainfall and yield of wheat. *Australian Journal of Experimental Agriculture and Animal Husbandry* **11**, 236–42.

Sharma, B. R., and Chaudhary, T. N. (1984). Nitrogen uptake by wheat in relation to water regime and depth of N placement in a loamy sand soil: measurement and model simulation. *Journal of Soil Science* **35**, 141–8.

Smika, D. E., and Greb, B. W. (1973). Protein content of winter wheat grain as related to soil and climate factor in the semiarid Central Great Plains. *Agronomy Journal* **65**, 433–6.

Storrier, R. R. (1962). The availability of mineral nitrogen in a wheat soil from southern New South Wales. *Australian Journal of Experimental Agriculture and Animal Husbandry* **2**, 185–92.

Storrier, R. R. (1965). The leaching of nitrogen and its uptake by wheat in a soil from southern New South Wales. *Australian Journal of Experimental Agriculture and Animal Husbandry* **5**, 323–8.

Strong, W. M. (1982). Effect of late application of nitrogen on the yield and protein content of wheat. *Australian Journal of Experimental Agriculture and Animal Husbandry* **22**, 54–61.

Strong, W. M. (1986). Effect of nitrogen applications before sowing compared with the effects of split applications before and after sowing for irrigated wheat on the Darling Downs. *Australian Journal of Agricultural Research* **26**, 201–7.

Strong, W. M., and Cooper, J. E. (1980). Recovery of nitrogen from various depths in a cracking clay. *Australian Journal of Experimental Agriculture and Animal Husbandry* **20**, 82–7.

Tennant, D. (1975). A test of a modified line intersect method of estimating root length. *Journal of Applied Ecology* **63**, 995–1001.

Walter, C. J., and Barley, K. P. (1974). The depletion of soil water by wheat at low, intermediate and high rates of seeding. Transactions 10th International Congress. *Soil Science, Moscow*, **1**, 150–8.

Weier, K. L., and MacRae, I. C. (1993). Net mineralisation, net nitrification and potentially available nitrogen in the subsoil beneath a cultivated crop and a permanent pasture. *Journal of Soil Science* **44**, 451–8.

Wiersum, L. K. (1967). Potential subsoil utilisation by roots. *Plant and Soil* **27**, 383–400.

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