THE INFLUENCE OF SOIL, CLIMATIC AND MANAGEMENT FACTORS ON NITROGEN ACCRETION BY ANNUAL *MEDICAGO* SPECIES IN A SEMI-ARID ENVIRONMENT OF SOUTH AUSTRALIA

A thesis submitted

by

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DEDICATION

To my wife, Zeineb, for her patience and understanding

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STATEMENT

This thesis has not been previously submitted for a degree at this or any other University, and to the best of my knowledge it contains no material previously published or written by another person except where due reference is made in the text.

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SUMMARY

A survey of farmers' fields was undertaken in the district of Mallala to evaluate the contribution of annual *Medicago* species to soil total nitrogen under a semi-arid environment. The diversity of annual medic history in cereal rotation was the criteria for selecting the study area.

Ninety-five paddocks in pasture were soil sampled during spring. Soils were transitional between red-brown earth and solonized brown soil and mean annual rainfall varied from 325 to 400 mm. Pastures were designated medic-dominant or grass-dominant within the 25m x 25m sampling area from which 16 randomly located soil cores were drawn and bulked.

Cropping history of each site and for the 10-year period (1965-1974) was obtained from the farmers. Soils were analysed for physical and chemical properties. Correlations between pairs of measured variables and linear regressions were used to identify the degree of interaction between variables. Principal component analysis was also performed on the data in order to simplify the description of the interrelationships between soil, management and climatic factors in relation to soil total nitrogen of the 0-5 and 5-10cm depth intervals.

Variation in soil total nitrogen between sampled sites was positively associated with the frequency of years of pasture (FYRP) and negatively correlated with the frequency of years of crops (FYRC). A combination between a management factor (FYRP) and a climatic factor (May - October rainfall), defined as accumulated seasonal rainfall on pastures (ASRP), led to a significant improvement of the coefficient of determination of the regression of soil total nitrogen on FYRP (from $R^2 = 0.28^{***}$ to $R^2 = 0.42^{***}$).

Estimates of nitrogen increment in the 0-10cm layer of soil under medic-dominant pastures were derived from linear regressions of soil total nitrogen on ASRP and were found to vary between seasons with an average mean annual increment of 200 kg ha^{-1} N in a mean rainfall year (248 mm, May - October). The corresponding figure for the grass-dominant pastures was low and poorly defined. Principal component analysis indicated that differences in soil total nitrogen under medic-dominant pastures were positively associated with the ASRP, soil lime, total phosphorus and negatively associated with bulk density. There was a lack of correlation between soil total nitrogen and available phosphorus and soil total nitrogen and clay content of the surface soil.

The split of soils into solonized brown soils and red-brown earths showed the same associations between soil total nitrogen and the measured variables except that red-brown earths are lime-free in the surface soil and clay was more closely associated with soil total nitrogen.

The acetylene reduction assay was used to measure nitrogenase activity as an estimate of rate of nitrogen fixation in nodulated *Medicago truncatula* (cv. Jemalong) in the field on several occasions during the year. The effects of varying the density of sowing from 0 to 1000 kg ha^{-1} seed, and date of sowing were investigated, as the survey had indicated that dominance of the pastures by medic was of primary importance to soil nitrogen accretion. Acetylene reduction activity increased with plant density during the vegetative phase of plant growth and reached an optimum at about flowering. Beyond flowering acetylene reduction activity decreased more rapidly at the medium and high plant densities than at the low ones. Results were interpreted in terms of measured effects on dry matter production and plant population.

The effect of phosphate supply and grass-medic competition on acetylene reduction activity of *Medicago truncatula* (cv. Jemalong) was investigated using a glasshouse experiment. The experiment was aimed at clarifying the result from the survey of a lack of correlation between soil total nitrogen and soil available phosphorus.

Acetylene reduction activity, shoot dry weight, root dry weight and nitrogen content of medic roots all responded strongly to rate of phosphate supply and all had the same optimum phosphate level (160 ppm P). There was no significant plant x community interaction on acetylene reduction activity of medic grown in association with ryegrass. It was concluded that none of the hypotheses under test could be sustained and survey result is likely to be an indirect effect of the influence of phosphorus in crop years.

1.0 INTRODUCTION

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The nitrogen content of soils of many regions in the world remains a factor limiting crop yields. Nitrogen fertilizers may increase yields in those regions where environmental conditions are adequate for nitrogen uptake and utilization by plant communities (Russell 1968a). In some Australian cereal growing regions with a Mediterranean type of climate, the use of legume pasture leys as a means of providing nitrogen to cereal crops is considered to be safer and more profitable than fertilizers because of the steady build-up of soil nitrogen reserves and subsequent nitrogen release to crops, the lower cost of cereal production, the integration of cereal and animal production and the diversification of farmers enterprises and incomes (grain, wool, livestock, and sometimes pasture seeds).

In Australia, striking progress in cereal production has been made since the introduction of legume pasture leys in cereal rotations (Donald 1965). Many studies have clearly demonstrated the beneficial effects of subterranean clover *(Trifolium subterraneum)* on soil nitrogen (N) accumulation and subsequent cereal production (Donald and Williams 1954, Williams and Lipsett 1961, Russell 1960). Field surveys (Donald and Williams 1954, Williams and Lipsett 1961) and long-term field experiments (Russell 1960, Watson 1963, 1969, Greenland 1971; Jenkinson 1976) have been used to study soil N status with varying management practices. The estimated amounts of N fixed by subterranean clover leys varied from 50 to 300 kg ha^{-1} $year^{-1}$. Nutman (1976) reported the amounts of N fixed (kg N ha^{-1} $year^{-1}$) by several forage legumes and grain legumes, and mentioned that forage legumes generally fix more N than grain legumes. But, so far, there is a lack of detailed comparative studies between the amounts of N fixed by legume-based pastures and grain legumes.

Wheat yields have more than doubled in many areas of the Australian cereal belt from 1930-40 to 1950-60. These areas generally lie in the higher rainfall zones of the wheat belt (500 mm or more annual rainfall) where subterranean clover grows profusely on acid soils. However, cereal production extends to regions of much lower seasonal rainfall (300 to 400 mm) where growth and persistence of subterranean clover is almost always unsatisfactory.

In the lower rainfall regions with neutral to alkaline soils annual medics *(Medicago* spp.*)* grow well in most seasons. One may ask whether the introduction of annual medics into cereal rotations has the same effects as does subterranean clover on soil fertility build-up and cereal production. There are few data in the literature to help answer this question. It seemed useful, therefore, to study the contribution of annual medics to soil N and to determine the factors in the environment (soil, management and climate) that may be linked with the build-up of soil N under semi-arid conditions. For this purpose, a survey of 106 pasture paddocks of varied annual medic history was conducted in the district of Mallala (South Australia) in 1974. This survey aimed at examining the relation between soil total N and the frequency of annual medic leys in the rotation over a number of seasons and under farm conditions (Part 3.0). Hypotheses derived from the results of the survey were tested using a field experiment (Part 4.0) and a glasshouse experiment (Part 5.0). The field experiment looked at the effects of sowing density and sowing date on the rate of nitrogen fixation (N_2-fixation) by barrel medic *(Medicago truncatula,* cv. Jemalong*)* throughout a growing season (1975). The glasshouse experiment investigated the effect of phosphate supply and competition from grasses on growth and N_2-fixation by barrel medic. Nitrogen content of herbage was estimated by analyses of plants and N_2-fixation activity in the plants was measured by means of the acetylene reduction assay.

2.0 LITERATURE REVIEW

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While differing greatly from other great soil groups of the world, the soils characteristic of the Mediterranean climates nevertheless share the almost universal deficiency in N. Under continuous cropping soil N declines (Greenland 1971) owing to losses in a number of ways.

2.1 DEPLETION OF SOIL NITROGEN

2.1.1 Leaching

Leaching of available soil N into deeper subsoil layers is one of the processes of N loss from the arable layer of soil. The magnitude of leaching depends on nitrate accumulation in the soil, the incidence of heavy rains and soil texture (Allison 1966). Since nitrate follows the movement of water, it is evident that the degree of water percolation is the principal factor controlling loss of nitrate by leaching. From the agricultural point of view, the significance of this downward movement of nitrate depends on crop species. According to Greenland (1971), subsoil accumulations of nitrate in the cereal zones of Australia often remain within the boundaries of the root zone since rainfall intensity is generally quite low. The same author suggested that loss of N by volatilization is more important in the cereal belt of Australia.

2.1.2 Volatilization

Volatilization of soil N is due primarily to the evolution of ammonia, especially from alkaline soils and when N fertilizers such as ammonium nitrate or urea are added to the soil (Simpson 1968) or from areas receiving urine from grazing animals (Watson and Lapins 1969). The loss of ammonia by volatilization from ammonia-based N fertilizers was estimated to be 15 - 25% of the quantity applied (Allison 1966).

In Australia, Watson and Lapins (1969) mentioned losses of N from the soil-plant system of the order of 45 kg for every 100 kg of herbage N ingested by grazing sheep. Volatilization of ammonia from urine was the main source of loss. Denmead, Simpson and Freney (1974) reported that N was lost as ammonia at an annual rate of 100 kg ha^{-1} from grazed lucerne pastures near Canberra. The loss of N by volatilization was reported to be greater under warm conditions and from bare ground (Watson and Lapins 1969; Clarke 1970; and Denmead *et al.* 1974).

2.1.3 Biological denitrification

Denitrification of soil N consists of the biological reduction of soil nitrate and nitrite to volatile gases by anaerobic bacteria. With soil water near field capacity and in micro-sites of low oxygen partial pressure, nitrate is used by denitrifying organisms in place of oxygen as a hydrogen acceptor. Anaerobic organisms use soil organic matter as source of energy for the reduction of nitrate. The main pathway of denitrification involves the following sequence of reductions (Broadbent and Clark 1965): $NO_3^- \longrightarrow NO_2^- \longrightarrow NO_2 \longrightarrow N_2$. However, Stanford, Legg, Dzienia and Simpson (1975) reported the reduction of NO_3^- to NH_4^+. Nitrous oxide (N_2O) may also be produced from hydroxylamine (NH_2OH) or from NH_4^+ by oxidation by *Nitrosomonas europaea* (Yoshida and Alexander 1970). Ritchie and Nicholas (1972) suggested the presence of an active nitrite reductase system in *Nitrosomonas europaea*.

The effects of soil vegetation and soil conditions on denitrification were investigated by Burford and Greenland (1970) who found that under an annual pasture of wimmera ryegrass *(Lolium rigidum)* and subterranean clover, losses of N as N_2O were greater in mid-winter at periods of highest soil water content and lowest soil temperature (5 to 15°C).

These conditions corresponded with an early stage of plant development. Stefanson (1972) measured gaseous losses of N from 4 different soils using sealed growth chambers held at 20°C temperature. He observed that the amounts of N_2O and nitrogen gas (N_2) evolved increased as a function of soil water content and of plant growth as well as the form of N fertilizer applied to the soil plant system, the oxygen tension, and soil texture. The rate of N loss was 1-15 mg N/kg of soil per week when nitrate N was applied as fertilizer, and 1-4 mg when ammonium N was applied. Greenland (1971) pointed out that denitrification can be an important source of N loss especially from soil newly opened from pasture. Bartholomew (1964a) and Allison (1966) indicated that 5 to 15% of the available N can be lost during a single growing season.

Apart from these losses due to biological denitrification, gaseous loss of N can stem from either direct oxidation of ammonium N or chemical reduction of nitrites. Ammonium oxidation in extracts of *Nitrosomonas europaea* in solution culture was observed by Yoshida and Alexander (1970) and by Ritchie and Nicholas (1972). The first named authors suggested that the same kind of oxidation can also take place in soil. The non-biological reduction of nitrite was reported by Bulla, Gilmour and Bollen (1970), who were able to detect by gas chromatography the evolution of N_2, N_2O and NO following the chemical decomposition of nitrites present in sterilized soils incubated at 25°C.

2.1.4 Water and wind erosion

Water and wind erosion are other contributors to soil N depletion. The magnitude of these losses depends on the intensity and frequency of adverse climatic factors as well as on soil properties, mainly soil topography, infiltration rate and aggregate stability. The practice of fallowing in arable areas can enhance soil erosion and N loss from the surface of light textured soil.

2.1.5 Cropping

History has shown that soil N deficiency is accentuated by continuous cropping without restitution of nutrients. Donald (1965) observed that on an Australia-wide basis, heavy cropping led to soil N exhaustion accompanied by a serious decline in wheat yields, for example, from 0.9 t ha^{-1} in 1870 to 0.5 t ha^{-1} in 1890. Greenland (1971) observed in a permanent rotation trial at the Waite Agricultural Research Institute a decline of soil N content of the order of 2.2 t ha^{-1} N under continuous crops of wheat for the 38-year period between 1925 and 1963. Jenkinson (1976) reported that there was a steady loss of 36 kg N ha^{-1} from unmanured plots at Rothamsted Experimental Station, under the continuous wheat experiment over the period 1852-1967, but that N losses were greater (41 kg ha^{-1} $year^{-1}$) from plots which had received P, K and Mg. In Tunisia, under climatic conditions similar to those prevailing at the Waite Institute Essafi (1964) compared different crop rotations for a period of 25 years (1936-1960) and found that under continuous crops of wheat and without any restitution, soil total N decreased from 0.106 to 0.089% while soil organic carbon decreased from 1.02 to 0.86% in the 0-20 cm depth of soil. Such studies provide good experimental evidence that continuous cultivation of soil over a long period of time results in a gradual loss of soil N and ends in an undesirable decline in cereal production.

The advent in the 1860's of the dry farming system based on the alternation of crops and bare fallow periods was effective in providing more available N to the crop. Improved yields resulted. Obviously, under this farming system the increases in yield were not only due to higher mineralization of soil N but also to better carry over of soil water and to the reduction of weeds. In South Australia, French (1963) found that soil mineral N increased by 26 kg ha^{-1} during a fallow year. However, French (1966) reported that about 59% of wheat yield response to fallow could be ascribed to variation in additional soil water at sowing. According to the literature, long periods of soil cultivation and fallowing have led to depletion of soil total N associated with a degradation of soil structure, soil erosion and loss of organic matter. Clark and Marshall (1947) reported that after 16 and 20 years of fallow-wheat rotations total N in the surface 10 cm of red-brown earths decreased from 0.158 to 0.094% and from 0.222 to 0.135% respectively. Cornish (1949) stated that, during the period from 1896 to 1941, wheat yield, in South Australia declined as a result of intensive cropping systems based on continuous wheat or fallow-wheat or fallow-wheat-oats. Cornish attributed the decline in soil fertility and the loss of soil structure to these cropping systems.

In many other cereal growing regions where bare fallow was used as a means of increasing that fraction of soil total N which is available to crops, studies reported losses as high as 0.8% of the total N per year (Martin and Cox 1956). In north-western New South Wales, Hallsworth, Gibbons and Lemerle (1954) mentioned losses of N reaching 2% per year from wheat soils. Williams and Lipsett (1961) pointed out that continuous cultivation of soil led to a breakdown of organic matter with loss of 20 to 50% of carbon, nitrogen and sulphur and loss of 17% of organic phosphorus after 40 to 50 years. So although fallowing increases the rate of N mineralization and releases more available N for crop use, its long term effect undoubtedly consists of exhaustion of the soil N reserve.

2.2 ACCUMULATION OF SOIL NITROGEN

2.2.1 Rainfall

Nitrogen in the atmosphere is carried down in rain largely as NO_3-N and NH_4-N. Hauck (1971) quoted values going from 0.8 kg ha^{-1} N to 57 kg ha^{-1} N per year. The highest values were recorded in temperate regions and in humid tropics. Steyn and Delwiche (1970) reported that for a seasonal total precipitation of 617 mm, rainwater contained about 1 kg ha^{-1} N per year. In Australia (Queensland), Probert (1976) reported values of 1.2 to 2.7 kg ha^{-1} of NH_4-N per year. Jenkinson (1976) reported that the mean annual amount of mineral N carried down in rain was at Rothamsted over the period 1889-1903, 4.4 kg ha^{-1} and that there was a trend towards larger values. Considering the variability and distribution of seasonal rainfall in the semi-arid areas, the contribution from rain to soil N balance appears to be of little importance in agricultural systems compared with that of other sources of N input. In South Australia, at the Waite Agricultural Research Institute, the total amount of N (Total N + nitrate N) added in rain water varied from 1.6 kg N ha^{-1} in a dry year (1967) to 2.5 kg N ha^{-1} in a wet year (1968) (Barley 1976, unpublished data).

2.2.2 Nitrogen fertilizers

To mitigate soil N depletion, agriculturalists have recourse to the use of N fertilizers. The use of N fertilizers combined sometimes with superphosphate may arrest the decline in soil total N. At Rothamsted Experimental Station total N of the 0-23 cm depth interval of plots under continuous wheat and receiving N fertilizer did not change significantly during the period 1865-1966 (Jenkinson 1976). Further, in many Mediterranean cereal growing regions the use of N fertilizer may accentuate the variability in cereal yields caused by large fluctuations in amount and distribution of rainfall within and between seasons. In South Australia, Russell (1967) suggested that N fertilizers are likely to be profitable where:

- the soil is sandy or has a poor history of legume pasture sowing;
- the seasonal (winter) rainfall exceeds 250 mm;
- the spring (September, October) rainfall exceeds 65 mm;
- weather conditions are mild during the heading stage.

As can be seen, these conditions, apart from the first, are unknown before the fertilizer is applied, and unpredictable, except in statistical terms. Consequently the skill and practical knowledge of a farmer have to be taken into consideration when such fertilizer is to be used. Use of N fertilizers has remained to this day a hazardous and expensive proposition in the low rainfall areas of the cereal belt.

An alternative to the use of N fertilizers is the use of "legume" N by the introduction of legume-based pastures into the rotation. In the semi-arid regions annual medics (*Medicago* spp., to be referred to as medics) can be a valuable source of soil N and organic matter. The advantages of medics in cereal rotations are multiple: (i) build-up of soil total N and consequent increases in cereal yields and grain protein; (ii) lower and steady availability of legume N which is claimed to adjust the supply to the season somewhat, avoiding excessively leafy crops; (iii) good legume growth may improve soil structure and soil water holding capacity through the accumulation of soil organic matter; (iv) reduction in cereal production costs, due to less intensive cultivation and lower fertilizer costs; (v) reduction in losses due to cereal root pathogens especially nematodes and take-all; (vi) higher returns from increased stocking capacities (wood production, livestock, etc.);

(vii) annual legume seed production can provide average gross margins comparable to wheat (Bicknell 1973).

2.2.3 Asymbiotic nitrogen fixation

The role of free-living organisms such as bacteria and blue-green algae in N_2-fixation in the soil was not until recently well investigated. According to Stewart (1966) asymbiotic N_2-fixation is higher in Indian paddy fields than in arid Australian soils. The mean value reported was respectively 34 and 3 kg ha^{-1} N per year. In California, Steyn and Delwiche (1970) found that over a period of one year asymbiotic fixation of N was higher in winter and varied from 2 kg ha^{-1} N per year in arid uncultivated land to 5 kg ha^{-1} N per year in irrigated perennial lawn grass. Under a natural *Agropyron-Koeleria* grassland Paul, Meyers and Rice (1971) observed that asymbiotic N_2-fixation was highest in medium textured soils of virgin grassland and amounted to 1 kg ha^{-1} N per year. Englund (1975) found no blue-green algae activity in the arid regions of Tunisia, where in cultivated regions, the maximum N fixing rate was higher under olive trees than in wheat fields. Witty, Day and Dart (1976), using the acetylene reduction assay estimated that under continuous wheat the contribution from blue-green algae to soil N varied from 19 to 23 kg N ha^{-1} per year, and that algal fixation over the season was associated with soil moisture.

These studies and others (Jensen 1965, Allison 1965) produced evidence that the contributions from free-living organisms (*Azotobacter*, *Clostridium*, blue-green algae, etc.) to soil N status is generally small and is relatively greater under wet conditions. In practice, the amounts of N fixed asymbiotically are not of agricultural importance in the cereal zones of the semi-arid areas of the Mediterranean climatic regions mainly because of low surface soil moisture through much of the season and especially during hot weather conditions in summer.

2.2.4 The introduction of improved annual legume pastures into the rotation and their effects on soil nitrogen

In ancient times, agriculturists had already an empirical knowledge of the beneficial effects of legumes as green-manure (Dart 1973). The adoption of ley farming based on legume pasture-cereal crop rotation has replaced the extensive cropping on fallow in most cereal-growing regions of Australia since the 1930's and early 1940's (Donald 1965; Matz 1973 and Webber 1973). Subterranean clover and/or medics are the most commonly used annual legume pastures in cereal rotations in the Mediterranean or winter-rainfall environment.

(i) The lift in soil fertility through improved subterranean clover pastures in high rainfall areas of the southern cereal belt

Many studies made in New South Wales, Victoria and South Australia have shown that increases in soil total N accounted for by subterranean clover vary from 40 to 350 kg ha^{-1} N per year according to growth conditions, availability of nutrients and management factors. In New South Wales, under 817 mm average rainfall (Crookwell district), Donald and Williams (1954) found that soil total N rose by 40 kg ha^{-1} N for each 120 kg ha^{-1} of superphosphate applied on subterranean clover leys. Donald and Williams pointed out that soil total N level appeared to increase linearly with the length of pasture ley. At Rutherglen in Victoria, Mullaly,McPherson,Mann and Rooney (1967) observed the same trend of increasing soil total N under subterranean clover (cv. Mt.Barker) leys over a period of 8 years. Similar relationships between soil total N and years under pasture were reported by Russell (1960) and Watson (1963). McLachlan (1968) found that soil total N under a 15 year old subterranean clover pasture was higher (0.164%) where stocking rate was high than where the stocking rate was low (0.101%). However, Simpson and Bromfield (1974) found higher soil total N build-up with light grazing pressure (from 4.4 to 6.8 sheep ha^{-1}) than with high (from 13.2 to 20.4 sheep ha^{-1}), although mineral N was greater under heavy grazing pressure than under light grazing pressure. However, "high" and "low" stocking rates are relative terms depending on the amount of feed available in the pasture. Watson (1969) mentioned that about 75% of the ingested N could be excreted in sheep urine.

Such studies show that, when properly established and managed, subterranean clover can play an important role in raising soil fertility and in improving agricultural productivity in regions of comparatively high seasonal rainfalls (500 mm or more) and on acid soils (pH 5.0 - 6.5). Persistence and growth of subterranean clover are inadequate in regions of lower annual rainfall (less than 400 mm) and on neutral to alkaline soils, probably owing to the low survival of *Rhizobium trifolii* in alkaline soil, the failure of subterranean clover to set sufficient seed for adequate regeneration (Rossiter 1966), the poor ability of subterranean clover to utilize phosphorus in soils of high lime status (Trumble and Donald 1938), and the low availability of zinc and manganese in alkaline soils (Higgs 1958).

In the cereal growing zones receiving between 250 and 400 mm rain per year wheat production is not only affected by soil conditions but also by very irregular winter and spring rainfalls. So, as far as soil fertility and soil structure are concerned, the need in these regions for a soil stabiliser appears to be of prime importance.

(ii) Utilization of improved medic pastures in the semi-arid areas

Both subterranean clover and medics are important pasture legumes in southern Australia. While subterranean clover is dominant on acid soils in the higher rainfall regions (more than 500 mm rainfall per season) medics are better adapted to alkaline soils of relatively heavier texture in areas of low seasonal rainfall (between 200 and 450 mm). The impact of subterranean clover pastures on soil fertility and cereal-livestock production has been studied by many authors since 1938 (Trumble and Donald). The principal idea revealed in these studies is that with judicious farm management the use of topdressed subterranean clover pastures in rotation with cereals could bring about remarkable improvement in both cereal production and livestock numbers. However, although medics possess the same ability to increase soil fertility, their adoption in cropping systems by cereal growing farmers has been rather slower, and scientists too have been slow in quantifying the contribution of medics to soil N.

Quinlivan (1965) reported that only 3% of the total area of improved pastures in Western Australia was based on medics. On Yorke Peninsula, South Australia, Counties Daly and Fergusson represent a large part of the medic country, but the increase during the period 1959-60 to 1968-69 in area sown with improved medic pastures (topdressed with superphosphate) was from 24,650 ha to 26,850 ha in County Daly, and from 34,160 ha to 36,326 ha in County Fergusson (Webber and Matz 1970). In County Gawler in the Lower North of South Australia, topdressed pastures (mainly medic pastures) increased very slightly from 9,356 ha to 10,770 ha during the period 1952-53 to 1961-62. In the district of Mallala (County Gawler), production statistics showed no increase in improved pastures between

1956-60 and 1961-65. In fact, the 6050 ha (1957-60) and 5746 ha (1961-65) of improved pastures in the district of Mallala (where a soil fertility survey was carried out in 1974, see Section 3.0 of the present study) represent 7% of the average area used for agricultural and pastoral purposes. Thus, the areas of improved medic pastures in South Australia still remain low in proportion to the potential medic land in this state. This slow adoption of improved medic pastures could be partly attributed in the past to the lack of successful varieties for all the different soil types and rainfall zones. However, in the past few years an increasing interest in medics has been shown by cereal farmers not only in southern Australia but also in the Mediterranean regions of North Africa (Tunisia, Algeria, Libya) and the Middle East following the availability of new cultivars such as Harbinger *(Medicago littoralis)*, Paragosa *(Medicago rugosa)*, Cyprus *(Medicago truncatula)* and Jemalong or Barrel 173 *(Medicago truncatula)*. These cultivars have different requirements as far as climatic conditions, soil types and plant nutrient availability are concerned. Thus, a principal hurdle to the expansion of improved medic pastures appears to have been overcome. It now seems appropriate to look at the factors affecting the performance of these medics.

(iii) Factors affecting the growth of medics

Three major groups of factors have direct effects on medic growth:

- Climatic factors: rainfall, temperature, light.
- Soil factors: physical, chemical and biological.
- Farm management practices: rotation, fertilizer application, grazing pressure, weed control (herbicide, cultivation, grazing pressure), and pest control.

(a) Climatic factors The distribution of both subterranean clover and medics is related to the length of the growing season. But since the length of growing season is highly associated with mean annual rainfall it is obvious that for medics as for subterranean clover early maturing strains will appear and develop in the low rainfall areas whereas late maturing varieties will dominate in relatively higher rainfall zones.

Rainfall To an extent greater than subterranean clover, medics appear to be susceptible to waterlogging. This may in part explain the absence of medics in high rainfall zones (500 mm or more) where more frequent waterlogging is likely to occur. Moreover, within the medics *Medicago littoralis* and *Medicago truncatula* are reported to be more susceptible to waterlogging conditions than *Medicago polymorpha* and *Medicago intertexta* (Robson 1969). This low tolerance of medics to waterlogging has been attributed to fungal attack or to manganese toxicity or low oxygen supply. Another factor in water supply is the reliability of the rains. Weathering of medic pods over summer and autumn leads to a break of seed dormancy and enables medic seeds to absorb water after early autumn rains. However, rainfall is not always reliable throughout the growing season and drought can occur at any stage of plant development. The occurrence of a drought period after early autumn rains leads to heavy losses of medic seedlings and thereafter poor stands of medics during the season because of the depletion of readily germinable seed. Nevertheless, some medic varieties such as *Medicago minima* and *Medicago truncatula* (cv. Jemalong) are reported in the literature to be more tolerant of temporary drought (Crawford 1962, Robson 1969). Mathison (1973) mentioned that there are differences in speed of germination and rate of radicle extrusion among medic species.

From the agricultural point of view fast germination could be either a disadvantage or an advantage. It would be a disadvantage if an early false break of season occurred because seedling mortality is more likely to be high, or an advantage when the opening rains are late because then fast germination will permit medic plants to overcome competition from annual grasses. Similarly in the low seasonal rainfall areas (<300 mm annual rainfall) where self-regeneration of medics in the pasture phase of the ley-farming system depends upon soil seed reserves, the earlier the germination of medics the better the growth and the higher the proportion of medic seed entering the soil each pasture phase.

Temperature Owing to their Mediterranean origin, medics grow mainly in winter and early spring when the temperature is cool to mild. Amor (1966) reported that Harbinger and Jemalong cultivars produced more winter herbage in the Victoria mallee, than common barrel medic and Cyprus. Common barrel medic came from a strain found at Noarlunga in South Australia, whereas Barrel medic 173 or Jemalong was selected in the Forbes district of New South Wales. Thus, it is not surprising that Jemalong produced more winter herbage than common barrel medic in the Victorian mallee where winter temperature is less suitable for the growth of common barrel medic than for that of Jemalong. Kleinig (1965) observed a low emergence of barrel medic seedlings under conditions of low soil temperature. Although medics require a cold period for flower initiation, temperatures below 10^{o}C slow down the rate of pasture growth as is the case in zones of high altitude in North Africa. Millikan (1961) found that winter conditions with mean monthly maximum temperatures varying from 9 to 19°C favoured shoot growth of Jemalong at the expense of root growth, but he observed that the mean daily increase in dry weight of Jemalong (tops + roots) was less in winter than in summer when the mean monthly maximum temperature varied from 19 to 33°C.

The growth of self-regenerating legume species, such as medics, is influenced by high temperatures because of the effect of high temperature on seed coat permeability. Quinlivan (1971) reported that seed coat impermeability or hardseededness limited the use of medics as pastures in areas of high rainfall and long growing seasons with short and cool dry summer periods in southern Australia. On the contrary, better regeneration and break down of hardseedness was observed in areas of long and warm dry summer period. It appears that the diurnal temperature fluctuations between day and night have an effect on the integuments of the seeds which, following continuous expansions and contractions, become loose and more permeable. Therefore, high temperatures are important for the self-regeneration and subsequent growth of medics.

Light Light limitation in subterranean clover arises from either interplant competition at high density per unit area or in mixed swards owing to shading of subterranean clover by the taller growing grasses (Donald 1963). The interception of light by taller growing grass species (e.g. ryegrass) at the expense of shorter annual legumes leads to a poor growth of the legume species. This diminution of growth occurs also after more or less severe defoliation of legume-based pastures. In New Zealand, Harris and Thomas (1973) pointed out that less frequent cutting at 8 cm of a white clover (*Trifolium repens*) and ryegrass sward enhanced the disappearance of white clover from the sward. Sinclair (1973) observed that cutting subterranean clover plants 1 cm above the soil surface produced a depression of bacterial N fixing activity for several days. It appears, therefore, that recent photosynthate is a prerequisite for nodulation and symbiotic N_2-fixation. No data on light and shading effects are available for medics.

(b) Soil factors

Physical properties Ecological studies have shown that medics prefer soils of medium to heavy texture with a high content of lime (Trumble 1939; Rossiter 1966, and Lazenby & Swain 1969). Nevertheless, cultivars differ in their adaptability to various soil types. Soil physical characteristics which aggravate either waterlogging or drying out affect medic growth and persistence. In the field, medics persist poorly on very light sandy soils which are drought-prone, lime-free, structureless and subject to drift during the summer. On solodized solonetz soils with a loamy sand or sandy loam topsoil, the tendency of the surface soil to set hard on drying usually affects the emergence of annual legume seedlings. On this type of soil, losses of seedlings are often associated with either lack of water in the surface soil and the resulting mechanical impedance of the soil on seedling emergence, or waterlogging following sporadic heavy rains due to the shallowness of the surface soil overlaying a heavy clay subsoil.

Chemical properties According to the natural distribution of medics in Australia and around the world, and from a few experimental studies of the behaviour of the medics under different environmental conditions, it is clear that medics are essentially adapted to neutral and alkaline soils (Amor 1965; Robson 1969). The principal limiting factor to the growth of medics on acid soils is believed to be the inability of *Rhizobium meliloti* to survive in these soils. This susceptibility of the bacteria to soil acidity is probably due to effects of hydrogen ions *per se*, to aluminium and manganese toxicity or to calcium and molybdenum deficiency (Robson 1969). The possibility that calcium is needed for both nodulation and *Rhizobium* multiplication was clearly shown in the study made by Robson and Loneragan (1970) concerning the effect of calcium carbonate on the growth of barrel medic on acid soil of pH 4.6. The failure of medics to persist to maturity on red-brown earths with a pH of 5.7 was linked with molybdenum deficiency (Cameron and Newman 1958). Kleining (1965) attributed the poor growth of barrel medic seedlings on acid soil to fungal attack. However, *Medicago minima* and *Medicago laciniata* were reported to be more tolerant to soil acidity (pH<6.5) than *Medicago polymorpha* (Rossiter 1966).

Nitrogen availability in soil is unlikely to limit the growth of well nodulated medics. However, it is often found, in practice, that a light application of N fertilizer at seeding helps the growth of the seedlings before nodulation starts. The effect of combined N on nodulation and N_2-fixation of legumes will be reported and discussed in Section 2.4.

Phosphorus is a major factor limiting plant growth in Australia due to the poverty in phosphorus of most Australian soils. Accordingly phosphorus availability has been reported to affect medic growth directly. French and Rudd (1967) reported that the more superphosphate applied the higher the yield of medic pastures and that medic yields were greater where superphosphate was applied to both crop and pasture rather than wholly to the crop. Rudd (1972) pointed out that the level of soil available P (kg ha^{-1} in the 0-10 cm depth interval 0.5M $NaHCO_3$ extractable) that is required to produce 90% of the maximum medic yield varied from 32 to 38 for sandy soils, 41 for loamy mallee soils and 47 for dark-brown cracking clay soils. Rossiter and Kirton (1956), in a comparative study of the effects of different sources of phosphate on the growth of several legumes grown in pots observed that 44 days after sowing, barrel medic yielded as much as subterranean clover when supplied at rates of P varying from 5 to 150 kg ha^{-1}, but at the high rate, phosphorus depressed top growth of barrel medic in the late winter trial (mean daily temperature of 16^{o}C under glass). At this high rate of P, there was a decrease in nodule numbers associated with a decline in soil pH from 6.9 to 6.1. Millikan (1961) reported that phosphorus deficiency reduced the total growth of barrel medic but that there was no significant season x phosphate level interactions. Asher and Loneragan (1966, 1967) found that dry matter production of many species growing in flowing solution cultures, did not increase in proportion to the increase in phosphorus concentration of the solution; however the relative growth rate of barrel medic and of subterranean clover increased as phosphorus concentrations increased from 0.04 to 1 μM, but at higher phosphorus levels neither legume showed any response.

Most of these studies deal with the effect of phosphorus on the growth of barrel medic in comparison to that of subterranean clover. It is desirable to extend these comparative studies on the effect of phosphorus to other medic cultivars in order to clarify our understanding of the effect of phosphorus on the growth of medic species in different environments (soil type, pH, calcium content, rainfall etc.)

Calcium deficiency occurs mainly in acid soils which have not received phosphorus fertilizer regularly, for superphosphate contains calcium phosphate and gypsum. But as medics commonly grow well on neutral to alkaline soils, calcium deficiency in medics is rarely encountered, except perhaps in red-brown earths with a poor superphosphate history. Loneragan, Snowball and Simmons (1968) reported that fresh weight of barrel medic was more affected by low concentrations of calcium in solution culture than that of grasses or cereals. They observed a 50% increase in the growth of barrel medic when calcium concentration in solution was increased from 100 μM to 1000 μM. Like phosphorus, calcium carbonate reduced barrel medic growth at low soil phosphate levels whereas at high phosphate levels calcium carbonate enhanced growth. Olsen (1953) reported that phosphate solubility in calcareous soils is at a minimum in the range of pH of 7.0 to 7.5. This interaction between calcium, phosphate and soil pH is an example of the complexity of plant nutrition showing the interdependent effects of various factors on plant growth.

The effect of sulphur on medic growth has not yet been widely studied. This may well be due to the fact that soils in the semi-arid areas of the cereal belt are unlikely to be naturally deficient in sulphur. Robson (1969)* stated that *Medicago minima* is less susceptible to sulphur deficiency than *Medicago polymorpha* or *Medicago orbicularis*. This could be due partly to a greater penetration of *Medicago minima* roots into the subsoil where they can reach some of the sulphur which has been leached in sandy soils. French, Clarke, Rudd, Seeliger and Lewis (1975) mentioned that pasture responses to sulphur are most likely in sandy soils when total autumn and winter rainfall exceeds 200 mm.

Molybdenum deficiency is more common on acid than on alkaline soils. In South Australia the amounts of molybdenum in the 0-15 cm depth interval were found to vary from less than 0.1 ppm in deep sands to 2 ppm in red-brown earths (Russell 1960). Here too the interdependence of mineral nutrient effects is very complex. Indeed, Anderson and Spencer (1950a) found that the response of subterranean clover plants to molybdenum was much greater where soil sulphur status was low and where no fertilizer N was added to the soil. Molybdenum is now believed to be involved in the reduction of nitrogen (N_2) to ammonia by the enzyme nitrogenase (Dilworth 1974). This agrees with the statement of Bouma (1969) that molybdenum is required by plants self-sufficient in N at higher rates than those required by grasses. According to Rossiter (1966), molybdenum requirements for N_2-fixation may be higher in medics than in subterranean clovers.

Other trace elements were found to be essential for the growth and nodulation of leguminous plant species and for the efficiency of symbiotic N_2-fixation. Cartwright and Hallsworth (1970) reported a depressing effect of low copper supply on the yield of dry matter of subterranean clover nodule bacteroids and they

*quoting Hilder and Spencer: *J.Aust.Inst.Agric.Sci.* 20:17(1954).

observed a slower development of nodules of subterranean clover (cv. Mt.Barker) as copper stress increased. With regard to cobalt, Powrie (1960) found in a field experiment in South Australia that 560 g ha^{-1} of cobaltous sulphate ($CoSO_4.7H_2O$) applied to a grey siliceous sand improved the yield of subterranean clover (cv. Mt.Barker) by increasing the size of the nodules and their survival. In the same experimental area, Powrie (1964) observed that lucerne plants which had received 280 g ha^{-1} of cobaltous sulphate fixed higher amounts of N per unit fresh weight of nodular tissue than those which had not received cobalt fertilizer. According to the data of McKenzie (1957) cobalt appears to be present in sufficient amount in the red-brown earths of South Australia.

Biological properties include *Rhizobium* strain and population, rhizobial activity, insects parasitic on root nodules, and fungi. Nodule formation on the roots of leguminous species depends on the extent of root hair infection by soil bacteria. The rhizosphere population of effective strains of *Rhizobium* for the nodulation of medics determines the extent of medic growth and establishment on different soil types. Dart and Pate (1959), using a glasshouse experiment, compared the nodulation and growth of barrel medic grown in sand and inoculated with either effective or ineffective strain of *Rhizobium*. These authors found that nodulation, shoot dry matter yield, leaf development and top:root weight ratio were much higher in plants inoculated with the effective strain. They mentioned that nodules formed by the effective *Rhizobium* strain remained red and in active growth throughout the growing period whereas those formed by ineffective strain had a red pigmentation life of only 5 days. *Rhizobium meliloti* which nodulates medic species is not active in acid soil and may not survive (Robson and Loneragan 1970). Low and high soil temperatures inhibit the infection of the root by the bacteria and prevent nodule formation. However, medics are not all able to utilize the same strain of *Rhizobium* for nodulation. *Medicago rugosa* (cv. Paragosa) which prefers high rainfall areas and black earths is nodulated by a specific strain of *Rhizobium* different from that required by barrel medic.

(c) Farming management factors

Rotation is one aspect of farming management which can affect the establishment, growth and persistence of medic pastures. The use of medics in rotation in the semi-arid areas of the cereal belt of South Australia is considered to be a successful operation in spite of the vicissitudes of climate. However, it has been observed in practice that a period of more than 2 successive years of cropping or cultivation is followed by poor regeneration of medics due to seed reserve depletion (Matz 1973). On the other hand, long term pastures (more than 3 years) increase the percentage of weeds (Winn 1965). Short crop rotation systems tend to be more stable and consequently are essential for good regeneration and maximum pasture production.

Fertilizer application Usually in the semi-arid areas, to ensure good growth of medics phosphorus dressings are recommended either as small annual applications or as large dressings now and then. The amounts to be applied depend on whether the superphosphate programme is in an establishment phase or in a maintenance phase and on the severity of the deficiency to be corrected.

However, as soil N and phosphorus increase, invading grasses, mainly annuals, tend to dominate the pasture (Donald and Williams 1954; Watson 1963, 1969; Rossiter 1966 and Kohn 1975). Annual legumes and associated grasses will compete for light, nutrients, and available water.

Grazing pressure The frequency and intensity of grazing also determine the proportion of grass in the pasture. Understocking usually leads to grass dominant pastures with a decline in annual legumes due to shading and competition from the grasses whereas a moderately high stocking rate eliminates taller plants and favours more prostrate plants such as subterranean clover or medics (Rossiter 1966). To date, there is a lack of published studies concerning the extent of weed control in medic pastures by varying stocking rate and rotational grazing.

Weed control The use of selective herbicides is indicated when other methods fail to control weeds as in the case of weeds like soursob *(Oxalis pes-caprae)*, barley grass *(Hordeum leporinum)* and ryegrass *(Lolium rigidum)*. However, herbicides are not often used for pasture treatment during the winter growth of medics or subterranean clover owing in part to diminished efficiency following rainfall and leaching of the active ingredients.

Tillage practice for crop seedbed preparations after autumn rains usually help the control of weeds, especially early germinating broadleafed weeds like crucifers and milk thistle *(Celibum marianum)*. By eliminating these early germinating species, shading of cereal seedlings can be suppressed. In Tunisia, it was found that a single plant of milk thistle can shade out 1 m^2

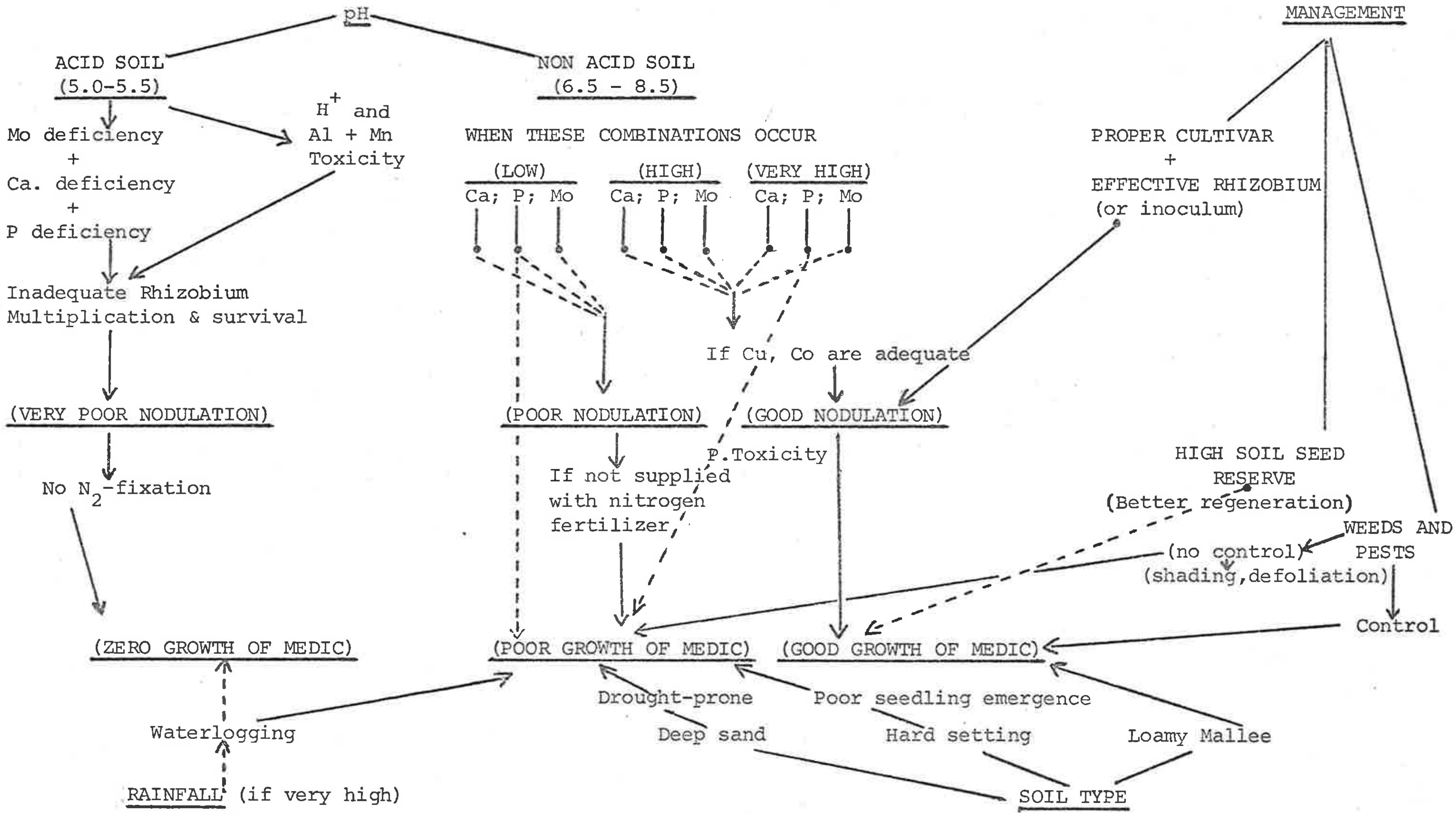
of wheat seedlings (CIMMYT Report 1974). The same shading of legume pasture seedlings can occur at the early stage of pasture growth if these kinds of weeds were not eliminated the year before by good seedbed preparation for cereal crops.

The methods of weed control mentioned above are complementary and consequently they can be used respectively during the pasture phase and crop phase to control the proliferation of weeds which are able to survive either herbicide treatments, grazing pressure or soil cultivation.

Pest control is as important as weed control. In South Australia, the most common insect which occasions defoliation and poor growth of medics is Sitona weevil *(Sitona humeralis)* (Moulden 1973). While the adults attack the leaves of medics, the larvae attack the root nodules and cause severe reductions in nodule numbers. Moulden (1973) observed in irrigation bench cultures higher survival of Sitona weevil larvae in loam than in sand. Matz (1973) estimated that a 50% reduction in winter feed production of medics could be caused by the attack of insects, namely Sitona weevil, red-legged earthmite *(Halotydeus destructor)* and lucerne flea *(Sminthurus viridis)*. Recently, spotted alfalfa aphid and blue-green aphid are threatening medic pastures. The use of insecticides provides a good control of many of these insects. Alternatives not yet fully investigated include developing pasture plants resistant to some of the above mentioned pests and the use of biological control methods.

By way of summary of the effects of the different factors on the growth of medics, I have developed Diagram 2.1 which certainly could be improved with the advance of research.

Diagram 2.1 SUMMARY OF EFFECTS OF SOIL pH, NUTRITIONAL FACTORS AND ENVIRONMENTAL CONDITIONS ON MEDIC GROWTH



2.3 ASSESSMENT OF SOIL FERTILITY BY SOIL SURVEY

2.3.1 Methods

Soil tests have been developed for the identification of nutrient deficiencies in soils and have been widely used for fertilizer recommendations. Nutrient concentration in soil is measured by different methods. Most chemical methods consist of the use of extracting solutions which provide an estimate of the availability of a nutrient in the soil and determine the potential of the soil to supply the measured nutrient. Biological methods are at times more effective in assessing soil nutrient levels because they involve the use of higher plants as a component of the whole soil-plant system. However, the assessment of nutrient levels in a soil is often made by extractants which are less time-consuming than biological methods (Williams 1967). The value of a method for the measurement of soil nutrient status is usually determined through the goodness of the correlation between nutrient level and plant yield. Thereby, it has been found that measuring the total amount of a nutrient element is often of little use for the prediction of crop need, whereas determination of some "available" form of the nutrient gives better correlation. Nevertheless, the availability of an element such as N is ephemeral for it is dependent upon soil microbiological activity and environmental conditions. This is further complicated by the fact that soils are heterogeneous in space and nutrients can move vertically and or laterally depending on topography and rain intensity. In soil testing it is unanimously admitted that the intensity of soil sampling and the depth of sampling constitute the operational factors which govern the accuracy of the estimation obtained. Therefore, it appears that there are 3 principal factors which need to be taken into consideration in any soil testing for nutrients status:

- Soil physical properties and topography
- The chemical fraction of the nutrient and, accordingly, the depth of sampling
- The season at which soil sampling is to be accomplished.

2.3.2 Soil sampling in relation to

Soil topography and texture. The selection of sampling sites within one area presents the first difficulty in soil survey for the micro-topography of the area could account for much of the variability between individual samples. Small elevations or depressions of a few cm only are usually avoided by soil samplers for the reason that nutrients often desert this kind of site following leaching or lateral displacement caused by seasonal rains.

The diversity of soil within each sampling unit has always been a perplexity in soil survey. Peterson and Calvin (1965) stated that the intensity of sampling depends on the magnitude of the variation within the soil population. Estimation of the variability of individual samples drawn from a given soil population is necessary in order to determine the number of soil cores to be taken to ensure a satisfactory estimate of the population. This variability is generally assessed by taking at random a certain number of samples from each site and computing the coefficients of variation of the individual samples or sub-samples. Once the degree of variation among sub-samples is known, the intensity of sampling is chosen in such a way that the sampling error is reduced to an acceptable level. Yates (1971) pointed out that the standard error of the estimate of the mean of a large population from a random sample is inversely proportional to the square root of the number of units in the sample following the equation:

$$\text{Standard error } (\bar{y}) = \frac{1}{\sqrt{n}} \sigma$$

Vallis (1973) reported that the degree of variation due to soil heterogeneity between samples within one site increased rapidly with increasing distance between samples. However, Hauser (1973) stated that in heterogeneous soils the variation in nutrient levels within a small area (1 m^2) is nearly the same as that within a bigger area of the same site (1 hectare). This supports the idea of Hemingway (1955) that if soil variability from point to point is considerable, intensive sampling to produce a reliable mean may not always reflect the true nutrient status of soil. The same author found that in fields of uniform soil type the distance between sampling sites had no influence on the sampling error. Hauser (1973) reported that as the number of sub-samples increases the percentage error variance decreases with the factor $\frac{1}{\sqrt{n}}$. Hauser observed that many soil testing laboratories recommended between 15 and 40 sub-samples per composite sample in field survey work.

<u>Nutrient form in the soil (depth of sampling)</u> Nitrogen exists in the soil in organic and inorganic forms. The latter form is usually absorbed by plants and includes nitrate, nitrite and ammonium N. The availability of this mineral N form varies with seasonal conditions, soil types and the rates of mineralization and immobilization processes. Further, the variation in soil N content within a field and between fields is related to farming management practices of fertilizers application and cropping history. In regions where legume pastures are included in the rotation, stocking rate and grazing pressure have a superimposed effect on N status of the soil. For this reason sampling error and sample variations in soil tests for N measurements may be expected to vary greatly. Vallis (1973) studying soil total N changes with time of 5 pasture sites compared 3 types of sampling (simple random, stratified random and repeated sampling of the same set of sites) and

found that from 150 to 1000 soil cores were needed to measure a mean change in soil N of ± 50 kg N ha^{-1} in a field, but repeated sampling of the same set of sites (as close as possible to the original position) gave more precise estimates of changes of soil total N than did the two other types of sampling. Beckett and Webster (1971) reported for N and organic matter, that the coefficients of variation of individual samples drawn from the top soil layer of 0.01 ha fluctuated between 10 to 20%, but they found that the variance within 1 m^2 was already equal to half of the variance within the whole field. This is interesting because if fertility differences between several fields are to be measured it will be less expensive and much faster to take a large number of soil cores within a small area than to take few samples distributed over the whole field.

Although lateral variation of soil N is important, vertical variation is so great that it must be kept at a controlled level by sampling each time and each site to the appropriate depth. Changes in soil bulk density with water content and cultivation should be considered in a sampling scheme. Vertical variation in soil N content arises also from soil variability in texture and structure with depth. Deep leaching of N in light textured soils may lead to high variance of soil N estimates from season to season. The following example of % total N variation with depth illustrates the importance of depth in soil sampling:

Sand over clay soil, South Australia (Potter 1970).

Depth (cm)	% total N
0 - 2.5	0.031
2.5 - 7.5	0.012
7.5 - 15	0.005

The question of depth of soil sampling depends on whether the crop is deep rooted or not and also on the soil profile characteristics (Graham 1975).

In spite of its difficulty, soil testing for N is useful in that crop yields, mainly those of cereals, are commonly a function of N level of soils as determined by soil analyses. Thus important improvements of crop yields and protein production can be achieved by first measuring the concentration of N and programming fertilizer N needs accordingly.

Phosphorus availability in Australian agricultural soils depends both on soil parent material and on farm management practices. Here too, the intensity of sampling is related to the soil type and nutrient concentrations in the soil. Hemingway (1955) reported that, over 50 fields of widely differing soil types, 24 soil cores drawn from each field within increasing areas (0.004 ha, 0.4 ha, 2.0 ha and 12 ha) gave a standard deviation from the mean of the order of ± 20 to ± 30%. This was in unfertilized fields, whereas in areas which have received phosphorus the sampling error was greater and varied between ± 40 to ± 45%. There was no increase in the error as the area sampled increased. The higher sampling error in fertilized soils could be partly explained by uneven distribution by seed/fertilizer drills.

As in the case of N, the depth of sampling must be kept constant for applied phosphate remains usually within the top layer of soil. According to Williams (1967), even the depth of ploughing in cultivated areas can affect the soil test results if samples are drawn from the cultivated zone only.

<u>Sampling period</u> Levels of available nutrients in soils vary with the season owing to the biological activity which in turn depends on water

and temperature factors, among others. In the Mediterranean climate, the hot and dry summer conditions reduce bacterial activity whereas, in autumn, after the opening rains and while soil temperature is still high, mineralization processes increase rapidly the quantity of available N which can be extracted.

In practical terms soil sampling in summer or after long dry periods is to be avoided. Collection of samples from light textured soils using the ordinary sampling tools (augers, tubes) is not practical, for the lack of cohesion between soil particles lets the soil slide back into the hole. In contrast, heavy textured soils are hard to dig under these conditions. Unless improvements are made in the sampling tools, collection of soil samples under these dry conditions is not strictly accurate.

2.4 THE ACETYLENE REDUCTION ASSAY FOR MEASUREMENTS OF BACTERIAL NITROGENASE ACTIVITY

2.4.1 Agent responsible Despite the high concentration of nitrogen (N_2) in the atmosphere (78%) only a few organisms possess the ability to fix and convert this N into reduced forms which are available to plants. Bacteria, blue-green algae and actinomycetes represent the N-fixing organisms known to date. The organisms may be classified into groups according to the oxygen requirement for the accomplishment of atmospheric N_2-fixation.

Bacteria:

- Obligate aerobes: include the family of Azotobactereacea *(Azotobacter)*
- Facultative aerobes: including *Klebsiella pneumoniae*
- Obligate anaerobes: including *Clostridium pasteurianum*
- Blue-green algae: including *Anabaena, Nostoc*

2.4.2 Products of nitrogen fixation

Enzymologic experiments with $^{15}N_2$ indicated that ammonia (NH_3) and amino-acids were the first stable products of the biological N_2-fixation by micro-organisms (Hardy, Holsten, Jackson and Burns 1968, Postgate 1970, Dilworth 1974). The reduction of N_2 to NH_3 was found to be catalysed by an enzyme complex called nitrogenase (N-ase) which was extracted from both free-living organisms and legume nodule bacteria. Dilworth (1966) and Shöllhorn and Burris (1967) observed that N_2-fixation was inhibited by acetylene (C_2H_2) which was reduced to ethylene (C_2H_4). Since then it has been established that the enzyme N-ase reduces C_2H_2 to C_2H_4 in the same way it reduces N_2 to NH_3 following the reaction:

$$N_2 + 6H^+ + 6e^- \xrightarrow{\text{N-ase}} 2\ NH_3$$

$$3\ C_2H_2 + 6H^+ + 6e^- \xrightarrow{\text{N-ase}} 3\ C_2H_4$$

Because C_2H_2 has similar molecular dimension to N_2 it has been reported to be a competitive inhibitor of N_2 reduction (Trinick, Dilworth and Grounds 1976). But Hwang, Chen and Burris (1973) reported that C_2H_2 acts as a non-competitive inhibitor. Acetylene reduction (AR) has become an accepted method of measuring N-ase activity and of estimating N_2-fixation.

2.4.3 Principle and characteristics of the acetylene reduction assay

Usually, C_2H_2 is exposed to the material under test in a closed incubation chamber. After recorded intervals of time, gas samples are withdrawn from the incubation chamber by means of syringes and introduced into a gas chromatograph for the determination of the C_2H_4 produced. The gas mixture inside the incubation chamber varies with the N fixing organism being tested. Generally for aerobic fixing systems the air in the assay chamber is flushed out and replaced by a gas mixture of Ar : O_2 : CO_2 in the proportion of 0.8 : 0.2 : 0.04 (Hardy, Burns and

Holsten 1973) in order to eliminate the competition between N_2 and C_2H_2. However, for these aerobic systems, incubation of samples under a gas mixture of air and C_2H_2 i.e., in the presence of N_2, was reported to be practicable (Hardy *et al.* 1973; Sinclair 1974). Trinick *et al.*, (1976) studied the factors affecting the reduction of C_2H_2 by root nodules of *Lupinus* species and found that, provided a sufficiently high (0.05 - 0.1 atm.) C_2H_2 concentration is used, C_2H_2 reduction (AR) can be satisfactorily measured in air.

2.4.4 Expression of results (C_2H_2 : N_2 ratio) N-ase activity measured by AR may be expressed in terms of moles of C_2H_2 reduced or of C_2H_4 produced. But as it can be seen in the N-ase reaction, the theoretical ratio between moles of C_2H_2 reduced and moles of N_2 fixed is 3 to 1 (Hardy *et al.*, 1968). However, in empirical determinations this ratio was found to vary with both micro-organisms and kind of sample used for the test. Bergerson (1970) found for soybean nodules, ratios varying from 2.7 to 4.2. Sinclair (1973) measured in white clover a ratio of 3.7, whereas Roughley and Dart (1969) reported an average ratio of 2.6 for subterranean clover. Steyn *et al.*, (1970) found for soils taken from a native grassland site a ratio of 4.1 but for soils taken from a fallow site the ratio was 6.9. A figure for medics has not been published.

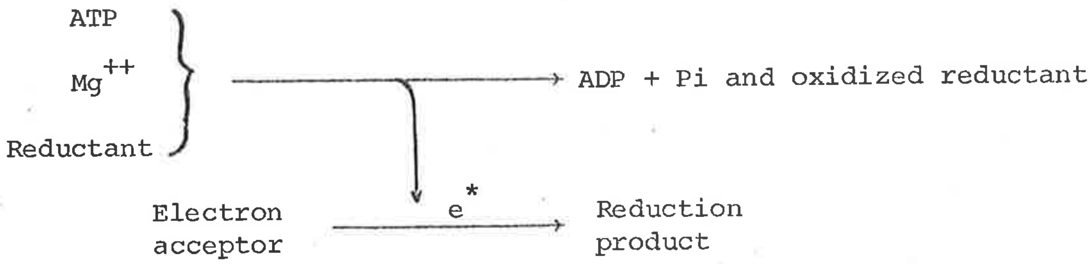
The variation of the C_2H_2/N_2 ratio with different environmental conditions and different legume species emphasize the need for the determination of the proper conversion ratio of C_2H_2 to N_2 if quantitative estimates of N_2-fixation by a specific legume or free-living organisms are pursued under specific environmental conditions. The theoretical ratio of 3:1 cannot be considered as a standard conversion ratio for interpreting AR in terms of N_2-fixation.

2.4.5 Factors affecting nirogenase activity and the acetylene reduction assay.

Nitrogenase requirement and reaction: N-ase requires a source of electrons, ATP and Mg^{++} (Dilworth 1974). The supply of energy for N-ase activity comes from the hydrolysis of ATP to ADP and Pi (orthophosphate). Hardy *et al.*, (1973) presented the N-ase reaction as follows:

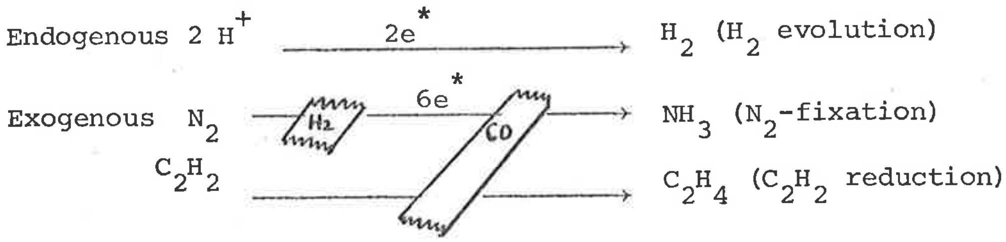
N-ase Reaction

(a): Electron activation



Electron acceptors may be endogenous protons or they may be exogenous acceptors such as N_2 or C_2H_2.

(b): Substrate reduction



H_2 inhibits only N_2 reduction (Dilworth 1974), whereas CO inhibits both N_2-fixation and AR (Hardy *et al.*, 1973). Recent studies showed that molybdenum was directly involved in H_2 evolution and N_2-fixation. Ljones (1974) suggested that N_2 forms a complex with molybdenum on the Mo-Fe protein fraction of N-ase before reduction takes place. N-ase has been resolved into 2 protein fractions: the Mo-Fe protein and the Fe protein. The latter fraction showed more sensitivity to oxygen tension

(Drozd and Postgate 1970a; Dilworth 1974). Cole (1976) reported that both component proteins are inactivated by oxygen.

Effects of oxygen and acetylene partial pressure Oxygen was found to affect N-ase activity differently according to the strain of bacteria involved in N_2-fixation. Although legume nodules and the associated *Rhizobium* require O_2, O_2 has long been known to inhibit N_2-fixation even in aerobic organisms such as *Azotobacter* (Bergersen 1971; Dilworth 1974). Postgate (1972) stated that aerobic N fixing organisms switch off their N-ase activity when exposed to a high atmospheric oxygen pressure, particularly in carbon or phosphate limiting conditions. Bergersen (1971) reported that O_2 is required for bacteroid respiration and the consequent provision of ATP for N_2-fixation, but without leghaemoglobin the diffusion of O_2 through the dense nodule tissue would be completely inadequate to meet the ATP requirement. Yates (1972a) pointed out that oxidation of the electron donor to N-ase in *Azotobacter chroococcum* could account for the non-competitive inhibition of AR by oxygen. However, the optimal oxygen partial pressure (pO_2) for a complete inhibition of N_2-fixation or AR depended mainly on population density of the bacteria under test (Dilworth 1974).

Although authors in the literature suggested that pO_2 in AR assay should duplicate that under which natural N_2-fixation occurs, one could object that, under field conditions, neither pO_2 nor population density of bacteria is constant in the soil microenvironments or during the growing season. We still do not know at what depth of soil maximum N_2-fixation by root nodule bacteria occurs. Nor whether this maximum of bacterial activity lies always at the same depth of soil throughout the season or whether it varies according to environmental conditions and root growth. O_2 partial pressure under field conditions varies with both depth of soil and root growth of the plant host as well as with soil water content. Sinclair, Hannagan and Risk (1976) found that, under turfs, 80% of AR activity of soil cores was found in the surface 7.5 cm of soil. Under field conditions the shortage of O_2 in the rhizosphere of legume root nodules due to waterlogging or poor soil structure may be linked with poor legume nodulation and a lack of N_2-fixation.

Acetylene partial pressure in assays for N-ase activity should be adjusted to saturate N-ase to the same extent that is saturated by ambient N_2 for the rate of AR is proportional to C_2H_2 partial pressure at levels less than that required to saturate the enzyme (Hardy *et al.*, 1973). Acetylene partial pressure (p C_2H_2) or Km C_2H_2 for nodulated soybean roots was reported (Hardy *et al.*, 1968) to be about 0.007 atmosphere. However, Bergersen (1970) found that a decrease in p C_2H_2 from 0.1 atmosphere to 0.05 atmosphere led to an inhibition of C_2H_4 production by detached soybean nodule when incubated at the same pO_2 of 0.5 atmosphere. A p C_2H_2 of 0.1 atmosphere has often been used in studies of N-ase activity (Bergersen 1970, Steyn and Delwiche 1970, Paul *et al.*, 1971, Trinick *et al.*, 1976).

Effect of combined nitrogen, light and defoliation Combined N (inorganic) has been reported in the literature to have inhibitory effects on both nodulation of leguminous plants and symbiotic N_2-fixation. Pate and Dart (1961) observed that low levels of ammonium nitrate (~10 ppm N) applied to barrel medic plants at sowing increased primary-root nodulation, whereas at the highest level of ammonium nitrate (220 ppm N) the number of both primary-root nodules and lateral-root nodules decreased. At this high N level they found that ammonium nitrate had depressed nodule activity and symbiotic N_2-fixation as early as the first pigmentation stage of first nodules. However, they mentioned that there were marked differences in the nodulation response of bacterial strains to added ammonium nitrate. Mustapha (1969) using AR assay observed that application of calcium ammonium nitrate fertilizer at a rate of 90 kg N ha^{-1} to white clover decreased the rate of AR activity or N_2-fixation to 20-30% of that of the control but did not eliminate it. Day, Neves and Döbereiner (1974) observed in intact soil-plant cores with *Paspalum notatum* system that addition of 10 ppm NH_4^+-N inhibited N-ase activity within 2 h, whereas 10 ppm NO_3^--N had the same effect within 4 h. Dilworth (1974) reported that inhibition of N-ase synthesis in N-fixing cultures by ammonia (NH_3) varied with the micro-organism involved and was directly related to the population density. This agrees with the report of Keister and William (1976) who found that ammonium chloride even at a high concentration did not inhibit AR activity by pure cultures of *Rhizobium* sp. 32H1 and *Rhizobium japonicum* 3I 1683. Inorganic N was reported to reduce N-ase activity of soybeans (Ham, Lawn and Brun 1976). The outlined results among others (Matherson and Murphy 1976) produce evidence concerning the inhibitory effect of combined N on N-ase activity. Presumably, under field conditions a high mineralization of soil N at the onset of nodule formation of legume-based pastures will decrease nodule growth and restrict symbiotic N_2-fixation.

Light, by controlling photosynthesis in plants, controls the rate of carbohydrate supply for the heterotrophic bacteria whose activity was found to be related to light intensity (Wilson and Wagner 1935). This photosynthetic effect of light on N_2-fixation was reported by Virtanen, Moisio and Burris (1955) who found that detached nodules from pea plants kept in the dark for 24 h had less leghaemoglobin and showed lower capacity for fixing N than nodules from illuminated plants. Furthermore, Butler, Greenwood and Soper (1959) reported that decreasing the light intensity to approximately 25% led to a marked loss of nodules of white clover and that after 3 days of shading there was a fading of the pink colour of the nodules. With the advent of the AR assay more sophisticated investigations concerning the effect of light on bacterial N-ase activity have been reported. Bergersen (1970) observed in a 28 h period that N-ase activity of bacteroids associated with soybean plants was much higher in the light than in the dark and that the activity of nodulated roots was greater and more variable than that of detached nodules. Chu and Robertson (1974), using a glasshouse experiment, found that the reduction of light intensity to 15% of that of the normal daylight, though it decreased the number and weight of nodules, did occasion neither an obvious change in nodule colour nor an immediate decrease in AR activity of white clover plants. It seems that light was not decreased to very limiting conditions. Halliday and Pate (1976) brought further evidence that light is a prerequisite for symbiotic N_2-fixation. Apart from the fact that the last named authors observed a diurnal fluctuation in AR activity of 12 weeks old seedlings of white clover, they found that shading of the leaves of the main crown depressed N-ase activity in nodules on all parts of the root system. Of great interest is the fact that besides the effect of light on photosynthesis and thus the supply of carbohydrates to N-fixing systems, there seems to exist an additional non-photosynthetic effect of light on the nodulation process. Lie (1971) found that exposure of the shoots or the roots of pea plants to far-red light (730 mμ) inhibited nodulation which, on the contrary, was abundant in red light (560 mμ). This led the author to suggest that, as is the case beneath forest canopies, shading of legumes in mixtures of grasses and legumes not only reduces plant photosynthesis but also increases the proportion of far-red light received by shorter plants (legumes) and consequently inhibits nodule formation or new-formed nodules. It can be concluded that artificial shading of plants may not give a true indication of what is happening under field conditions. This effect of far-red light on the nodulation process could partly explain why Chu and Robertson (1974) who used sarlon netting to shade white clover plants did not observe any effect of shading until 6 days after the treatment began.

Defoliation of legume plants was reported to affect N-ase activity of root nodules more severely than shading does. Mustapha (1969) reported that defoliation of white clover plants led to a decrease in AR activity of noduled roots just 30 minutes after treatment, and it took 3 weeks before the defoliated plants recovered their full activity. This rise in AR activity probably paralleled the active re-growth of white clover plants (new leaves, new roots and nodules) as was observed by Butler *et al.*, (1959) on defoliated white clover plants. The same phenomenon due to plant defoliation was reported by Hardy *et al.*, (1968); Gibson (1971); Chu and Roberston (1974); Halliday and Pate (1976); and Ham *et al.*, (1976).

The effect of defoliation on the rate of N_2-fixation was attributed to a breakdown of nodule leghaemoglobin which controls O_2 flux into the nodules and to photosynthate deficiency as well as to injury. From a practical point of view, heavy grazing and trampling of swards of legumes by animals could have the same effect in reducing N-ase activity of root nodule bacteria and consequently N_2-fixation.

<u>Temperature effects</u> Although above ground temperatures affect symbiotic N_2-fixation by controlling plant growth and respiration, soil temperatures in the rhizosphere have been reported to be more important

as far as bacterial activity is concerned. Mustapha and Mortenson (1968) observed that N-ase extracted from *Clostridium pasteurianum* was inactivated at about $0^{o}C$. The same inactivation of detached nodules of different legume species during storage at $0^{o}C$ for 30 minutes was observed by Mustapha (1969). However, Dart and Day (1971) pointed out that a 3 day storage at $2^{o}C$ in the dark before testing for AR activity, did not affect N-ase activity of medic (cv. Jemalong) or of *Medicago sativa* whereas subterranean clover (cv. Mt.Barker) lost two-thirds of its initial activity. The same effect of low temperature below $5^{o}C$ on AR activity of *Alnus glutinosa* nodules was observed by Waughman (1972). Nevertheless, Gibson (1971) reported that *Rhizobium* strains of subarctic zones were more resistant to cold than those coming from temperate regions.

At higher levels of temperature N-ase activity of nodulated roots of soybeans was found to reach its maximum between $20^{o}C$ and $30^{o}C$, whereas temperatures greater than $30^{o}C$ decreased N-ase activity (Hardy *et al.*, 1968; Gibson 1971). For subterranean clover (cv..Tallorook) optimum N_2-fixation was reported at about $25^{o}C$ (Gibson 1971). For medic (cv. Jemalong), Dart and Day (1971) observed that N-ase activity increased rapidly between $5^{o}C$ and $20^{o}C$ and decreased also rapidly above $30^{o}C$ temperature. The same authors reported that N-ase activity of *Medicago sativa* continued to increase until $35^{o}C$ but not beyond.

Under field conditions, the effects of soil temperature on rhizobial activity can be confounded with soil water (Vlassak, Paul and Harris 1973). Diurnal changes in AR activity of white clover seedlings appeared to run parallel with changes in soil temperature (Halliday and Pate 1976). In view of these studies, it appears that both low and high temperatures have adverse effects on N-ase activity of *Rhizobium* strains and are related to other environmental conditions of the symbiosis.

Effects of Water Both water excess and deficiency can lead to a reduction in bacterial N-ase activity and N_2-fixation as reported by many studies. According to Sprent (1971), reduced AR activity of soybean nodules immersed in water was due to a very low supply of O_2, for the same nodules were found to recover their full rate of activity after shaking them at $pO_2 = 0.8$. Further studies by Sprent (1971) showed that a decrease in the water content of detached nodules to 80% of normal fresh weight led to a decrease in AR activity of these nodules. Even a 10% loss in fresh weight reduced the rate of AR by half. Sprent concluded that the effects of water stress on O_2 uptake and AR are causally related either via supplies of ATP, reductant or both.

Likewise, Schwinghamer, Evans and Dawson (1970) reported very low rates of AR by excessively wet legume nodules. Also, La Rue and Kurz (1973), found that AR activity in intact pea plants was inhibited if the sand-vermiculite soil supporting the plants was too wet. However, Van Straten and Schmidt (1975) observed that wetting detached soybean nodules decreased their rate of AR, but wetting nodules still attached to portions of roots had no inhibitory effect. They concluded that nodule injuries caused by detachment of nodules from roots are responsible for low AR activity rather than simple impairment of gas exchange under wet conditions.

In the case of soil samples, Paul *et al.*, (1971) mentioned that soil cores from *Agropyron-Koeleria* grassland showed an increase in their N-ase activity when soil moisture was increased to field capacity, with the medium textured soils having the highest fixation rates.

Thus, it appears from these studies that a soil water content above field capacity decreases bacterial N-ase activity by decreasing gas exchanges between soil atmosphere and aerobic nodule bacteria. However, it is possible that owing to the slight solubility of C_2H_4 in water, low evolution of C_2H_4 is detected in wet conditions. Also, it is likely that in the field, besides soil temperature, soil texture and soil structure intervene to reduce or emphasize the effects of soil moisture levels on N-fixation activity of the bacteroids.

Other inhibitors of the AR assay Apart from high O_2 partial pressure, carbon monoxide (CO) was found to be a competitive inhibitor of the AR as well of N_2-fixation itself (Hardy *et al.*, 1971). Cyanide inhibits AR (Dilworth 1974). Inhibition of N-ase activity by endogenously produced ethylene in bean roots has recently been observed by Grobbelaar, Clarke and Hough (1971). However, despite the fact that C_2H_4 is produced in small amounts by certain plants and bacteria, inhibition of AR caused by C_2H_4 production has been seldom reported, but may cause error in estimating N-ase activity. Adsorption of C_2H_4 to soil surfaces, resulting in lower AR rates is possible (Steyn and Delwiche 1970).

2.4.6 Advantages and disadvantages of the assay

Advantages (i) AR assay is sensitive and useful for detecting biological N_2-fixation; (ii) Its sensitivity is reported to be respectively 10^3 and 10^6 times that of N_2^{15} and Kjeldahl methods; (iii) Gas chromatography methods are simple and specific for C_2H_4 as it is easily separated from the other hydrocarbons involved; (iv) As a test for N-ase activity measurement, AR is a rapid and practical method since assays could be achieved at a rate of up to 20 h^{-1}; (v) AR is a non destructive method because under certain conditions the same sample can be retested.

Disadvantages Apart from the chemical nature of the acetylene reaction and the explosive nature of the gas C_2H_2, it is rather difficult during incubation of samples with C_2H_2 to match exactly the environmental conditions under which natural N_2-fixation occurs. For this reason, the theoretical ratio of 3 molecules C_2H_2 reduced to 1 molecule N fixed is not absolute and will vary according to the conditions of AR test. Calibration of this ratio for the system under test is needed. Furthermore, C_2H_4 may come from other sources (plants, bacteria) than the chemical reduction of C_2H_2 or, on the contrary, could be adsorbed to soil surface. Under field conditions the integration of the results of AR assay over a season is very difficult and almost impossible because of the high variability in bacterial N-ase activity with light intensity, temperature, soil water content and stage of plant growth.

2.5 CONCLUSION

Soil N is subject to continuous losses due to crop uptake, volatilization, denitrification, leaching and top-soil erosion. Possibilities for overcoming the depletion of soil N reserves have been outlined. Considerable progress has been made in Australia, especially in the high rainfall areas of the cereal belt, to counteract the exhaustion of soil N by the inclusion of subterranean clover pastures in the rotation. A wide range of studies have dealt with the amount of N fixed by subterranean clover pastures during a single growing period and over a number of years, and most of the studies have emphasized the role of superphosphate and/or grazing management in the build-up of soil fertility. Although some studies have looked at the effects of the botanical composition of the pasture on soil N increment (Donald and Williams 1954; Watson 1963) none was involved in the investigation of the effect of sowing density of legume pasture on N_2-fixation. Comparatively little has been published concerning the contribution of medics to soil N in the semi-arid areas of the cereal growing regions. In Victoria, Meagher and Rooney (1966) and Mullaly *et al.*, (1967) have published some results although these studies have not fully taken into account the long term effects of medic leys, farming management, soil factors and pasture composition. The following questions arise:

- How much N are medics able to fix per hectare per year?
- What are the limiting factors to this fixation?
- Of what value is the N fixed to the ensuing crops?
- What is the pattern of N_2-fixation within a single growing season?
- What is the influence of the sowing density on N_2-fixation by medics?
- How does N_2-fixation vary with the seasons?

Our present study aims to assess the effects of medic leys on soil fertility build-up under different rotations, to look into the effects of sowing density, sowing date and stage of growth of medics on AR activity, and to investigate the effect of phosphate supply and competition from grasses on growth, AR activity and N content of Jemalong medic plants.

3.0 RESULTS AND DISCUSSION OF THE SOIL FERTILITY SURVEY

3.0 SOIL FERTILITY SURVEY

3.1 INTRODUCTION

The aim of this study was to evaluate by means of a soil survey the effects of medic leys in the rotation on soil fertility build-up in a semi-arid environment.

Estimation of changes in soil N are difficult since the small increments involved must be measured against the background soil N level and its inherent variability. The increments can be magnified by measurement over a number of years but long term experiments, especially under farm conditions, are rare indeed. Survey techniques where a time-based factor can be introduced are a means of approaching the problem, although the compression of the years has its price in the lack of precision in the result, as the following pages will reveal. For all that, the method can be quite powerful as was demonstrated by Donald and Williams 20 years before (1954).

3.2 CHARACTERISTICS OF THE SURVEY AREA

The choice of the district of Mallala to conduct the present survey was based on the diversity of medic history in cropping systems in this area which is only 60 km from Adelaide. Lying between 34^{o} and 34^{o}30' latitude south, the survey area is 18 km from the coast of St. Vincent's Gulf. It is a flat plain of pleistocene sediments about 100 m above sea level in the Hundred of Grace, County Light, South Australia.

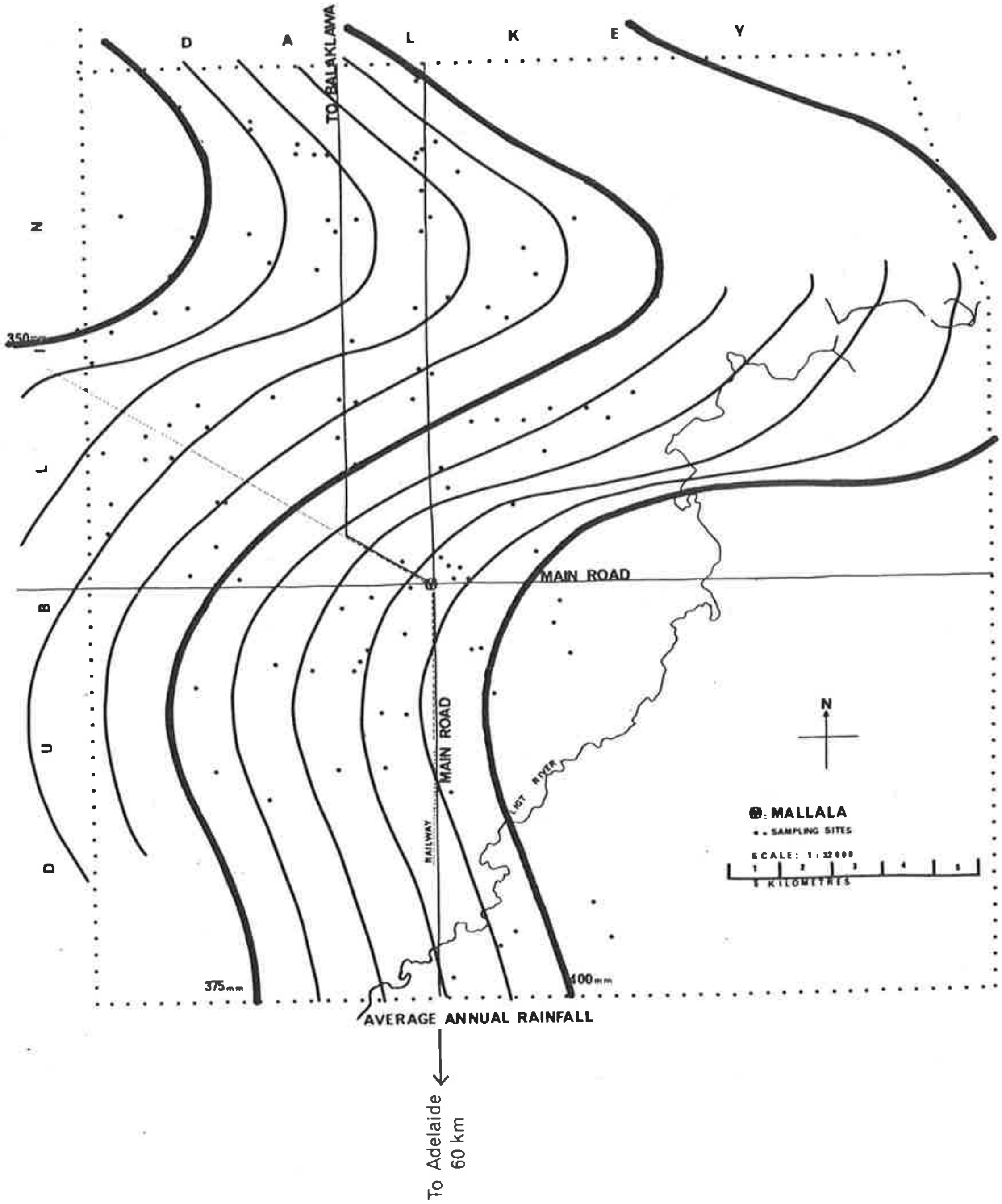
Soils are sandy loams to loams in the surface horizons with increasing amounts of clay and lime in the subsoil. Originally the survey area was recommended for its relative uniformity of soils but analysis, especially for the presence or absence of lime in the A horizon, showed that the soils fell into two groups grading into one another. They are mainly solonized brown soils (Gc2, Gn) transitional to associated red-brown earths (Dr2.23) (Northcote 1970). Solonized brown soils have lime in the surface horizon, a good structure and although light in texture they are not prone to wind and water erosion unless they are over-cultivated. Their water holding capacity varies with the nature and position of the limestone layer. The red-brown earths have a medium textured topsoil, predominantly loam, over a heavier subsoil. Lime is present only in the lower subsoil from 30cm depth. These soils are well-drained with good water holding capacity but they may become badly structured under heavy cropping and can be easily eroded (French *et al.*, 1969). Soils in the survey area are neutral to alkaline in the 0-10cm depth interval.

Annual rainfall in this district averages 375 mm to 405 mm with the significant falls occurring between April and October (Figure 3.1 and Table 3.1). In this semi-arid environment rain is needed throughout the growing season but there are 2 critical periods for pasture and cereal production. The first period is April-May and the second is September-October. For Mallala, over a period of 83 years, 54% of the years have received 75 mm of rain or more during April-May and the same area has about 50% chance of receiving 75 mm or more rain in September-October (Bureau of Meteorology 1972).

The average length of growing season is 5 to 6 months in winter and spring. Severe frosts are rare during the growing season. A drought year occurs about once every 5 or 6 years, according to local farmers. In general, the environmental conditions in the survey area

Figure 3.1: RAINFALL ISOHYETS IN THE STUDY AREA.

Taken from "The Climatic Survey of the Light Region of South Australia" (Australia 1972) which gives the 350, 375 and 400 mm isohyets only. Other isohyets were interpolated. Sampling sites are dotted in.



AVERAGE ANNUAL RAINFALL

Table 3.1: Average monthly rainfall (mm) and temperature (°C) Mallala P.O. (South Australia)

	J	F	M	A	M	J	J	A	S	O	N	D	Annual
Rainfall[1]	18	18	18	36	48	50	45	47	42	36	25	22	405
Average Max. Temp.	30.6	30.0	28.9	22.5	18.6	16.7	15.3	16.1	19.7	22.8	26.4	28.6	23.0
Average Minimum Temp.	16.1	15.7	14.9	12.3	9.8	7.7	6.7	6.8	8.3	10.1	12.3	15.0	11.3
Mean Temp.	23.3	22.9	21.9	17.4	14.2	12.2	11.0	11.5	14.0	16.5	19.3	21.8	17.2

[1]The mean monthly rainfall applies to the period 1925-1974 (inclusive).

are suitable for the growth of medics. Although common barrel medic and burr medic have colonized these soils for more than 20 or 30 years, it is still possible to find fields of unimproved pastures dominated by such grasses as barley grass, Wimmera ryegrass and brome grass (*Bromus* spp.) together with soursob, mustard *(Sisymbrium orientale)*, three-cornered jacks *(Emex australis)*, wild turnip *(Brassica tournefortii* Gonan), onion weed *(Asphodelus fistulosis)*.

Like other areas of the cereal belt of South Australia the survey area was subjected to heavy cropping for cereal production for more than 80 years until the 1950's (Donald 1964, Webber 1968, Webber, Cocks, Jefferies 1976) during which cereal yields fell steadily. Although the adoption of new varieties, mechanization and use of superphosphate temporarily brought about an increase in cereal yields, there was still a continuous loss of soil structure and soil depth due to wind and water erosion. A new farming practice based on the inclusion of pasture leys in the rotational systems was developed in the early 1950's. Accordingly, cereal rotations changed progressively from fallow-wheat to fallow-wheat-pasture and fallow-wheat-barley-pasture. Webber (1968) mentioned that pastures in 1964 were poor and generally not legume-dominant but a mixture of volunteer species namely barrel medic, woolly burr medic, wimmera ryegrass and barley grass. Moreover, according to a report of the Reserve Bank of South Australia (1967) improved pastures in the survey area in 1967 were rare and represented only 7% of the agricultural and pastoral area of the Mallala district. Thus until 1965, unimproved pastures and rotations with low frequency of pasture leys prevailed in the survey area. Mr. Peter March, a pioneer in the utilization of improved medic pastures (new varieties and superphosphate) corroborated that prior to 1965 pastures were generally grass-dominant. This is also the view of the farming community generally. But by 1974, the survey of farmers' fields (95 paddocks) to be described later revealed that 63% of the surveyed pastures were dominated by good medic stands. It follows that a drastic change in farming management systems occurred in the period between 1965 and 1974. This is a vital point in the design of the survey. There is also a trend, which has continued, towards shorter rotations involving suitable varieties of medics. Rotations include:

Fallow - crop - pasture

Pasture - crop - crop

Pasture - crop - pasture - crop.

Depending on autumn rainfall, market expectations and market prices, crop can be either wheat or barley. Wheat is generally sown in May-June whereas if the opening rains are late barley is preferred because of its shorter growing cycle. Single superphosphate (8.6% P, 10 to 12% S, 20% Ca) is commonly applied to cereal crops at a rate of 100 to 120 kg ha^{-1}.

Fallowing is carried out in August-September if winter rains have exceeded 100 mm (Webber 1975). In fallowing, after the initial cultivation subsequent shallow cultivations are carried out each time sufficient rain has fallen in order to keep the surface soil open and free from weeds. The depth of the fallowing does not exceed 7-8cm.

Livestock raising, mainly of Merino sheep, is integrated in the farming systems for meat and wool production and recently farmers have become interested in the production of medic seed for overseas markets.

The average farm size in the survey area is about 500 ha.

3.3 METHODS AND MATERIALS

3.3.1 Cropping history survey

Elaboration of the survey After several visits to the Mallala district the survey area was delimited and the farmers were identified with the help of Mr. A. Mitchelmore, the regional officer of the Department of Agriculture and Fisheries. Preliminary contact with the farmers was made to ask for their cooperation and to explain the purpose of the soil fertility survey to be undertaken.

Almost all paddocks in pasture in the area were included in the survey which covered 95 sites spread over 28 different farms (i.e., an average of 3.4 paddocks per farm). Also, 11 "reference sites", as natural as possible, were included in the survey for the purpose of comparing soil fertility in cultivated and uncultivated sites. These reference sites had not received any phosphorus or N fertilizers as far as farmers could remember and medics were almost completely absent. Scrubs used for sheep camping were avoided as reference sites. All sites were located on a map of the Hundred of Grace (scale 1:32000) and on an aerial photograph (Plate 3.1). Figure 3.1 shows the scatter of the selected sites over the area with regard to rainfall isohyets, and the aerial photo features the different paddocks included in the survey and the number of sites sampled within each paddock.

Management histories. The farms selected included a wide range of cropping histories. The rotation at each site was obtained from the farmers for the period 1965-1974. Farmers had difficulty in remembering rotations over the 10 years and memory aids such as "drought year", "year-of-the-dry-finish", "year-of-quotas", "low wool prices", in addition to their own such as "year-I-got-the-new-tractor" were helpful. A questionnaire handed to the farmers and collected a few

Plate 3.1. AERIAL PHOTOGRAPH SHOWING THE DISTRIBUTION OF SAMPLING SITES OVER THE SURVEY AREA AND PADDOCKS WHICH WERE IN PASTURE IN 1974.

days later was designed to provide most of the other information of interest, namely the date of introduction of improved medic leys into the rotation, the amount and type of fertilizer applied, cereal yields and rainfall records. A copy of the questionnaire is presented in Appendix I.1.

3.3.2 Soil sampling

Within each site a sampling area (25m x 25m) was identified as typical of the paddock and of its cropping history. Typical is used in a sense that the sampling site need not be a true arithmetical average of the paddock but it needs to have experienced the cropping history of the paddock. From each site 16 soil cores were taken using a sampling tube 5cm in diameter, and the cores were divided into 2 depths, 0-5cm and 5-10cm. The 16 core positions were defined at random and pegged down. This number of soil cores was estimated to give, within a 95% confidence interval, a coefficient of variation of ± 10% of the true mean (based on French, unpublished data). For each depth, soil cores were mixed to form composite samples for each site which were air-dried on the same day of sampling. Soil samples included any litter on the surface soil but not standing vegetation. Sites were sampled in the spring (i.e. September 18 to October 8, 1974).

Pasture composition at each site before soil sampling was recorded. The proportion of area covered by medics or grasses was estimated visually and the pasture was classified either as medic-dominant pasture or grass-dominant pasture. The numbers of sites of each category of pasture were respectively 60 and 35 sites.

3.3.3 Soil analysis

The following determinations were made on each composite sample and all results were expressed on the oven-dry basis.

Total Nitrogen (%)

The percent total N was determined on air-dried soils ground to pass through a 1 mm sieve. For each composite sample a subsample of 5g was digested for 2 h with concentrated H_2SO_4 using Se as catalyst. The ratio of K_2SO_4 to H_2SO_4 was 3:10 (Bremner 1965). Each soil sample was digested and distilled in duplicate. Values of N content measured by the Kjeldahl method will be referred to as total N with the recognition that the values obtained by Kjeldahl method do not include nitrates or forms of N compounds not broken down in the short digestion time.

Mineralizable Nitrogen (ppm)

Nitrogen mineralization potential was determined on the same samples. 10g of air-dried soil (≤1 mm) was added to 30g acid washed sand and the mixture placed in a 250 ml plastic bottle containing 6 ml distilled water. After aerobic incubation at 30°C for 2 weeks samples were analysed for exchangeable ammonium + nitrate + nitrite following the procedure outlined by Bremner (1965).

Soil Available Phosphorus (ppm)

Available P refers to extractable P soluble in sodium bicarbonate ($NaHCO_3$). Subsamples of 2g sieved soil (≤1 mm) were placed in 250 ml plastic bottles and mixed with 200 ml of 0.5M $NaHCO_3$. After shaking overnight on a rotary shaker, the mixture was filtered and about 75 ml was centrifuged at 3000 rpm. 30 ml of the centrifuged solution was treated with 0.06g activated charcoal (Norit A + Nucha C) and shaken for 1 h to clarify the solution of coloured organic materials. The charcoal was previously freed of $PO_4^{\equiv}$ by several extractions or washings with 0.5M $NaHCO_3$ until the filtrate contained less than 1 ppm PO_4-P. Immediately after filtering, a 20 ml aliquot was transferred into a 50 ml volumetric flask and then the pH was adjusted using P-nitrophenol indicator and 8N H_2SO_4. Colour development technique was that described by Olsen, Cole, Watanabe and Dean (1954).

Total Phosphorus (ppm)

This was determined on 2g of dried soil after digestion for 45 minutes with 6 ml boiling 70% $HClO_4$ (Olsen and Dean 1965). Colour development was as for available P.

Organic Carbon (%)

Determination of the amount of organic carbon in each composite sample were made on 1g finely ground soil ($\leq$0.5mm) following the method of Walkley and Black (1965). The conversion to percent carbon was made using the correction factor of 1.33 which allows for an incomplete oxidation of the inert forms of carbon (Black 1965).

Calcium Carbonate (%)

Soil $CaCO_3$ was determined for the 0-5cm layer on a 5g subsample (2.5g for 5-10cm layer of soil), following the volumetric calcimeter method outlined by Allison and Moodie (1965).

Soil pH

Hydrogen ion concentration in soil was measured by pH meter on a 1:5 soil suspension in water after standing for 1 h with intermittent mixing and vigorous stirring immediately before measurement.

Bulk Density (g cm^{-3})

Bulk density was estimated for each composite sample from dry weight of the composite sample and its volume calculated from the dimensions of the soil sampling tube.

Clay content (%)

The clay content of each composite sample was determined on 50g air-dried soil by the sedimentation method using a Bouyoucos hydrometer after dispersion with calgon and NaOH (Piper 1950, Day 1965).

3.3.4 Statistical Methods

The statistical approach adopted in the present study aimed at:

1) Identifying the components of the environment that are associated with high soil total N in the survey area (Section 3.4.4).
2) Ascertaining whether there were any patterns in the relationships between soil total N and the farming management systems based on cereal rotations with legume pasture leys (Section 3.4.3).
3) Understanding the interrelationships between soil properties, farming management practices and climate in relation to soil N accumulation under the semi-arid environment of the study area (Section 3.4.6).

Statistical analysis was performed using a statistical package for the social sciences (Nie, Hull, Jenkins, Steinbrenner and Bent, 1975). Bivariate analysis was used for a preliminary assessment of the degree of association between variables. Pearson correlation coefficients were computed and allowance for a two-tailed test of significance was made. The interrelations of 2 variables were examined by means of simple linear regressions whereas complex relations holding between more than 2 variables were studied using multivariate analysis, primarily principal component analysis. The latter analytical method aims to summarize most of the variation in a multivariate system in fewer and new independent variables called components. Each component represents a linear combination of all original variables and it is ranked according to its eigenvalue. The eigenvalue of each component is proportional to the variance in the original data which it accounts for and the set of coefficients that specify each component comprise its eigenvector. Of all possible linear combinations of the original variables, the first principal component accounts for the greatest variance. The components are uncorrelated with each other and arranged in order of decreasing variance (Marriott 1974; Nie *et al.*, 1975). There is no assumption about the interrelations between the original variables included, so this multivariate technique simply describes how the original variables are linked and may simplify the interpretation of the complex biological system under investigation. In the present study principal component analysis was based on correlation matrices without involving any rotation to the principal axis.

3.4 RESULTS AND DISCUSSION OF THE SOIL FERTILITY SURVEY

In this study of soil fertility 3 major groups of factors are considered:

1) Soil properties.
2) Farming management factors.
3) Climatic conditions (Rainfall).

Soils were divided into two groups based on the presence of lime. Soils containing more than 0.05% $CaCO_3$ (50 sites) are referred to as "solonized brown soils" and those containing 0% $CaCO_3$ (45 sites) are referred to as "red-brown earths" (Appendix I.3). The division of pastures into medic-dominant and grass-dominant was based on the assessment of the botanical composition of the pasture in 1974 only. No information was available for the other nine years considered in the survey.

Appendix I.2A gives the classification of sites between medic-dominant and grass-dominant pastures, with soil characteristics for each site, and annual rainfall.

Although the N content of soils in the survey area prior to 1965 was not known and although variables such as temperature, grazing pressure, N losses were not measured, no less than 71% of the variation in total N in the surface of the red-brown earths was associated with one managerial factor and one soil physical property as follows:

Soil total N (%) = 0.00011 (ASRP) + 0.0019 (% Clay) + 0.009

$R^2 = 71\%^{***}$ and where:

Soil total N (%) = Total N held in the surface 5cm of soil under medic-dominant pastures (23 sites)

ASRP = Frequency of years of pasture as embodied in ASRP (to be further explained later)

% Clay = Clay content of the surface 10cm of soil.

In view of the limitations to the survey just mentioned, accounting for 71% of the variance with two variables was considered most encouraging to a further analysis of these relationships. Soil total N is represented here as the dependent variable and other measured variables (except %C and mineralizable N) as independent variables although it is recognized that cause and effect cannot be defined by correlation but intuitively inferred. Finding a significant co-efficient of determination does not in itself establish a causal relation between the dependent variable and the independent variables, for one or more of the measured variables may be independently associated with a third possibly unknown or unmeasured variable. This may be the case in the present survey where the independent variables are not orthogonal or mutually independent.

Before presenting the results for the interactions between the measured variables, the distribution of individual variables is discussed below. The frequency distributions and the results are based, in the first instance, on the botanical composition of the pasture with some reference to soil type.

3.4.1 Soil Properties

Soil Total Nitrogen. In cultivated soils (95 sites) total N varied from 0.043% to 0.267% and was higher in the 0-5cm than in the depth below (Figure 3.2C) with the 2 horizons well correlated (r = 0.74). By comparison, none of the reference sites (uncultivated) had less than 0.1% total N in the surface 5cm of soil and four of the 11 had total N contents above 0.2% (Appendix I.2B). A high proportion of soils under grass-dominant pastures contained between 0.06 to 0.12% total N in the 0-5cm depth interval (Figure 3.2B) while most soils under medic-dominant pastures contained between 0.14 and 0.2% total N in the same depth interval (Figure 3.2A). The same pattern of distribution of soil total N was found in the 5-10cm layer of soil. Under either medic-dominant or grass-dominant pastures soil total N for both depths was higher in the solonized brown soils than in the red-brown earths.

Soil Available Phosphorus

Unlike soil total N, available P is not normally distributed (Figure 3.3a). About 50% of the sites contained more than 20 ppm in the surface 5cm of soil but most of the sites (83) had less than 20 ppm in the second depth. Similarly, most of the reference sites contained less than 20 ppm throughout the top 10cm of soil (Appendix I.2B). This suggests that much of the added superphosphate must stay in the top 5cm, the cultivation depth for most of the area. The variation in available P in the sampling area:

		Minimum	Maximum	Mean	Std. deviation
Medic-dominant pastures	0-5cm	6	81	24	15
	5-10cm	1	28	8	7
Grass-dominant pastures	0-5cm	10	89	31	17
	5-10cm	1	55	15	11

Available P was generally higher under grass-dominant pastures. This association may identify frequently cropped sites with greater use of superphosphate and poor regeneration of medics.

Soil pH

Soil pH varied from 6.6 to 8.0 in the 0-5cm depth interval and almost the same range of soil pH was found in the second depth interval (Figure 3.3b). The standard deviations of the means were respectively 0.45 and 0.35. The surface soil of the reference sites was more alkaline than that of the pasture sites (Appendix I.2B) but the split of the 95 pastures into medic-dominant and grass-dominant did not reveal significant differences in soil pH. As expected soil pH was higher in the solonized brown soils than in the red-brown earths (Appendix I.3).

Figure 3.2 Frequency distribution of soil total N in the survey area

No. of Sites	Soil depth (cm)	Mean soil total N (%)	Std. deviation ±
A = 60	0-5	0.165	0.034
	5-10	0.111	0.027
B = 35	0-5	0.123	0.031
	5-10	0.096	0.040
C = 95	0-5	0.150	0.039
	5-10	0.106	0.033

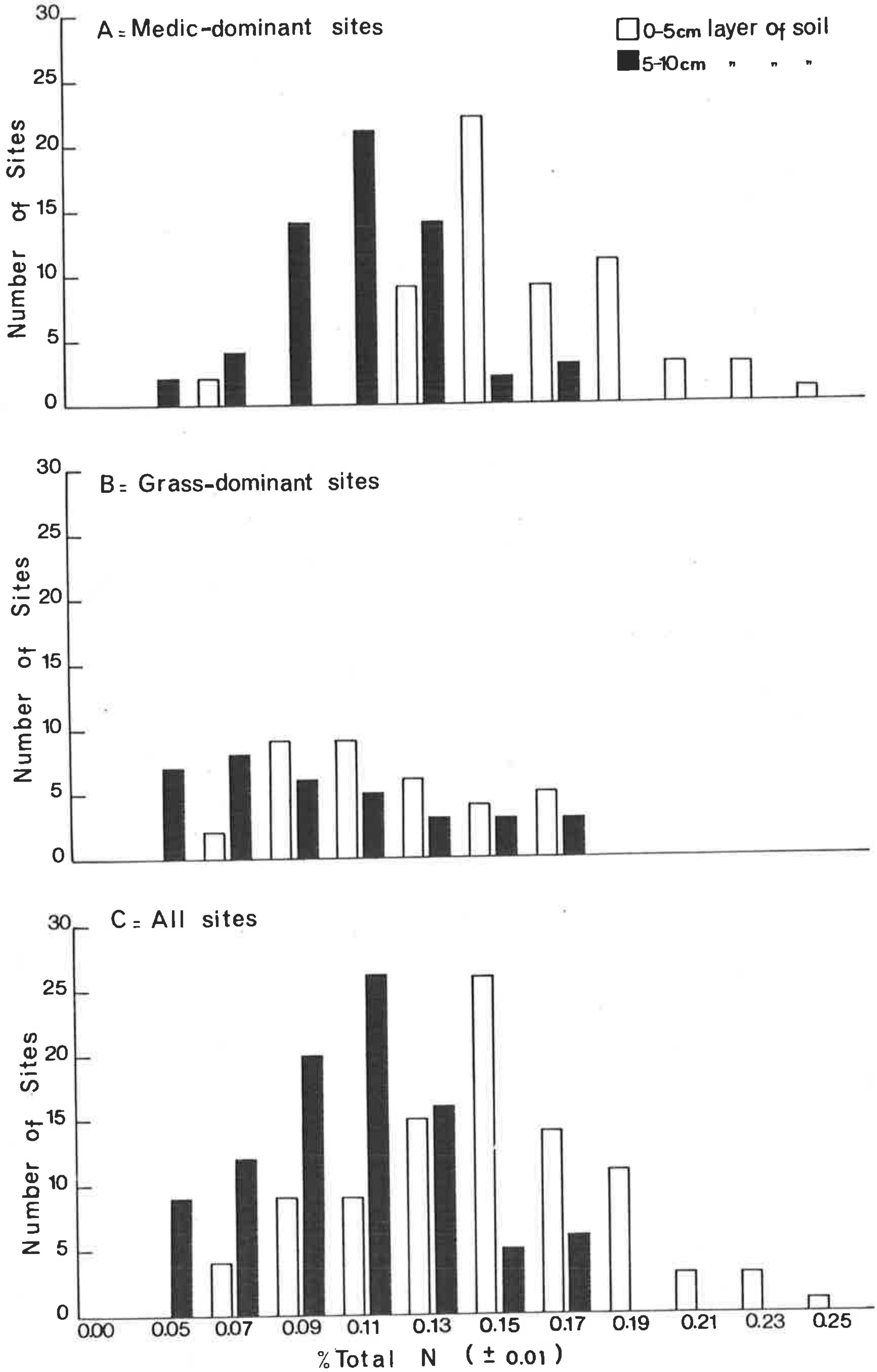

A = Medic-dominant sites
0-5cm layer of soil
5-10cm " " "
B = Grass-dominant sites
C = All sites
Number of Sites
30
25
20
15
10
5
0
0.00
0.05
0.07
0.09
0.11
0.13
0.15
0.17
0.19
0.21
0.23
0.25
% Total N (± 0.01)

Figure 3.3 Frequency distributions over all sites (95) for:

A - Available P (ppm)

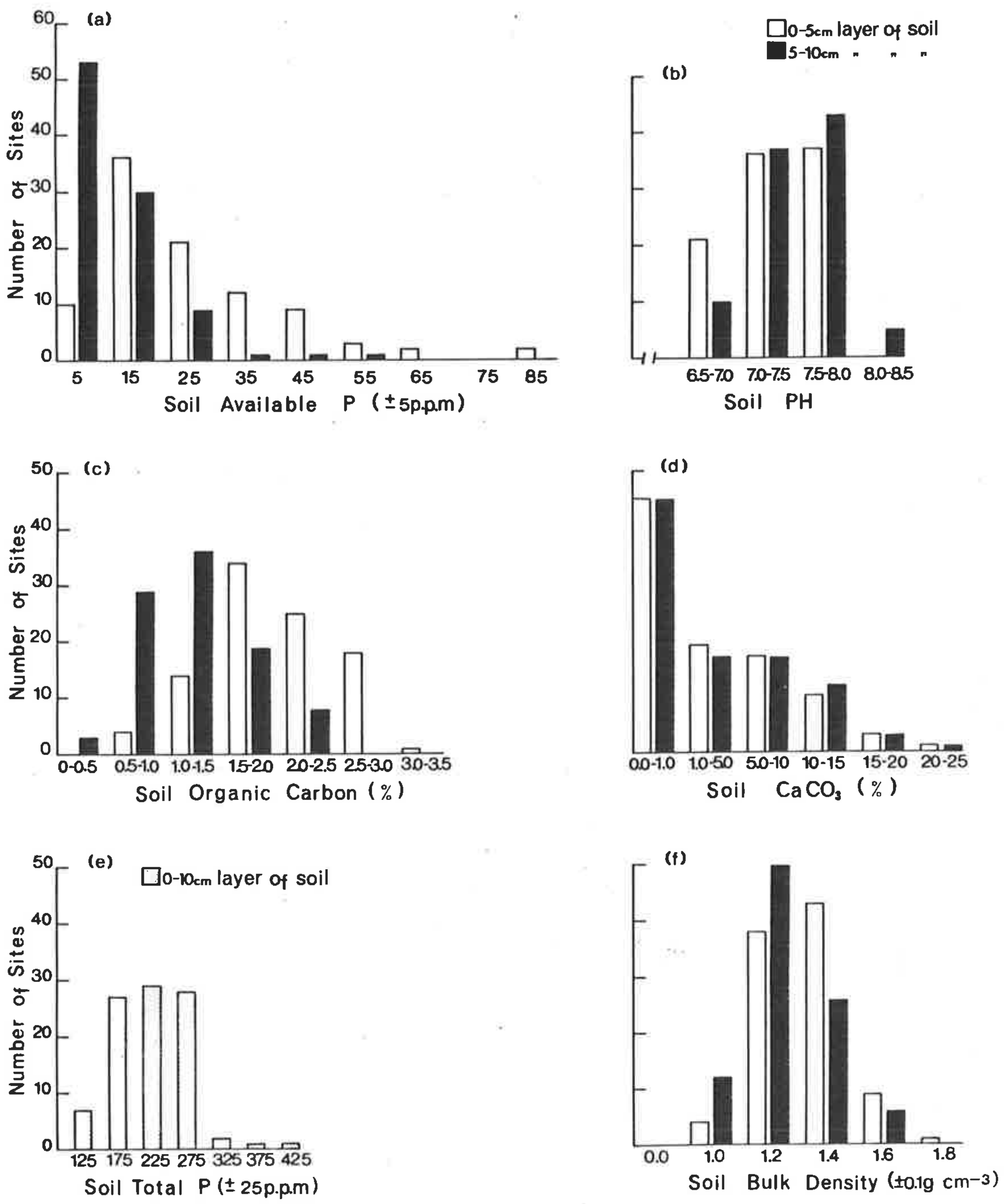
B - pH

C - Organic C (%)

D - Lime

E - Total P (ppm)

F - Bulk density (g cm^{-3})



0-5cm layer of soil
5-10cm " " "
(a)
Number of Sites
Soil Available P (±5p.p.m)
(b)
6.5-7.0 7.0-7.5 7.5-8.0 8.0-8.5
Soil PH
(c)
0-0.5 0.5-1.0 1.0-1.5 1.5-2.0 2.0-2.5 2.5-3.0 3.0-3.5
Soil Organic Carbon (%)
(d)
0.0-1.0 1.0-5.0 5.0-10 10-15 15-20 20-25
Soil CaCO3 (%)
(e)
0-10cm layer of soil
125 175 225 275 325 375 425
Soil Total P (± 25p.p.m)
(f)
0.0 1.0 1.2 1.4 1.6 1.8
Soil Bulk Density (±0.1g cm-3)

Soil Organic Carbon

Organic carbon varied from 0.3 to 3.4% with most soils containing 1.5 - 2.5%C (Figure 3.3c). In the reference sites the range was 1.1 to 3.6%C. Over all sites %C was higher in the 0-5 cm layer than in the 5-10cm layer and was not closely associated with the botanical composition of the pastures (Appendix I.2).

Soil $CaCO_3$

For this study, % $CaCO_3$ is referred to as lime. The distribution of lime in cultivated soils is not symmetric but positively skewed (Figure 3.3d). Almost 50% of the pasture sites contained no lime at either depth but when present lime ranged up to more than 15%. Of 11 reference sites 10 had lime in the top 10cm (Appendix I.2B).

Soil Total Phosphorus

Soil total P varied from 130 ppm to 420 ppm with 60% of the pasture sites containing between 200 and 300 ppm (Figure 3.3e). Reference sites varied from 160 ppm to 425 ppm total P (Appendix I.2B). There was bigger variation in soil total P under grass-dominant pastures than under medic-dominant pastures as indicated by the means and standard deviation which were respectively 221 ± 69 and 228 ± 45. Total P in the 0-10cm depth interval was higher in the solonized brown soils (257 ± 51) than in the red-brown earths (191 ± 34) (Appendix I.3).

Soil Bulk Density

Over all sites bulk density varied between 0.94 and 1.79 g cm^{-3} (Figure 3.3f). The surface 5cm of soil had a mean bulk density of 1.33 g cm^{-3} which was higher than the corresponding mean (1.27 g cm^{-3}) for the 5-10cm depth interval. Soils of the cultivated areas had higher bulk density than those of the reference sites where bulk density values ranged from 0.98 to 1.35 g cm^{-3} with a mean of 1.15 g cm^{-3} for the 0-5cm layer of soil and 1.16 g cm^{-3} for the 5-10cm depth interval (Appendix I.2B). The bulk density at both depths was lower where lime was present (Appendix I.3).

Clay content

Most of the sites (90%) had light textured 0-10cm horizons which contained from 10 to 36% clay with 42% of the sites between 20-36% clay (Appendix I.2).

Soil Mineralizable Nitrogen

Mineralizable N was higher (and more variable) in 0-5cm than in the 5-10cm layer and higher under medics than under grass (Figures 3. 4A,B,C). Reference sites were similar to medic-dominant pastures.

3.4.2 Cropping Histories and Cultural Practices

Within the 10-year period for which details of cropping history have been collected, the number of years of pasture leys is lower than the number of years of crops. The latter does not include the fallow phase which sometimes comes after a pasture ley and preceeds a wheat crop.

The variation in the frequency of pasture and crop in the sampling area (1965-74):

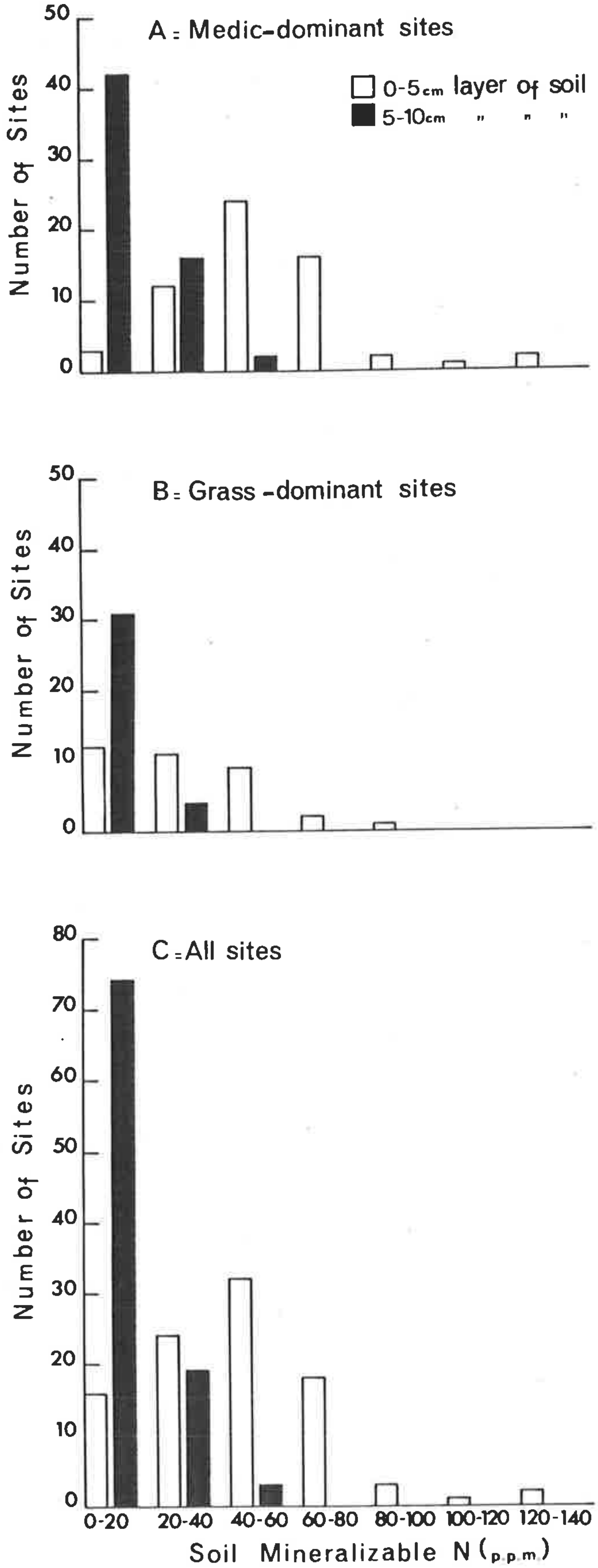
	Minimum	Maximum	Mean	Std.deviation
No. of years of pasture	1	6	3.7	1.1
No. of years of crop	3	7	4.7	0.9

Figure 3.4 Frequency distributions of Mineralizable N (ppm)

A = 60 sites

B = 35 sites

C = 95 sites



A = Medic-dominant sites
0-5cm layer of soil
5-10cm " " "
Number of Sites
50
40
30
20
10
0
B = Grass-dominant sites
Number of Sites
50
40
30
20
10
0
C = All sites
Number of Sites
80
70
60
50
40
30
20
10
0
0-20
20-40
40-60
60-80
80-100
100-120
120-140
Soil Mineralizable N (p.p.m.)

3.4.3 Relationships between soil total nitrogen and botanical composition of pasture, frequency of pasture or crop years, and seasonal rainfall

Botanical composition of pasture

Soil total N was higher under medic-dominant pastures than under grass-dominant pastures (Figures 3.2B and C), but it must be remembered that N build-up occurred over a number of years and the botanical composition was recorded in the year of sampling only (1974). Plate 3.2 shows a medic-dominant pasture and a grass-dominant pasture with reference to the rotation for each site during the period 1965-74 and to soil total N measured in 1974. Note the effect of pasture management and long rotation with 3 or more years cultivation on the disappearance of medics from the pasture leading to low soil total N in 1974. The amount of hard seed present in the surface soil may be a better variable for the assessment of pasture botanical composition in the past 10 years (1965-1974). The relation between pasture botanical composition and soil total N was further assessed by considering the management history for each site over a period of 10 years (1965-1974).

Relationship between soil total nitrogen and the frequency of pasture leys

In the present study the number of years of pasture is referred to as the frequency of years of pasture (FYRP) and the number of years of crop as the frequency of years of crop (FYRC). Changes in soil total N occurring under pasture clearly vary with the botanical composition of the pasture, whether it was composed of leguminous species or grasses or of a mixture of legumes and grasses.

For medic-dominant pastures soil total N increased with the frequency of years of pasture (Figure 3.5A). The association between soil total N and the FYRP was stronger for the 0-5cm than for the depth below (5-10cm). The regression describing this relationship for the

Plate 3.2: A medic-dominant and a grass-dominant pasture in 1974.

Above: Self-regenerated medic-dominant pasture in 1974 on a solonized brown soil. The rotation for the period 1965-1974 was:

Medic-wheat-medic-wheat-medic-barley-medic-fallow-wheat-medic.

Soil total N (0-5cm depth interval) in 1974 was 0.229%.

For further details on soil properties and rainfall refer to Appendix I.2(a)site No.93.

Below: Grass-dominant pasture in 1974 on a red-brown earth. The rotation for the period 1965-1974 was:

†Grass + some medic-fallow-wheat-barley-grass-barley-fallow-wheat-wheat-grass.

Soil total N (0-5cm depth interval) in 1974 was 0.066%.

For further details refer to Appendix I.2(a)site No.6.

†Grass + medic was cut for hay and medic did not set seed.

surface 5cm of soil is shown in Figure 3.5A.

$$\text{Soil total N (\%)} = 0.11 + 0.015\ \text{FYRP}\ (R^2 = 28\%^{***})$$

This means that over one quarter of the overall variance in total N can be explained by this simple variate defined only over the last 10 years. The scatter of points above and below the fitted regression line indicate that other factors were also associated with soil total N variation under medic-dominant pastures.

For the 5-10cm depth interval, the linear relationship between soil total N and the FYRP has a low R^2 ($9\%^{*}$) although it is still significant at P level of 0.05. The regression equation for the second depth interval is as follows:

$$\text{Soil total N (\%)} = 0.087 + 0.007\ \text{FYRP}$$

In contrast, under grass-dominant pastures the regressions were not significant (Figure 3.5B).

Relationships between soil total nitrogen and the frequency of crops

Over all sites, soil total N and the frequency of years of crop (FYRC) were negatively associated in the 0-5cm layer ($R^2 = 12\%^{**}$) but there was no significant association at the second depth ($R^2 = 4\%$ non-significant at $P = 0.05$). The negative association in the surface 5cm of soil was due to a stronger correlation in the medic-dominant pastures ($R^2 = 23\%^{***}$ for the 0-5cm and $7\%^{*}$ for the 5-10cm),whereas in the grass-dominant pastures correlations were not significant. The relationship under medic-dominant pastures is shown in Figure 3.5C and is described by the regression equation:

$$\text{Soil total N (\%)} = 0.256 - 0.02\ \text{FYRC}\ (R^2 = 23\%^{***})$$

where soil total N (%) = for the 0-5cm depth interval.

Again there was a wide scatter of points due to other factors.

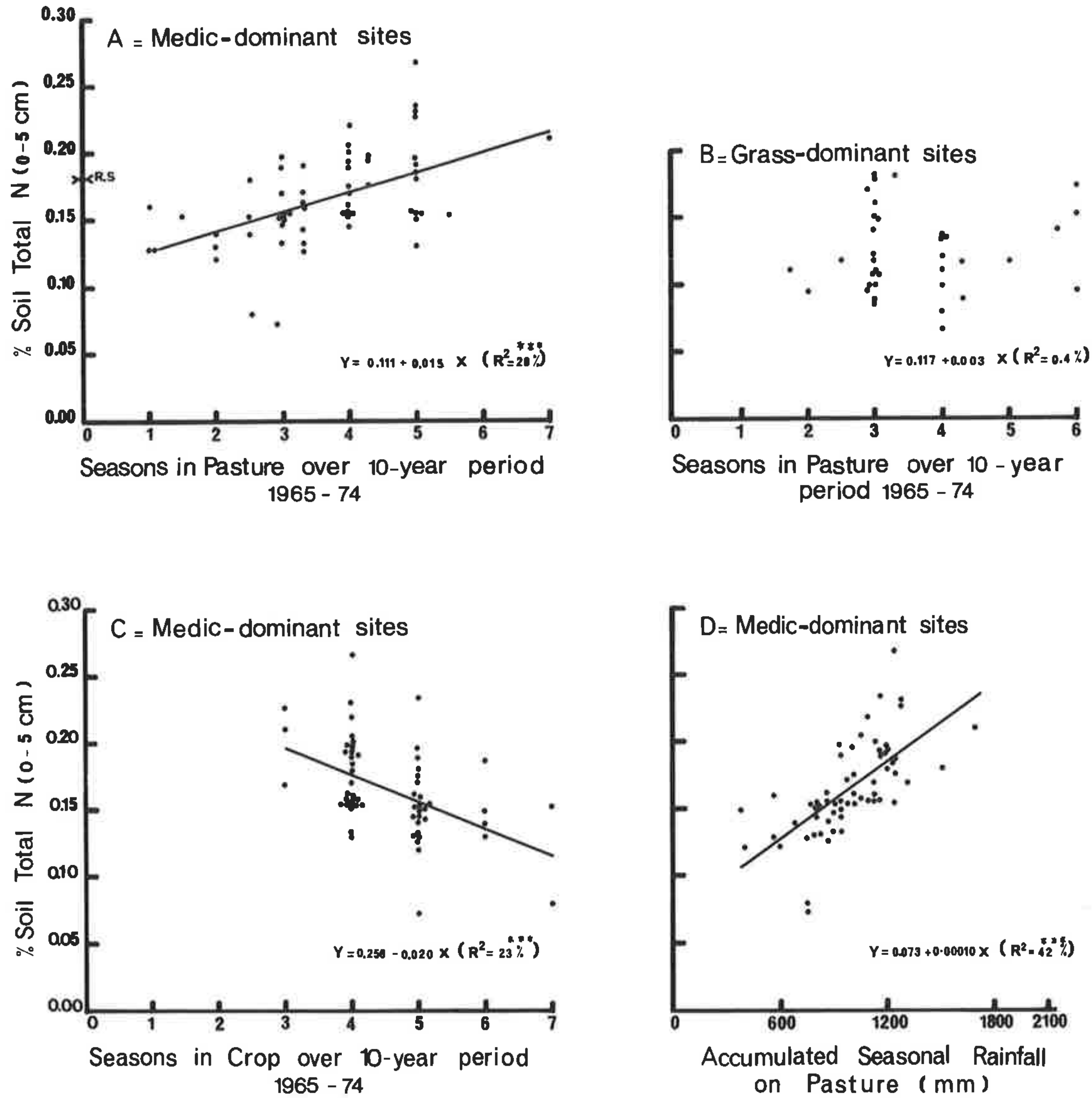
Figure 3.5 Linear regressions of soil total N (0-5cm depth interval) on:

A - Frequency of pasture leys under medic-dominant pastures (60 sites) during the period 1965-74.

B - Frequency of pasture leys under grass-dominant pastures (35 sites) during the period 1965-74.

C - Frequency of crops in medic-dominant sites (60 sites) during the period 1965-74.

D - Accumulated seasonal rainfall on medic pastures (60 sites).



A = Medic-dominant sites
% Soil Total N (0-5 cm)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
R.S
Y = 0.111 + 0.015 X (R² = 28%***)
0 1 2 3 4 5 6 7
Seasons in Pasture over 10-year period 1965 - 74
B = Grass-dominant sites
Y = 0.117 + 0.003 X (R² = 0.4 %)
0 1 2 3 4 5 6
Seasons in Pasture over 10 - year period 1965 - 74
C = Medic-dominant sites
% Soil Total N (0-5 cm)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Y = 0.256 - 0.020 X (R² = 23 %***)
0 1 2 3 4 5 6 7
Seasons in Crop over 10-year period 1965 - 74
D = Medic-dominant sites
Y = 0.073 + 0.00010 X (R² = 42 %***)
0 600 1200 1800 2100
Accumulated Seasonal Rainfall on Pasture (mm)

Relationship between soil total nitrogen and the accumulated seasonal rainfall on pasture

In an effort to make allowance for variation in the growth of medics due to seasonal variation in rainfall, the May-October rainfall received for each pasture phase was summed over the 10 year period (1965-74) for each site. The new variable was called accumulated seasonal rainfall on pasture (ASRP) and is in fact a combination of a management factor (FYRP) and a climatic factor (seasonal rainfall) as shown by the following equations:

$$FYRP = \Sigma_i^j \; P \qquad \text{------- (1)}$$

$$ASRP = \Sigma_i^j \; r_s . P \qquad \text{------- (2)}$$

where r_s = seasonal (May-October) rainfall for the pasture year (see Appenix 1.4).

P = 1.0 for a pasture year and 0.0 for a fallow or crop year.

i = 1965

j = 1974

For medic-dominant pastures, the combination ASRP of a management factor (FYRP) and a rainfall factor (r_s) had a much higher correlation with soil total N than existed between soil total N and the management factor FYRP alone (Figure 3.5D). For the 0-5cm depth interval, the regression equation became:

$$\text{Soil total N (\%)} = 0.073 + 0.00010 \text{ ASRP } (R^2 = 42\%^{***})$$

Likewise the coefficient of determination of the relationship between soil total N of the second depth interval (5-10cm) and the ASRP was much higher ($R^2 = 21\%^{***}$) than that found previously using FYRP as independent variable ($R^2 = 9\%^{*}$). The association between soil total N (5-10cm) and ASRP is described by the regression equation:

$$\text{Soil total N (\%)} = 0.059 + 0.000057 \text{ ASRP } (R^2 = 21\%^{***}).$$

A comparison of figures 3.5A and 3.5D clearly shows that FYRP is a discrete variable and ASRP is continuous. It was felt that this alone could cause a better correlation between soil total N and the cropping history variable (FYRP) and consequently the improvement could be an artifact of the method. However, change from discrete to continuous variable was also affected by feeding in sets of random numbers instead of r_s in equation (2) but the R^2 for the correlations was not improved in any of 3 attempts. We must conclude that the effect of accounting for seasonal rainfall, as embodied in ASRP is significant.

Yet again, there was no significant relationship between soil total N for either depth and ASRP for the grass-dominant pastures.

With the assumption that soil total N prior to 1965 and soil total N in 1974 were not correlated, our approach to be described below gives an estimate of the nett annual increment of soil total N under various rotations which involve both soil N gains and losses under farm conditions in the survey area.

Estimates of nitrogen increments under medics and under farm conditions derived from regression analyses of the survey data

From figure 3.5A and its regression equation:

$$\text{Soil total N (\%)} = 0.11 + 0.015 \text{ FYRP}$$

it is possible to calculate a mean annual increment of soil N (kg ha^{-1} year^{-1} N) under a medic-dominant pasture for the survey district using the equation:

$$\text{Mean annual N increment} = \frac{\Delta(\text{N}) \times \text{Soil depth} \times 10^4 \times 10^4 \times \text{bulk density}}{100 \times \Delta(\text{FYRP}) \times 1000}$$

where $\frac{\Delta(N)}{\Delta(FYRP)}$ = 0.015, the slope of the regression line with N in % and

FYRP in years.

Soil depth = cm

Bulk density = g cm^{-3}

Assuming a mean bulk density of 1.30 g cm^{-3} for the 0-5cm layer, the mean annual increment is 98 kg ha^{-1} N in this layer. A smaller annual increment of 46 kg ha^{-1} N per year can be calculated for the 5-10cm indicating, presumably, that most nodules are in the surface 5cm and cultivation does not greatly mix the two layers.

A similar calculation can be made using the variable ASRP (Figure 3.5D) and the regression equation for the 0-5cm layer:

Soil total N (%) = 0.073 + 0.00010 ASRP

The slope of the regression line $\frac{\Delta(N)}{\Delta(ASRP)}$ enables us to calculate as before a mean annual increment of soil total N per mm of seasonal rain on pasture. The calculated amount was found to be equal to 0.65 kg N ha^{-1} per mm of rain on pasture. This provides an estimate of the annual increment of soil N in the different pasture years. In fact, not only may a mean value for N increment be made for the district as a whole for a mean rainfall year, but also for maximum and minimum rainfall years, viz.,

Mean N increments

= 161 kg ha^{-1} N for mean rainfall year (248 mm)

= 230 kg ha^{-1} for maximum rainfall year (353 mm) (equivalent to 1968)

= 81 kg ha^{-1} N for minimum rainfall year (124 mm) (equivalent to 1967).

For the 5-10cm layer, the regression equation provided an estimated N increment of 0.37 kg N ha^{-1} per mm of rain on pasture. The variation in N increments between seasons were:

Mean N increment = 92 kg ha^{-1} N for mean rainfall year
131 kg ha^{-1} N for maximum rainfall year
46 kg ha^{-1} N for minimum rainfall year

Addition of the figures for the 0-5 and 5-10cm layers gives the total annual increments which become very significant indeed:

161 + 92 = 253 kg ha^{-1} N for mean rainfall year
230 + 131 = 361 kg ha^{-1} N for maximum rainfall year
81 + 46 = 127 kg ha^{-1} N for minimum rainfall year

These figures, derived as they are from the regression of soil total N on FYRP or ASRP are seen to represent the average nett effect in the district of replacing a crop year (sometimes fallow) by a medic pasture year. Thus they are seen to be the sum of both the N gains in the pasture year and the N losses in a crop year. An average cereal crop in the district exports about 30 kg of N in the grain and there are other losses as well due to leaching, denitrification, erosion and auto-oxidation. If these additional losses are estimated to amount to 20 kg ha^{-1} N (Greenland 1971) then by substraction of a value of 50 kg ha^{-1} N for crop removal and other losses from the above figures, estimates of N increments under medic pastures may be obtained for the farm situation in the Mallala district. These estimates would then become for

mean rainfall year	253-50	=	203
maximum rainfall year	361-50	=	311
minimum rainfall year	127-50	=	77

or in round figures, 80 to 300 with a mean of about 200 kg ha^{-1} N per year.

The split of soils into two groups revealed that, under medic-dominant pastures, and for the 0-5 depth interval, the estimated increment of N was higher (0.62 kg ha^{-1} N per mm of rain on pasture) in red-brown earths than in solonized brown soils (0.48 kg ha^{-1} N per mm of rain on pasture)

Thus before making allowance for N removal and loss in crop years (50 kg ha^{-1} N), the calculated mean N increments in the 0-5cm layer were:

154 kg ha^{-1} N in red-brown earths, and

120 kg ha^{-1} N in solonized brown soils.

These are derived from the following equations for the red-brown earths and solonized brown soils respectively:

$$\text{Soil total N (\%)} = 0.063 + 0.000096 \text{ ASRP } (R^2 = 37\%^{**})$$

$$\text{Soil total N (\%)} = 0.107 + 0.000074 \text{ ASRP } (R^2 = 28\%^{***})$$

Note that the intercept is markedly different for the two groups. Estimates for N increment in the 5-10cm layer were not calculated for the reason that regressions of soil total N on ASRP were not significant for that depth of soil, probably because there were fewer data in the regressions.

Solonized brown soils have higher mean soil total N levels than do the red-brown earths (page 91) but the above equations suggest that the rate of accumulation is higher in the red-brown earths. The intercept in the above equation for the solonized brown soil is higher than that for the red-brown earth.

Under grass-dominant pastures, and for the red-brown earths, there was a significant ($R^2 = 21\%^{*}$) relationship between soil total N of the 0-5cm depth interval and ASRP. Accordingly, the estimated increment of soil N was 0.29 kg ha^{-1} N per mm of rain on pasture, which gives an average annual increment of 22 kg ha^{-1} N (i.e., 72-50 kg ha^{-1} N). No estimate was derived for the 5-10cm layer, the regression for which was not significant.

The observed relationship between soil total N and the FYRP may be compared with the report of Mullaly *et al.*, (1967) who found a linear relationship between soil total N and the increasing number of consecutive years of medic ley. In our study, the botanical composition of the pasture was recorded in 1974 only, but it is very likely that medic-dominant pasture in 1974 implies good seed reserve in the soil and that the state of the pasture in 1974 reflects to some extent the nature of the pasture in earlier years.

Improvement of soil total N by medics has also been reported (Meagher and Rooney 1966) in long-term field experiments in Victoria. A mean annual soil N increment of 117 kg ha^{-1} in the surface 15cm of soil was reported after 4 consecutive years of mixed medic/grass pastures (Meagher and Rooney 1966). However, this kind of assessment of N accumulation in soils following several years of continuous medic + grass pastures and without involving any soil cultivation does not relate to changes in soil organic N under farm conditions where medic leys are separated by cereal crops and fallow periods and where consequently the nett accumulation of soil N may be quite different.

The association of N increments under medic-dominant pastures with variations in the seasonal rainfall agrees with other reports concerning the seasonal variation in N accumulation by subterranean clover pastures (Clarke 1970, Vallis 1972, Simpson, Bromfield and Jones 1974). According to Russell (1960) and Watson (1963), soil N accumulation is associated with the growth of the legume-based pasture. Presumably, the higher the seasonal rainfall the better the growth of medics and the higher the inputs of N into the soil. The effective seasonal rainfall or the effective medic growth from season to season might be a better variable than total seasonal rainfall. Nevertheless, the association of the amounts of N held in the topsoil with seasonal rainfall may have this biological meaning which needs to be tested, for this seasonal variation in N increments under medic-dominant pasture can have important implications for the ley farming system in South Australia. Cereal growers may need to vary their rotational system according to the N accumulated under medic pastures in the previous season; for fallowing after a good pasture year may increase the risks of N losses from the soil and may reduce the benefit of the N accumulated during the previous good pasture season.

The estimated mean annual increment of N under medic-dominant pastures (200 kg ha^{-1} N) in the surface 10cm of soil falls in the range of soil N increments by legume-based pastures (50-250 kg ha^{-1} N) reported in the literature. The survey area is likely to show high inputs of N because:

1) Low soil N levels of farmers' fields prior to 1965, especially as fallow-wheat rotations prevailed for more than 50 years and consequently where soil N reserves were depeleted (Norton 1973). Nitrogen accumulates at a much higher rate in soils of low N status than in soils of high N level (Watson and Lapins 1964, Simpson and Bromfield 1974, Jenkinson 1976).

2) The removal of N by cereal crops (grain + straw) may be higher than the amount of 30 kg ha^{-1} N per year especially in medic-dominant sites where high mineralization of soil N can occur in the pre-sowing period due to warm soil temperature and early seasonal rains. Indeed, the aerobic incubation results showed that soil under medic-dominant pastures has a high N mineralization potential with a mean of 54 ppm in the surface 5cm of soil. Losses of N under crops by denitrification could be also very high. (Stefanson and Greenland, 1970).

3) Stocking rate may be a covariate associated with the FYRP. Farmers having high stock numbers may need more frequent pasture leys. Stocking rate influences the botanical composition of the pasture and probably influences soil N accumulation (McLachlan 1968; Simpson *et al.*, 1974). The association between soil total N and the FYRP may need to be adjusted for inequalities in the covariate stocking rate which was not assessed in the present survey.

4) Pests and fungus attacks of medics in the low rainfall areas are not as frequent or severe as in high rainfall areas where subterranean clovers dominate (Moulden 1973).

The mean soil N increment of 200 kg ha^{-1} N per year in the top 10cm of soil indicates high potential of medics to build up soil N when they are well established and properly managed to avoid the proliferation of invading grasses following the initial increase in soil N level. Differences in soil total N between sites not accounted for above are obviously linked to other environmental variables.

3.4.4 Relationships between individual soil properties and soil total nitrogen

Although no assumption about the causative factors can be made from correlation and regression analysis which merely deals with associations, correlation and regression analysis were performed on the data in order to determine which of the measured soil characteristics are more closely associated with soil total N in the survey area. It should be mentioned that logarithmic values of lime were used throughout the statistical analysis. This transformation was needed because of the skewness of the distribution of lime values, there being many zero values. Likewise, logarithmic values of available P were used. Pearson correlation coefficients in Tables 3.3A,B,C, summarize the correlations between soil total N and individual soil properties and results of simple regression analysis are presented in Figure 3.6 and Table 3.2. All the analyses so far (Figure 3.6 and Table 3.2) suggest that the relationships between soil total N and individual soil properties were approximately linear (except for % C) and were generally closer for the 5-10cm than for the surface 5cm of soil. Moreover, these relationships were closer under grass-dominant than under medic-dominant pastures. The weak association between soil total N of the 0-5cm depth interval and total P of the surface 10cm of soil (Figure 3.6A) was partly due to 3 sites on the same farm which had received superphosphate regularly every year for the past 7 years (1968-1974) but where improved medic pastures were introduced only in 1972.

Of the 119 correlation coefficients, 80 are statistically significant at 1% level. The high level of intercorrelation suggests that some of the variables are measuring the same underlying soil properties. Correlation coefficients of Tables 3.3A,B,C, emphasize the closeness of association between soil total N and % C at both depths ($r = 0.77^{***}$ to 0.96^{***}) although it is higher in the second depth. This is not surprising, these 2 variables being closely associated in soil organic matter which itself is dependent on other soil, management and environmental variables. In soils of the survey area the C/N ratio was generally higher in the 0-5cm than in the 5-10cm depth interval and was higher under grass-dominant than under medic-dominant pastures as indicated below:

		Mean C/N ratio
Medic-dominant pastures	0- 5cm depth interval	13.0
	5-10cm " "	12.6
Grass-dominant pastures	0-5 cm " "	14.7
	5-10cm " "	12.2

Figure 3.6 Linear regressions of soil total N on:

A - Total P of the 0-10cm depth interval (95 sites)

B - Organic C of the 0-5cm depth interval (95 sites)

C - Mineralizable N of the 0-5cm depth interval (95 sites)

D - Linear regression of soil organic C on Total P (95 sites)

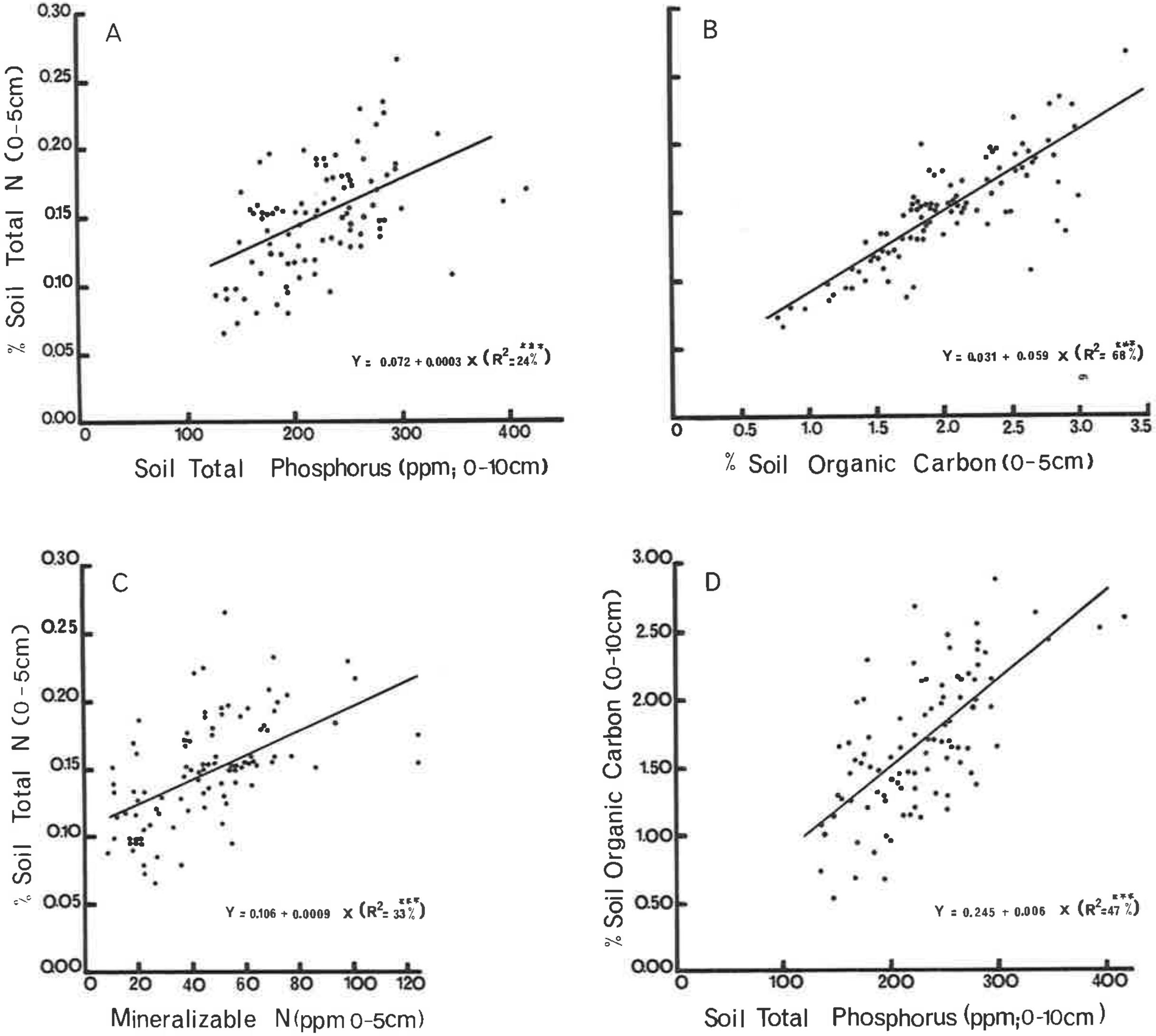

A
% Soil Total N (0-5cm)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
Soil Total Phosphorus (ppm; 0-10cm)
Y = 0.072 + 0.0003 X (R² = 24% ***)
B
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
% Soil Organic Carbon (0-5cm)
Y = 0.031 + 0.059 X (R² = 68% ***)
C
% Soil Total N (0-5cm)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
20
40
60
80
100
120
Mineralizable N (ppm 0-5cm)
Y = 0.106 + 0.0009 X (R² = 33% ***)
D
% Soil Organic Carbon (0-10cm)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
100
200
300
400
Soil Total Phosphorus (ppm; 0-10cm)
Y = 0.245 + 0.006 X (R² = 47% ***)

Table 3.2 Coefficients of linear regression of soil total N (%) on soil properties

Variable	Depth interval 0-5 cm: Coefficient b	Standard error of b	F	R^2	Depth interval 5-10 cm: b	Standard error of b	F	R^2
Lime (M)	0.020	± 0.004	31.85***	35	0.015	± 0.002	36.74***	39
Lime (G)	0.017	± 0.004	21.50***	40	0.030	± 0.003	100.30***	75
Total P(M)	0.0004	± 0.000	16.34***	22	0.0003	± 0.000	25.66***	31
Total P(G)	0.0003	± 0.000	24.07***	42	0.0005	± 0.000	104.33***	76
%org.C(M)	0.060	± 0.005	159.39***	73	0.053	± 0.004	162.27***	74
%org.C(G)	0.042	± 0.006	47.94***	59	0.071	± 0.004	378.07***	92
Mineralizable N(M)	290.3	±81.0	12.84**	18	104.7	±41.9	6.24*	10
Mineralisable N(G)	350.6	±88.1	15.82***	32	78.96	±27.5	8.26**	20
Bulk density (M)	- 0.148	± 0.032	21.91***	27	- 0.082	± 0.023	13.06***	18
Bulk density (G)	- 0.082	± 0.025	10.45**	24	- 0.180	± 0.025	51.02***	61
pH (M)	0.045	± 0.011	17.65***	23	0.039	± 0.009	17.37***	23
pH (G)	0.050	± 0.050	17.47***	35	0.059	± 0.017	12.65***	28

(M) = Medic-dominant pastures (60 sites)

(G) = Grass-dominant pastures (35 sites)

Table 3.3A Pearson correlation coefficients (r) for pairs of soil, management, and environmental factors (95 sites). A = 0-5cm depth interval; B = 5-10cm depth interval.

	Total N (A)	Total N (B)	Avail P (A)	Avail P (B)	C (A)	C (B)	Min.N (A)	Min.N (B)	PH (A)	PH (B)	Lime (A)	Lime (B)	Bulk D (A)	Bulk D (B)	Total P	Clay	Annual Rainfall
ASRP	0.32***	0.25*	-0.06	-0.07	0.28**	0.25*	0.32***	0.05	0.17	0.27**	0.26*	0.23*	-0.24*	-0.25*	0.25*	-0.11	0.19
FYRC	-0.35***	-0.19	0.30**	0.31**	-0.26*	-0.21*	-0.34***	-0.18	-0.17	-0.13	-0.18	-0.18	0.22*	0.12	-0.09	0.03	-0.08
Total N_A	1.00	0.70***	-0.15	-0.28**	0.82***	0.74***	0.58***	0.42***	0.51***	0.47***	0.58***	0.58***	-0.53***	-0.45***	0.49***	-0.00	0.37***
Total N_B		1.00	-0.02	-0.10	0.87***	0.91***	0.32**	0.39***	0.65***	0.52***	0.75***	0.73***	-0.69***	-0.63***	0.73***	-0.19	0.50***
Avail.P_A			1.00	0.55***	-0.09	-0.07	-0.30**	-0.06	-0.29**	0.01	-0.06	-0.11	-0.08	0.12	0.24*	0.01	-0.07
Avail.P_B				1.00	-0.13	-0.08	-0.26*	-0.18	-0.13	-0.18	-0.00	-0.02	-0.13	-0.02	0.34***	0.02	0.05
C_A					1.00	0.92***	0.36***	0.30**	0.57***	0.44***	0.70***	0.67***	-0.69***	-0.62***	0.68***	0.15	0.51***
C_B						1.00	0.32**	0.35***	0.67***	0.50***	0.76***	0.75***	-0.64***	-0.59***	0.66***	0.14	0.54***
Min.N_A							1.00	0.53***	0.34***	0.36***	0.35***	0.39***	-0.29**	-0.27**	0.23*	-0.04	0.24*
Min.N_B								1.00	0.35***	0.39***	0.36***	0.38***	-0.28**	-0.17	0.20	-0.06	0.14
PH_A									1.00	0.52***	0.74***	0.73***	-0.31**	-0.39***	0.48***	0.02	0.48***
PH_B										1.00	0.59***	0.59***	-0.28**	-0.17	0.33***	-0.20	0.28**
$Lime_A$											1.00	0.95***	-0.50***	-0.48***	0.70***	-0.03	0.44***
$Lime_B$												1.00	-0.47***	-0.44***	0.66***	-0.05	0.43***
Bulk D_A													1.00	-0.51***	-0.61***	-0.31**	-0.41***
Bulk D_B														1.00	-0.53***	-0.30**	-0.37***

Levels of significance: *significant at P = 0.05; **significant at P = 0.01; ***significant at P<0.01

Table 3.3B Pearson correlation coefficients (r) for pairs of soil, management, and environmental factors (Medic-dominant pastures : 60 sites).

A = 0-5cm depth interval; B = 5-10cm depth interval.

	Total N (A)	Total N (B)	Avail P (A)	Avail P (B)	C (A)	C (B)	Min.N (A)	Min.N (B)	PH (A)	PH (B)	Lime (A)	Lime (B)	Bulk D (A)	Bulk D (B)	Total P	Clay	Annual Rainfall
ASRP	0.65***	0.46***	-0.11	-0.19	0.49***	0.45***	0.46***	0.16	0.35**	0.37**	0.49***	0.47***	-0.38**	-0.39**	0.38**	-0.18	0.28*
FYRC	-0.48***	-0.26*	0.23	0.34**	-0.33**	-0.30*	-0.36**	-0.26*	-0.24	-0.19	-0.27*	-0.29*	0.18	0.16	-0.02	0.11	-0.06
Total N_A	1.00	0.74***	-0.07	-0.22	0.86***	0.80***	0.43***	0.27*	0.48***	0.37**	0.60***	0.59***	-0.52***	-0.33*	0.47***	-0.12	0.37**
Total N_B		1.00	-0.01	-0.25	0.81***	0.86***	0.24	0.31*	0.62***	0.48***	0.64***	0.62***	-0.50***	-0.43***	0.55***	0.00	0.38**
Avail.P_A			1.00	0.44***	-0.09	-0.09	-0.14	0.08	-0.25	0.16	0.00	-0.06	0.14	0.21	-0.22	-0.12	-0.00
Avail.P_B				1.00	-0.21	-0.14	-0.03	-0.08	-0.14	-0.11	-0.03	-0.02	-0.13	0.03	0.32*	-0.11	0.01
C_A					1.00	0.91***	0.23	0.11	0.51***	0.31*	0.60***	0.57***	-0.54***	-0.45***	0.53***	0.02	0.42***
C_B						1.00	0.22	0.21	0.66***	0.40***	0.66***	0.66***	-0.50***	-0.40***	0.53***	-0.02	0.47***
Min.N_A							1.00	0.50***	0.26*	0.22	0.31*	0.33**	-0.25	-0.15	0.20	-0.08	0.24
Min.N_B								1.00	0.23	0.33**	0.26*	0.30*	-0.24	-0.05	0.00	-0.05	0.06
PH_A									1.00	0.47***	0.71***	0.70***	-0.17	-0.35**	0.46***	-0.12	0.39**
PH_B										1.00	0.55***	0.57***	-0.16*	-0.01	0.29	-0.31*	0.19
$Lime_A$											1.00	0.95***	-0.31*	-0.32*	0.69***	-0.22	0.28*
$Lime_B$												1.00	-0.26*	-0.28*	0.65***	-0.26*	0.29*
Bulk D_A													1.00	0.24	-0.41***	-0.08	-0.36**
Bulk D_B														1.00	-0.36**	-0.15	-0.26*

Table 3.3C Pearson correlation coefficients (r) for pairs of soil, management, and environmental factors. (Grass-dominant pastures : 35 sites).

A = 0-5cm depth interval; B = 5-10cm depth interval.

	Total N (A)	Total N (B)	Avail P (A)	Avail P (B)	C (A)	C (B)	Min.N (A)	Min.N (B)	PH (A)	PH (B)	Lime (A)	Lime (B)	Bulk D (A)	Bulk D (B)	Total P	Clay	Annual rainfall
ASRP	0.07	0.09	-0.04	0.04	0.11	0.07	0.29	-0.08	-0.08	0.21	-0.01	-0.06	-0.16	-0.13	0.14	-0.05	0.07
FYRC	-0.04	-0.05	0.33	0.14	-0.09	-0.03	-0.21	0.09	-0.02	0.04	-0.02	0.02	0.19	0.01	0.03	-0.09	-0.19
Total N_A	1.00	0.69***	0.12	0.15	0.77***	0.70***	0.57***	0.47**	0.59***	0.50**	0.63***	0.61***	-0.49**	-0.52***	0.65***	0.27	0.59***
Total N_B		1.00	0.14	0.31	0.94***	0.96***	0.31	0.45**	0.69***	0.53***	0.88***	0.87***	-0.80***	-0.78***	0.87***	0.41*	0.67***
Avail.P_A			1.00	0.66***	0.13	0.11	-0.40*	-0.13	-0.26	-0.08	-0.08	-0.08	-0.19	0.16	0.36*	0.17	-0.20
Avail.P_B				1.00	0.32	0.25	-0.39*	-0.12	0.02	-0.10	0.23	0.17	-0.44**	-0.24	0.53***	0.06	0.10
C_A					1.00	0.93***	0.38*	0.47**	0.65***	0.51**	0.83***	0.79***	-0.80***	-0.77***	-0.87***	0.36*	0.70***
C_B						1.00	0.33	0.49**	0.67***	0.56***	0.87***	0.86***	-0.74***	-0.75***	0.81***	0.36*	0.68***
Min.N_A							1.00	0.37*	0.45**	0.47*	0.37*	0.45**	-0.20	-0.30	0.28	0.07	0.35*
Min.N_B								1.00	0.54***	0.39*	0.52***	0.49**	-0.23	-0.24	0.48**	-0.05	0.35*
PH_A									1.00	0.56***	0.78***	0.76***	-0.44**	-0.40*	0.53***	0.24	0.65***
PH_B										1.00	0.62***	0.59***	-0.32	-0.27	0.38*	-0.04	0.43**
$Lime_A$											1.00	0.96***	-0.66***	-0.64***	0.71***	0.21	0.66***
$Lime_B$												1.00	-0.67***	-0.62***	0.70***	0.25	0.64***
Bulk D_A													1.00	0.70***	-0.75***	-0.57***	-0.51**
Bulk D_B														1.00	-0.70***	-0.51**	-0.55***

Levels of significance: *significant at $P = 0.05$; **significant at $P = 0.01$; ***significant at $P<0.01$

There were other outstanding associations namely those between soil total N and lime, total P, and bulk density (negatively), lime correlating slightly better than the total P (Tables 3.3A,B,C). Total P includes soil organic P which has been reported (Donald and Williams 1954) to be correlated with soil organic N in soil organic matter for the more acid soils in higher rainfall areas and under permanent sub-terranean clover pastures. Unexpected results were the lack of correlation between soil total N and available P and soil total N and % clay.

The level of soil available P reflects to a certain extent the intensity of superphosphate application during the 10-year period 1965-1974. Application rates of superphosphate were obtained from farmers' records. The regression of soil available P of the 0-5cm depth interval on the amounts of superphosphate applied (kg ha^{-1}) is described as follows:

$$\text{Soil available P (ppm)} = 0.92 + 0.0537 \text{ Superphosphate} \quad (R^2 = 33.5\%^{***})$$

For the depth below (5-10cm) the relationship was poor ($R^2 = 9\%^{*}$). This may identify a common practice in the survey area of applying superphosphate in the top 5cm of soil together with cereal seed. From farmers' information about the cropping history of the different sites, it has been established that during the period 1965-1974 only 7% of the local farmers had applied superphosphate to the pasture leys at a rate approaching that usually applied to cereal crops (i.e., 100 kg ha^{-1}).

There was a high level of intercorrelation among several soil properties. High to moderate correlations existed between %C and lime (r ranges from 0.70^{***} to 0.75^{***}), %C and total P (r ranges from 0.66^{***}

to 0.68***), %C and soil pH (r ranges from 0.50*** to 0.57***), lime and soil pH (r ranges from 0.59*** to 0.74***), lime and total P (r ranges from 0.66*** to 0.70***) and negative correlations between %C and bulk density (r ranges from -0.59*** to -0.69***), lime and bulk density (r ranges from -0.44*** to -0.50***), % clay and bulk density (r ranges from -0.30*** to -0.57***).

3.4.5. Association between soil total nitrogen and the average annual rainfall

The average annual rainfall at the sites sampled, estimated from the average annual rainfall isohyets presented in Figure 3.1, varied from 345 mm to 402 mm. There was a significant positive correlation between rainfall and soil total N. The correlation coefficients were higher for soils under grass-dominant than under medic-dominant pastures (Tables 3.3B and C). As pointed out by Stevenson (1965), the association between rainfall and soil N accumulation is due to the effect of increasing rainfall on plant growth and, consequently, the production of larger quantities of raw material for synthesis of humic substances and stabilization of soil N.

3.4.6. Interrelationships between soil total nitrogen and soil, management and climatic factors

Interrelationships between the variables were examined using principal component analysis. This ordination technique standardizes all original variables (giving them zero mean and unit variance) and thus avoids prejudging their relative importance. Although this analytical method merely creates new variables (components) it may provide a simple description of the interrelationships between soil total N and soil, management and environmental factors and may reveal directly interesting features of the data.

In the present study, %C and mineralizable N were not included in the principal component analysis because they measure almost the same soil property as soil total N. In addition, only components having an eigenvalue greater than 1.00 were considered. That is, only components accounting for at least the amount of the total variance of a single variable were treated as significant (Nie *et al.*, 1975).

a). <u>Under medic-dominant pastures</u>

The first 3 principal components accounted for 65.5% of the variation in the original data and indicated that soil total N (dominant in the first component), available P (dominant in the second component) and % clay (dominant in the third component) were the 3 relatively independent soil variables at both depths within the study area (Table 3.4). For the 0-5cm depth the first component accounted for 38.4% of the variation in the data and was dominated by soil total N, lime (%$CaCO_3$) and the ASRP. It describes the trend of a high soil N accumulation under a high frequency of medic leys and in soils of high lime content. This component suggests that lime represents the solonized brown soils with a high pH and low bulk density. The latter soil property is related to the role of lime in maintaining aggregate stability and stabilizing soil structure (Greenland 1971). The role of lime in reducing the leaching of applied phosphate as was suggested by Russell (1960) could partly explain the association of total P with lime and soil total N. Also, more superphosphate may be needed on high pH soils. The second principal component which is orthogonal to the first accounted for 14.7% of the variation in the original data and describes the variation of soil available P. Soil total N (first component) and available P were thus not associated. This second component indicates a link between the availability of P in soil and the number of

Table 3.4 Principal component analysis of the various measured variables relevant to soil properties, management practices and environmental conditions (Medic-dominant pastures : 60 sites).

	0-5cm layer of soil				5-10cm layer of soil		
Element	Principal component 1	2	3	4	Principal component 1	2	3
Soil total N	.847	-.090	.052	-.200	.823	.006	-.183
ASRP	.757	-.277	-.050	-.310	.750	-.284	-.022
Available P	-.065	.736	-.343	-.395	-.141	.807	.209
pH	.718	-.091	.006	.570	.631	-.059	.445
Lime (% $CaCO_3$)	.830	.110	-.220	.297	.836	.145	.217
FYRC	-.491	.649	.050	.378	-.461	.661	-.030
Total P	.695	.474	-.153	.141	.687	.556	.014
Annual rainfall	.497	.264	.505	.147	.458	.236	-.375
Bulk density	-.581	-.316	-.308	.457	-.508	-.111	.552
% Clay	-.180	.094	.830	-0.116	-.199	.021	-.811
Eigenvalue	3.841	1.472	1.237	1.098	3.549	1.571	1.427
% of variance	38.4	14.7	12.4	11.0	35.5	15.7	14.3
Cumulative variance %	38.4	53.1	65.5	76.5	35.5	51.2	65.5

Components with Eigenvalues $\leq$ 1.00 are not included

The coefficients within each component represent the contribution from each of the original variables to that component, and their magnitude and the sign (of each coefficient) determine the contribution of each original variable to the variance of the component.

years of crop in the period 1965-1974. It may identify a farming practice concerning the application of superphosphate in crop years only (for when the FYRC was not included in the original variables, available P dominated the second component but was not linked with any other variable except weakly with total P). Soil analysis for available P showed that most sites in the survey area had less than 30 ppm P at both depths. This amount has been reported French *et al.*, (1975) for medics to be a standard level at which superphosphate addition can be reduced to a maintenance level. In view of this, the lack of association between soil total N and available P is surprising, but other hypotheses may be advanced to account for this result (see Part 5.0).

Surface-soil texture (% clay of the 0-10cm layer) dominated the third principal component which accounted for 12.4% of the variation in the original data. This component indicates that % clay varied considerably in the survey area independently of any other measured variables.

Since the 3 principal components are independent, the second and third components indicate that available P and % clay were almost unrelated to soil total N under medic-dominant pastures. The same results were obtained for the 5-10cm depth interval (Table 3.4). In addition, component II shows a tendency of available P to be correlated with total P, whereas component III indicates an inverse relationship between clay and bulk density. The latter component may describe the transition from red-brown earths to solonized brown soils with better subsoil structure and higher soil pH.

b). Under grass-dominant pastures

For the 0-5cm depth interval, 75% of the variance in the data was associated with the first 3 components (Table 3.5). This is higher than for medic-dominant pastures. The first component indicates that soil total N was not associated with the ASRP but with soil physical and chemical properties and average annual rainfall. This component suggests that total N in soils of poor legume history and in a semi-arid environment is associated with soil structure (more than with soil texture) and is also related to the average annual rainfall. The combination of good structure and high average annual rainfall may suggest better water entry into the soil and perhaps also lower losses of soil N by water erosion and/or denitrification. The latter process has been reported (Stefanson and Greenland 1970) to occur at a higher rate in badly structured soils when soil water content is maintained near field capacity. Greenland (1971) reported that losses of N as nitrous oxide from soils growing wheat can amount to 22 kg ha^{-1} in 3 weeks. The association between soil total N and free lime under grass-dominant pastures may stem from the fact that lime helps to stabilize humic substances in the form of organo-mineral complexes which become less prone to dispersion and subsequent removal and losses (Stefanson 1965, Greenland 1971). Greenland, Lindstrom and Quirk (1962) reported that in the lower north of South Australia, soils containing calcium carbonate have stable aggregates. The high correlation between lime and organic carbon (r ranges from 0.83^{***} to 0.86^{***}) suggests that lime promotes the accumulation of organic carbon which in turn stabilizes soil N. The second component describes the variation in the FYRC and (negatively) of the ASRP which are associated with soil total P and reflects the almost exclusive use of superphosphate in crop years only,

Table 3.5 Principal component analysis of the various measured variables relevant to soil properties, management practices and environmental conditions (Grass-dominant pastures : 35 sites).

	0-5cm layer of soil			5-10cm layer of soil			
Element	Principal component 1	2	3	Principal component 1	2	3	4
Soil total N	.791	.074	-.027	.970	.036	.001	-.022
ASRP	.113	-.737	.484	.141	-.869	-.157	.243
Available P	.047	.600	.680	.344	.254	-.568	.647
pH	.788	-.020	-.445	.563	-.147	.659	.103
Lime (%$CaCO_3$)	.883	.036	-.232	.874	.113	.264	-.041
FYRC	-.134	.882	-.256	-.062	.893	.190	.113
Total P	.848	.195	.252	.885	.089	-.181	.254
Annual rainfall	.776	-.189	-.297	.743	-.137	.239	-.032
Bulk density	-.819	-.019	-.359	-.830	-.040	.226	.209
% Clay	.435	.157	.407	.412	.076	-.494	-.675
Eigenvalue	4.242	1.787	1.461	4.359	1.687	1.271	1.068
% of variance	42.4	17.9	14.6	43.6	16.9	12.7	10.7
Cumulative %	42.4	60.3	74.9	43.6	60.5	73.2	83.8

Components with Eigenvalues $\leq$ 1.00 are not included

The coefficients within each component represent the contribution from each of the original variables to that component, and their magnitude and the sign (of each coefficient) determine the contribution of each original variable to the variance of the component.

where pastures are grass-dominated. The third component which accounted for 14.6% of the variation in the data was dominated by available P corroborating the non-association between soil total N and available P found under medic-dominant pastures.

3.4.7 Variables in the environment associated with soil nitrogen accumulation in absence and presence of free lime in the surface 10cm of soil

The 95 pasture sites fall into 4 major subdivisions:

	Medic-dominant pastures			Grass-dominant pastures		
	No. of sites	Total N (%)		No. of sites	Total N (%)	
		A*	B*		A	B
Sites without lime	23	0.141	0.090	22	0.110	0.072
Sites with lime	37	0.180	0.124	13	0.145	0.137

A* is the 0-5cm depth interval and B is the layer below (5-10cm)

a) In the absence of free lime

Under medic-dominant pastures. The first principal component which accounted for 31.5% of the variation in the data (Table 3.6) shows soil total N positively associated with the ASRP and negatively associated with available P and FYRC and to a lesser extent with total P. This component may identify the top (0-5cm) surface soil of the red-brown earths with a high bulk density and a low pH due to light texture (sandy loam). In this badly structured surface soil which often sets hard, soil total N increases with increasing number of pasture leys in the rotation. The second component which is orthogonal to the first suggests that in the absence of lime, soil total P and soil total N tend to be not correlated. The interdependence of soil total P and % clay in absence of lime may identify the need of a stabilizing agent for the

Table 3.6 Principal component analysis of the various measured variables relevant to soil properties, management practices and environmental conditions (Medic-dominant pastures : no lime).

Element	0-5cm layer of soil: Principal component 1	2	3	5-10cm layer of soil: Principal component 1	2	3	4
Soil total N	.878	.292	.117	-.417	.745	.323	.131
ASRP	.641	-.281	.591	-.747	.045	-.043	.494
Available P	-.564	.364	.622	.892	-.015	.122	.243
pH	.490	-.150	-.273	-.239	-.441	.476	.406
Lime (%$CaCO_3$)	.000	.000	.000	-.000	-.000	.000	.000
FYRC	-.688	.308	-.231	.772	-.088	.381	-.015
Total P	-.376	.739	.189	.636	.539	.074	.344
Annual rainfall	.434	.397	.338	-.246	.319	.681	-.055
Bulk density	-.344	-.789	-.004	-.140	-.641	.459	-.431
% Clay	.412	.603	-.567	-.043	.757	.056	-.507
Eigenvalue	2.831	2.104	1.349	2.667	2.140	1.175	1.050
% of variance	31.5	23.4	15.0	29.6	23.8	13.1	11.7
Cumulative %	31.5	54.8	69.8	29.6	53.4	66.5	78.1

Components with Eigenvalues $\leq$ 1.00 are not included.

The coefficients within each component represent the contribution from each of the original variables to that component, and their magnitude and the sign (of each coefficient) determine the contribution of each original variable to the variance of the component.

The number of sites with no lime in the 0-5cm and 5-10cm depth intervals is 23.

retention of phosphorus in the surface 10cm of soil. The third component describes the association of available P and ASRP and may identify farms in more easterly parts of the survey area where some farmers use medics for seed production and therefore topdress medic pastures regularly.

In the 5-10cm depth interval and in absence of lime soil total N shifts from component I to component II to be associated with soil texture (% clay) and soil structure (negative bulk density). Interpretation of the third and fourth components was more difficult and therefore considered of doubtful value in view of the small amount of variation with which they are associated. The third component represents largely annual rainfall and surprisingly this variable is not strongly associated with anything else. In short, for lime-free medic-dominant pastures, soil total N accumulation in the top surface soil layer was associated with the ASRP whereas in the depth below (5-10cm) the accumulation of soil N was related to % clay and bulk density (negatively).

Under grass-dominant pastures the absence of lime in 0-5cm depth interval revealed a tendency of soil total N to be associated with soil total P (Table 3.7, Component I) whereas the ASRP and soil pH (in component II and III) were dissociated from soil total N variation. The first component which accounted for 37.5% of the variation in the original data indicates that in absence of lime the retention of N compounds in the soil depends upon soil structure and % clay. Many studies in the literature (e.g., Stevenson 1965, Bremner 1965) reported an increase in soil total N with a decrease in particle size (fine clay). The association between soil total N and % clay in absence of lime may reflect the stabilizing effect of clay on soil organic matter. Probably

Table 3.7 Principal component analysis of the various measured variables relevant to soil properties, management practices and environmental conditions (Grass-dominant pastures : no lime).

Element	0-5cm layer of soil: Principal component 1	2	3	5-10cm layer of soil: Principal component 1	2	3
Soil total N	.887	.121	.130	.891	.097	.185
ASRP	.374	.676	-.422	.193	.861	-.135
Available P	.501	-.681	-.166	.382	-.551	-.354
pH	.096	.461	.709	-.288	.554	.687
Lime (% $CaCO_3$)	-.000	-.000	-.000	-.000	-.000	.000
FYRC	-0.380	-.649	.530	-.293	-.701	.541
Total P	.860	-.242	.160	.833	-.065	.227
Annual rainfall	.279	.548	.435	.312	.319	-.173
Bulk density	-.817	.082	.129	-.811	.045	-.065
% Clay	.745	-.253	.143	.804	-.124	.206
Eigenvalue	3.371	1.998	1.259	3.244	1.975	1.071
% variance	37.5	22.2	14.0	36.0	21.9	11.9
Cumulated %	37.5	59.7	73.6	36.0	58.0	69.9

Components with Eigenvalues $\leq$ 1.00 are not included.

The coefficients within each component represent the contribution from each of the original variables to that component, and their magnitude and the sign (of each coefficient) determine the contribution of each original variable to the variance of the component.

The number of sites with no lime in the 0-5cm and 5-10cm depth intervals is 22.

component I reflects a higher N accumulation in better-structured soils with higher clay contents because of the greater rainfall acceptance by these soils and the better water retention in the surface soil. Texture undoubtedly influences the tendency of the red-brown earths to form surface crusts restrictive to seedling emergence and plant growth, and ultimately influences the organic matter entering the soil and the N held in the surface layers of the red-brown earths. The association between soil total N and total P in lime-free soils under grass-dominant pastures could be linked with the association of the covariates % clay and % C with soil total P.

Similar results were obtained for the 5-10cm layer of soil.

b) <u>In the presence of lime</u>

<u>Under medic-dominant pastures</u>. Principal component analysis (Table 3.8) suggests that the results obtained for all medic sites (Table 3.4) were dominated by those of sites containing lime. Indeed, where lime was present under medic-dominant pastures, soil total N at both depths was strongly associated with the ASRP and strongly separated from % clay and available P. Lime was more closely associated with soil total N, presumably through medic growth, nodulation and N_2-fixation, than was soil pH which in fact varied within only a small range at both depths. The lack of association between soil total N and % clay in presence of lime may suggest that lime was the predominant factor related to soil physical conditions whereas in the red-brown earths (lime-free soils) % clay dominated soil condition. The method used for clay determination (no pre-treatment with acid to remove calcium carbonate) can be expected to give a lower recovery of clay in soils containing lime. However, according to Northcote (personal communication) the amount of clay not accounted for in presence of lime should be very low.

Table 3.8 Principal component analysis of the various measured variables relevant to soil properties, management practices and environmental conditions (Medic-dominant pastures : with 0.1% lime, or more).

Element	0-5cm layer of soil Principal component 1	2	3	4	5-10cm layer of soil Principal component 1	2	3	4
Soil total N	.784	-.223	-.050	.014	.620	.110	-.191	.336
ASRP	.787	-.169	-.284	-.137	.744	-.210	-.197	-.397
Available P	.238	-.036	.820	.127	.143	-.182	.818	-.172
pH	.132	.630	-.466	.235	.381	-.405	-.105	.668
Lime (% $CaCO_3$)	.594	.334	.017	.569	.578	-.307	.248	.262
FYRC	-.477	.505	.507	-.085	-.428	.608	.366	.340
Total P	.563	.329	.369	.254	.525	.108	.647	-.187
Annual rainfall	.371	.637	-.080	-.508	.490	.524	.110	.275
Bulk density	-.631	.160	-.285	.502	-.580	-.461	.305	.330
% Clay	-.078	.583	-0.037	-.260	.198	.631	-.075	.032
Eigenvalue	2.747	1.722	1.456	1.063	2.512	1.613	1.480	1.152
% variance	27.5	17.2	14.6	10.6	25.1	16.1	14.8	11.5
Cumulative %	27.5	44.7	59.3	69.9	25.1	41.3	56.1	67.6

Components with Eigenvalues $\leq$ 1.00 are not included.

The coefficients within each component represent the contribution from each of the original variables to that component, and their magnitude and the sign (of each coefficient) determine the contribution of each original variable to the variance of the component.

The number of sites with 0.1% lime or more in the 0-5cm and 5-10cm depth intervals is 37.

Under grass-dominant pastures. Soil total N at both depths was closely associated with the average annual rainfall, lime and total P (Table 3.9). The association of high lime, high total P and high average annual rainfall may be identified with the growth of grasses and consequently with enhanced soil organic carbon. As a result the C/N ratio of soil organic matter would be high, protecting soil total N from rapid mineralization and subsequent denitrification.

The close association between lime and total P under each category of pasture may identify a virgin relationship between these two soil variables, that is, a natural feature of soils containing lime. Diagram 3.1 summarizes the interrelations between soil total N of solonized brown soils and the measured variables and likely mechanisms involved.

3.5 ADVANTAGES AND DISADVANTAGES OF THE SURVEY

The advantages are:

(i) This survey allows assessment and comparison of the levels of soil total N under real farm conditions and farmers' practices.

(ii) It permitted inclusion of many variables relevant to soil properties, management practices and climate which could not be possible in a field experiment.

(iii) It provided relatively rapid data concerning the factors in the environment associated with soil N accumulation under farm conditions.

(iv) The survey permitted identification of certain factors that warrant detailed experimental investigations. Field experiments should follow such surveys in which a preliminary scanning of factors is made and hypotheses generated which can be tested in formal

Table 3.9 Principal component analysis of the various measured variables relevant to soil properties, management practices and environmental conditions (Grass-dominant pastures : with 0.1% lime or more).

Element	0-5cm layer of soil			5-10cm layer of soil		
	Principal component			Principal component		
	1	2	3	1	2	3
Soil total N	.669	.630	.083	.947	.111	.086
ASRP	.330	-.776	-.300	.523	-.648	.451
Available P	.184	-.214	.879	.651	.302	.391
pH	.597	.380	-.265	.665	-.122	-.245
Lime (% $CaCO_3$)	.893	-.079	-.047	.839	.130	-.144
FYRC	-.218	.379	.854	-.117	.946	.053
Total P	.763	-.338	.440	.865	.238	.177
Annual rainfall	.899	.043	-.193	.751	-.286	-.435
Bulk density	-.393	.784	-.227	-.725	-.084	-.405
% Clay	-.681	-.482	-.024	-.545	-.125	.745
Eigenvalue	3.802	2.304	1.954	4.889	1.612	1.392
% variance	38.0	23.0	19.5	48.9	16.1	13.9
Cumulative %	38.0	61.1	80.6	48.9	65.0	78.9

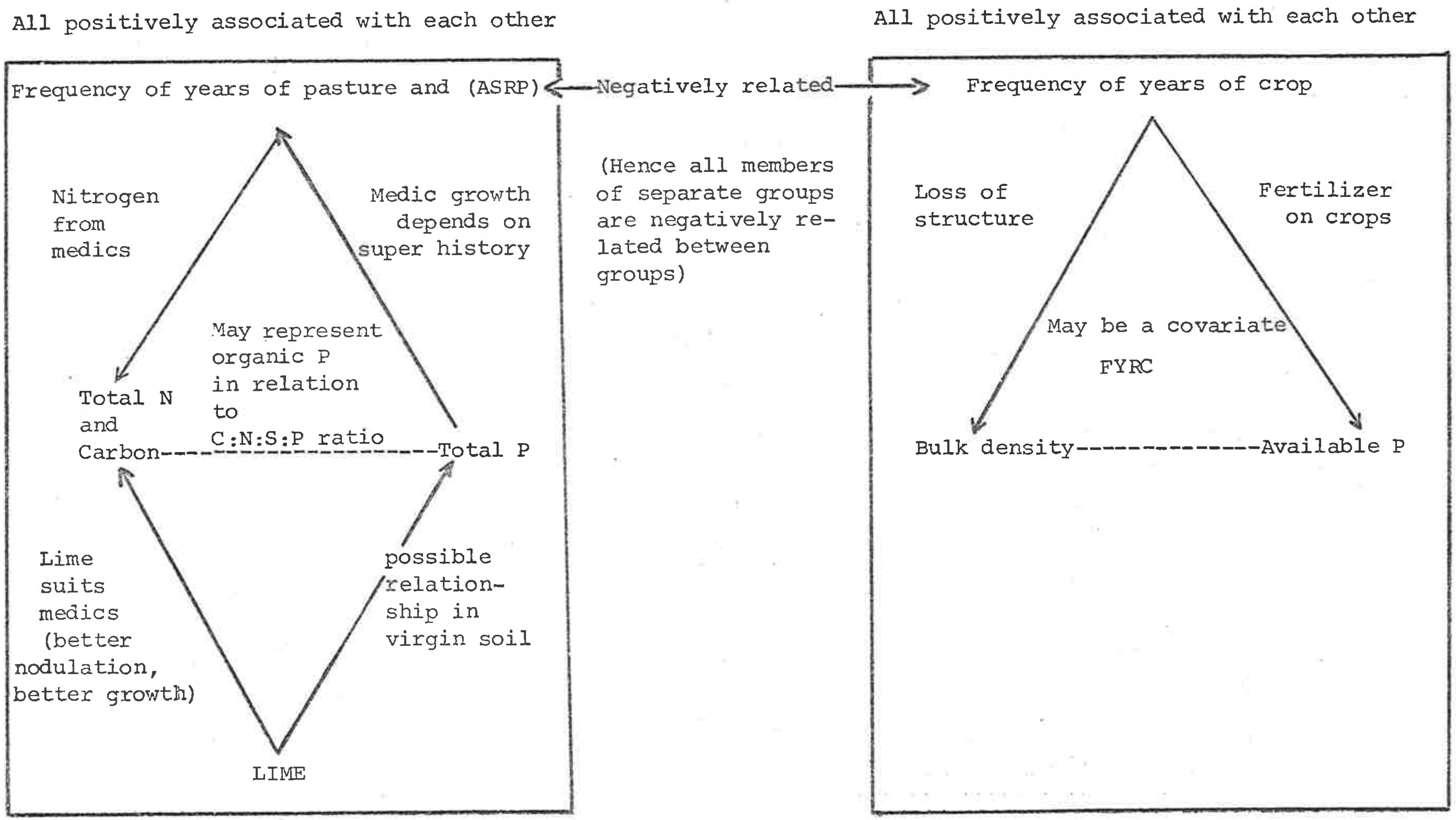
Components with Eigenvalues $\leq$ 1.00 are not included.

The coefficients within each component represent the contribution from each of the original variables to that component, and their magnitude and the sign (of each coefficient) determine the contribution of each original variable to the variance of the component.

The number of sites with 0.1% lime or more in the 0-5cm and 5-10cm depth intervals is 13.

Diagram 3.1 Summary of the interrelationships between soil total N and soil, management and climatic factors measured and likely mechanisms involved.

FOR CALCAREOUS SOILS



experiment. The higher build-up of soil N under medic-dominant than under grass-dominant pastures suggests further investigation of the possible relationship between N inputs and medic density. Part 4.0 of the present study looks at the variation in N_2-fixation by barrel medic induced by various medic sowing densities under field conditions. In part 5.0 the lack of association between soil total N and available P is investigated using a glasshouse experiment.

The disadvantages are:

(i) The survey describes the association between the measured variables but does not provide proof of the causative system underlying these associations.

(ii) The survey could mislead if important variables are not chosen. This survey showed that the botanical composition of the pasture, the cropping rotational system, seasonal rainfall and soil properties were important factors to consider in relation to soil N accumulation. Surveys such as this emphasize the need to include in the study as many variables as possible.

(iii) The disadvantages specific to this survey consisted of the unknown soil N background prior to 1965 and the need for good farm records.

(iv) Reification of the survey results may be difficult.

3.6 SUMMARY

The field survey covered a wide range of variation in soil total N and other measured variables. Simple regression analysis of soil total N on the number of years of medic pastures indicated that soil total N was associated with the FYRP.

Calculation of the amounts of N fixed in one season by a medic pasture showed large differences between seasons and ranged in the 0-10cm depth interval from 80 kg ha^{-1} N per year in a dry season (124 mm seasonal rainfall) to 300 kg ha^{-1} N per year in a very good season (353 mm seasonal rainfall), with an overall average of 200 kg ha^{-1} N per year. These estimates of N increment under medic-dominant pastures derived from a study of farmers' fields under semi-arid conditions are the first of their kind reported in Australian literature.

Solonized brown soils have higher mean soil total N levels than do the red-brown earths, but linear regressions of soil total N (0-5cm depth interval) on ASRP suggest that, under medic-dominant pastures, the rate of N accumulation is higher in the red-brown earths. Under grass-dominant pastures, there was a low average annual increment of soil N (approximately 20 kg ha^{-1} N) in the surface 5cm of the red-brown earths.

Ordination by principal component analysis showed that, 45 to 79% of the variation in total N in the soil in the survey area was associated positively with the ASRP, lime, total P and negatively with bulk density in the solonized brown soils, whereas in the red-brown earths % clay was also important. In both soil types total N varied independently from soil available P which was strongly associated with the number of years of crop. This is consistent with the results of the cropping history survey which revealed that superphosphate is generally applied to cereal crops only.

Soil factors were generally more closely associated with soil total N under grass- than under medic-dominant pastures.

3.7 CONCLUSION

The soil fertility survey provided estimates of the annual increments of soil total N under medics and different rotations as practiced under real farm conditions. A major problem in determining the trend of variation of soil total N with time was the unknown soil total N in 1965. However, the magnitude of the average annual increments in soil N under medic-dominant pastures denotes a rapid accumulation of soil N and suggests that soils in the study area are not approaching a steady-state condition. Ordination of the data by principal component analysis indicated how soil N accumulation varied in the survey area, and identified many aspects of the data that might be the subject of more intensive study. The results suggest that further surveys are needed to fill the gaps in our knowledge concerning the long-term trend of soil N accumulation under medics in relation to the current level of soil total N (1974) and to pasture management, namely grazing intensity or stocking rate, superphosphate application, and possible changes in the botanical composition of pasture.

4.0 THE FIELD EXPERIMENT

4.0 Effect of plant age and sowing density on acetylene reduction activity of *Medicago truncatula* at two different dates of sowing

4.1 INTRODUCTION

The field survey (1974) showed that high N status of soil was associated with a high proportion of medics in the pasture phase in 1974. Although the survey did not include detailed estimation of medic population at sampling, the hypothesis generated from the survey results for soil total N is that the higher the medic population per unit area the higher the inputs of N into the soils. So far no studies have been made with medics of the variation in N_2-fixation associated with various sowing densities. Most studies on N_2-fixation by legume-based pastures have concerned the estimation of annual increment of soil N under different growth conditions and management practices. There are a few papers on the rate of N_2-fixation by self-regenerating legume pastures throughout a life cycle of the plant (Brock 1973, Sinclair 1974, Halliday and Pate 1976), but these have involved clovers in pure stands or mixed with grasses. So far no such studies have been made with medics. Since in South Australia medics are used in ley farming systems for improving soil N and providing green fodder to grazing animals, more successful use of medic pastures for both purposes can result from knowing:

- Firstly, the effect of plant density on the rate of N_2-fixation.
- Secondly, the nature of variation in the rate of N_2-fixation by medics during the growing season.

A field experiment was conducted to test the hypothesis that N_2-fixation rate in barrel medic is a function of plant density per unit area, and to examine the variation in N_2-fixation at several stages in the life cycle of the plant. Nitrogen fixation was estimated by the acetylene reduction assay.

4.2 METHODS AND MATERIALS

Design and treatments

A randomized block design experiment with four replications was set out at the Waite Agricultural Research Institute. Each replicate consisted of 16 plots. The treatments were:

(a) Sowing densities: Seed was sown at 8 different densities, both greater and less than those normally found in practice under field conditions, (viz: 0, 1, 5, 20, 50, 100, 500, 1000 kg ha^{-1} seed).

(b) Sowing dates: April 4 and May 20, 1975.

Legume species: *Medicago truncatula* cv. Jemalong was used in both early and late sowing date.

Jemalong is referred to simply as medic.

Establishment of the experiment

The soil of the experimental area was Urrbrae loam classified as a red-brown earth (Dr 2.23 Northcote 1971) which is acid in the surface horizon (pH 5.5 - 6.0) and alkaline at depth. The amount of total N measured in the 0-10cm depth interval of soil of the different replications was as follows:

Replication No.	Soil total N (%)
I	0.12
II	0.13
III	0.12
IV	0.11

A basal application of 227 kg ha^{-1} of superphosphate (i.e., 50 kg ha^{-1} P) was made at sowing time.

Medic seed was broadcast on a 4.5m x 2m plot which was allocated at random to a treatment within each replication. All seed was inoculated with the commercial *Rhizobium meliloti* (Strain U45) and mixed with the standard rate of superphosphate before broadcasting. To facilitate the distribution of seed and superphosphate over the whole plot area, a slightly humid sand was added to the mixture before sowing.

The experiment was run jointly with Adem (1977) who studied the effects of seed rates and sowing date on medic growth and yield. Measurements of shoot dry matter and plant population were made by Adem and have been used for the interpretation of data obtained from AR assays.

Measurements

Plants were harvested and N-ase activity was measured several times during the season at approximately 28 day intervals (Table 4.1). For the early sowing treatments, 7 samplings were made throughout the growing period whereas only 6 samplings were done on the late sown plants at the same time interval (Table 4.1). It should be noted that after the second date, both early and late sowings were sampled during the same day. On each of these days harvesting and soil sampling started at 11.30 and were finished at 15.30 h.

At each sampling, the number of plants in each plot was counted and all herbage from the same plot was cut manually at ground level. Immediately after the medic had been harvested, 5 soil cores (5cm in diameter and 10cm in depth) were taken from each plot and N-ase activity of root nodule bacteria was measured by AR assay following the method described later in section 4.3. The interval between removal of plant tops and introduction of soil cores into the assay incubation jar was about 5 minutes.

Soil temperatures were recorded twice, 4 h apart on each sampling date at 2 different depths (2.5cm and 15cm) (Table 4.2).

Table 4.1 Dates on which plant dry matter and acetylene reduction measurements were made in the field experiment.

Date (1975)	Days from sowing Early sowing	 Late sowing
29-5	55	-
25-6	82	35
23-7	110	63
21-8	139	92
18-9	167	120
15-10	194	147
12-11	222	175

Table 4.2 Soil temperature and rainfall recorded at/or between sampling periods (1975).

Sampling date	Time h	Soil temperature (°C) at depth 2.5 cm	15 cm	Rainfall (mm) from previous sampling
29-5	11.30	24	15	110 (from 1 May)
	15.30	22	18	
25-6	11.30	18	11	12
	15.30	21	14	
23-7	11.30	18	12	98
	15.30	19	14	
21-8	11.30	18	13	73
	15.30	17	14	
18-9	11.30	23	16	45
	15.30	18	18	
15-10	11.30	21	17	85
	15.30	24	18	
12-11	11.30	34	20	70
	15.30	40	26	

4.3 PRELIMINARY INVESTIGATION WITH THE ACETYLENE REDUCTION ASSAY

4.3.1 Incubation technique assessed in the field

Soils were incubated in 2.2 litre glass jars having a rubber-lined screw-type lid of which a small section was removed exposing the rubber seal. Each jar was labelled with an appropriate number corresponding to the different plots and treatments. About 0.1 atmosphere of acetylene was used for the incubation of soil cores in presence of air following this procedure. Once the soil cores (5) were in place in the incubation jar, a wide-mouthed glass vial containing 0.8g of calcium carbide was introduced into the jar which was immediately sealed. After removing 10% of the volume of air present in the jar by means of syringe (plate 4.1), 10 ml of de-ionized water were added by another syringe to the vial containing the calcium carbide (CaC_2). The volume of acetylene evolved from the reaction of CaC_2 and water was equivalent to the volume of air removed previously. From then the time was recorded and the jar put into an insulated box and carried to the laboratory where ambient temperature was about 20^{o}C.

4.3.2 Length of incubation period

As we were interested in the rate of N_2-fixation operating in the plant at time of harvest, we assayed several incubation periods using different swards of medic grown on Urrbrae loam. Figure 4.1a indicates that as the time of incubation increased, the rate of AR decreased. Measurements of the ethylene (C_2H_4) produced at several times during a 24 h period revealed that after 4 h the rate of AR approached zero. During the first 40 minutes of incubation the rate was constant (Figure 4.1b). Acordingly a 30 minute incubation period was used in the main study.

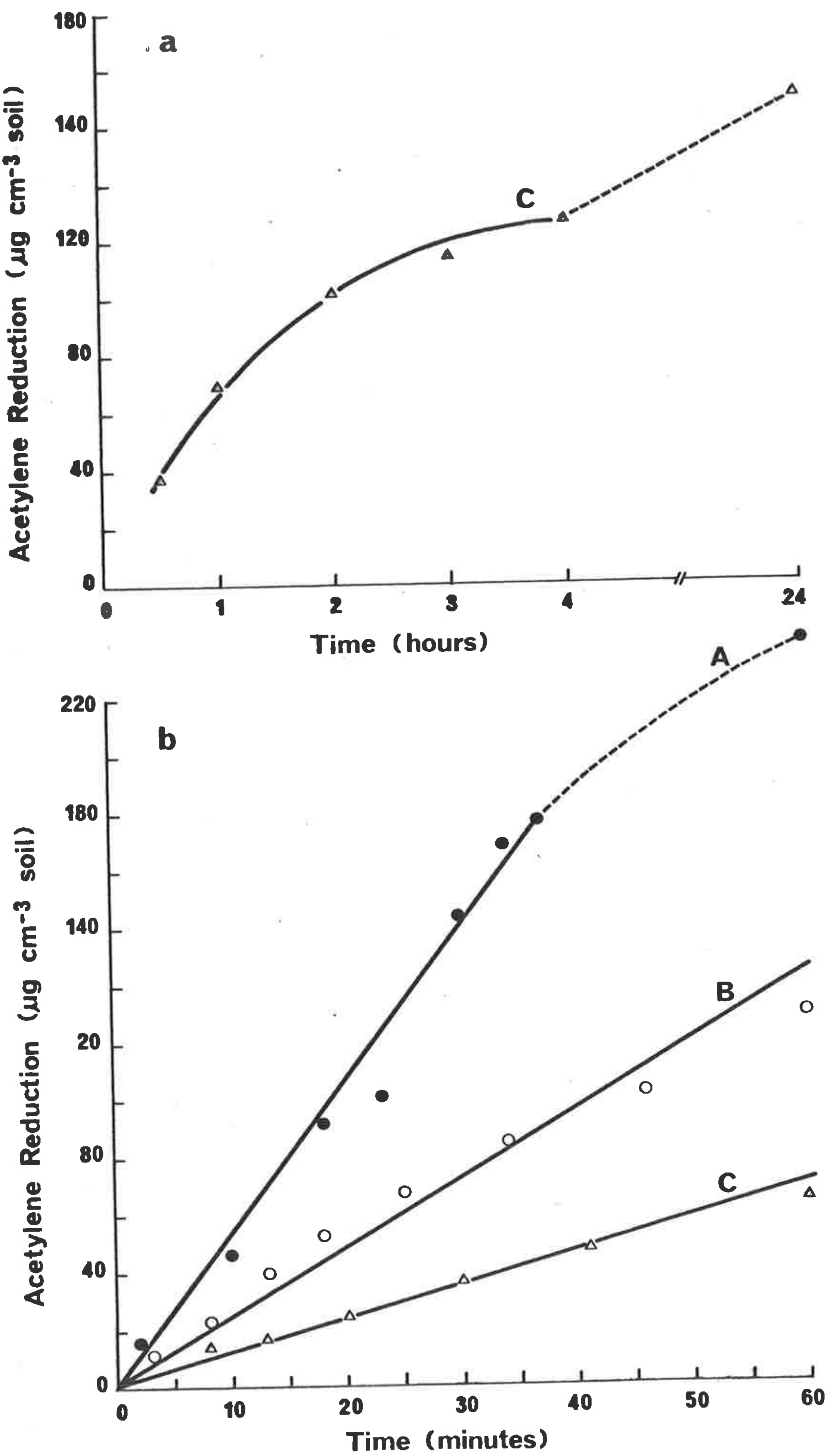
Plate 4.1: Incubation of soil cores in presence of acetylene generated from the reaction of water and calcium carbide. Operations were carried out in the field with a minimum of disturbance of soil cores prior to incubation.

Figure 4.1 Acetylene reduction in soil cores from the field experiment on Urrbrae loam as a function of time of incubation.

(a) Acetylene reduction recorded over long time intervals

(b) Acetylene reduction recorded over short time intervals

A,B,C represent various medic densities from the same field experiment.


a
Acetylene Reduction (µg cm-3 soil)
0
40
80
120
140
180
C
0
1
2
3
4
24
Time (hours)
b
Acetylene Reduction (µg cm-3 soil)
0
40
80
20
140
180
220
A
B
C
0
10
20
30
40
50
60
Time (minutes)

4.3.3 Acetylene reduction measurements

At the end of the incubation period, 0.5 ml of gas sample was withdrawn from the jar with a new syringe and needle and introduced into a Varian Aerograph Series 940 gas chromatograph fitted with a flame ionization detector and a Porapak R column. The C_2H_4 in the sample was determined on the basis of recorder peak heights in comparison to C_2H_4 standards, and the C_2H_4 in the incubation chamber calculated from the volumetric relationships involved.

4.4. RESULTS

4.4.1 Variation in acetylene reduction activity with sowing density

The results for the early sowing date indicated that there were 3 different aspects of variation of AR activity of soil cores with sowing densities (Figure 4.2, Appendix II.1).

- During the vegetative period of plant growth (i.e., 8, 12 and 16 weeks from sowing) AR activity increased with increasing medic sowing densities.
- At the pod setting and podfilling stages (i.e., 20 and 24 weeks AR activity tended not to vary according to the sowing density but showed peculiar variations at podfilling stage.
- Towards maturity (i.e., 28 and 32 weeks), AR activity was slightly higher at the low densities than at the medium or high ones. For the first 3 harvests, analysis of variance (Appendix II.1) showed a significant effect of sowing density on AR activity of soil cores but for the subsequent 4 harvests the effect of sowing density on AR activity of soil cores did not reach the significance level of P = 0.05.

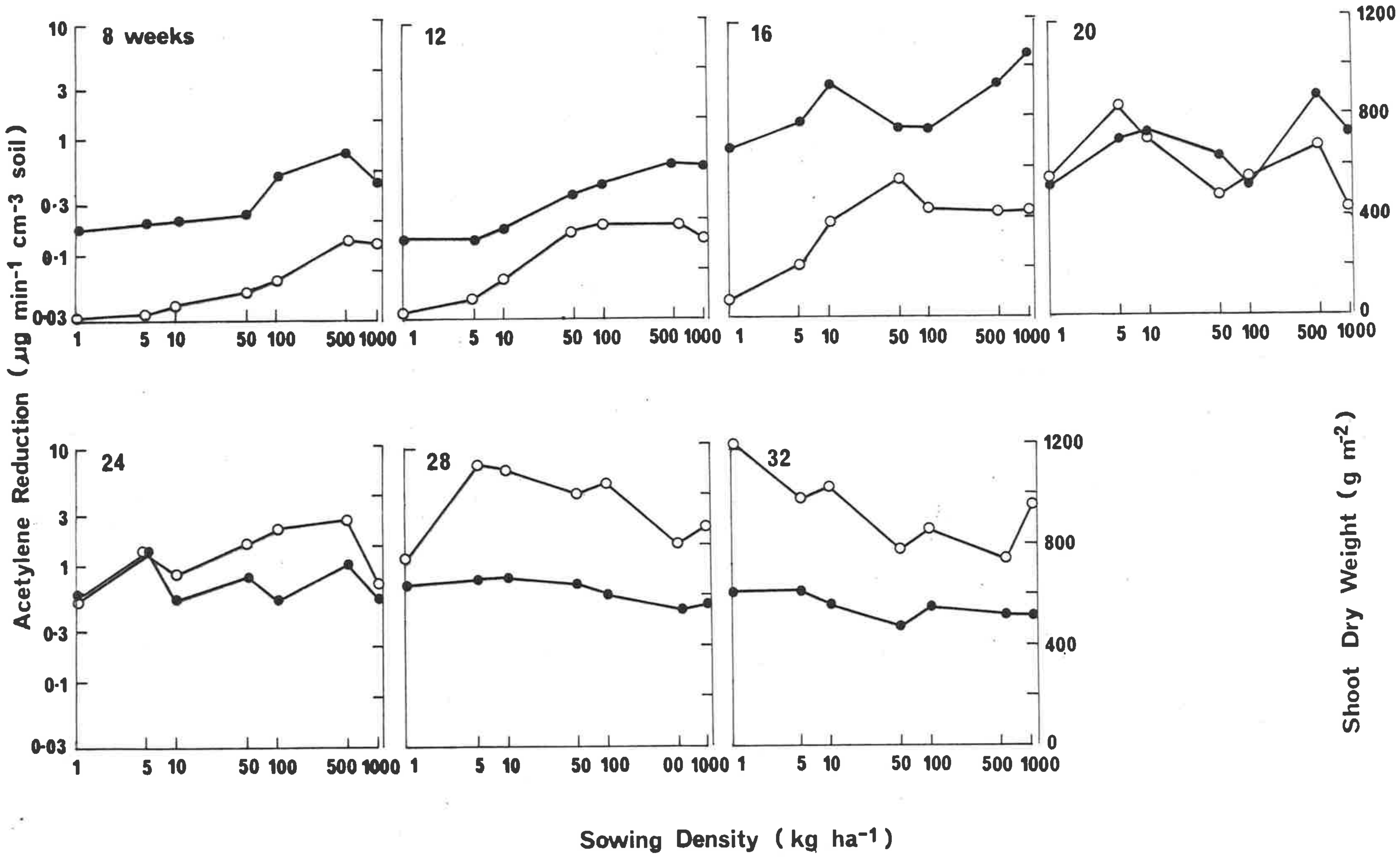
Over all sampling times, there was a significant effect of sowing density on AR activity of medic nodule bacteria (Appendix II.1).

Figure 4.2 Variation in acetylene reduction activity in soil cores at the various medic sowing densities (early sowing date)

● - Acetylene reduction (μg min^{-1} cm^{-3} soil)

O - Shoot dry weight (g m^{-2})

8, 12, 16, 20, 24, 28, 32 - represent weeks from sowing.


8 weeks
12
16
20
24
28
32
10
3
1
0·3
0·1
0·03
1200
800
400
0
1
5
10
50
100
500
1000
Acetylene Reduction (µg min-1 cm-3 soil)
Shoot Dry Weight (g m-2)
Sowing Density (kg ha-1)

For the late sowing date, over the first 5 harvests mean rates of AR activity increased steadily from low to high densities with the exception of a marked depression at harvest 3, the result, probably, of a temporary water stress (Figure 4.3). Except for the first and second harvest significant effects of sowing density on AR activity of soil cores were shown by analysis of variance for each harvest (Appendix II.2). The main effect of sowing densities across all times was highly significant.

The variation in AR activity with sowing density was almost parallel to the variation in shoot dry matter production by the various medic sowing densities (Figure 4.2). AR activity of soil cores was better correlated with the weight of dry matter present at the time of harvest during the vegetative period of plant growth than after flowering (Table 4.3a). AR activity slowed down when growth had ceased at medium and high densities and became greater at the low densities where swards were still growing (Figure 4.2). The same association between AR activity and shoot dry matter per unit area was observed in the late sown plants (Figure 4.3, Table 4.3b).

4.4.2 Variation in acetylene reduction activity with plant age

For the early sowing date and for all sowing densities there were 2 contrasting patterns in AR activity as the plants developed:

(i) During the vegetative phase of development, AR activity of soil cores at each sowing density increased with time from sowing to reach a maximum rate of AR activity at flowering, that is, at 16 weeks from sowing (Figure 4.4).

(ii) During the post-flowering phase there was a decline in AR activity at all sowing densities, but the decline was sharper at the high

Figure 4.3 Variation in acetylene reduction activity in soil cores at the various medic sowing densities (late sowing date)

● - Acetylene reduction (μg min^{-1} cm^{-3} soil)

0 - Shoot dry weight (g m^{-2})

5, 9, 13, 17, 21, 25 - represent weeks from sowing.

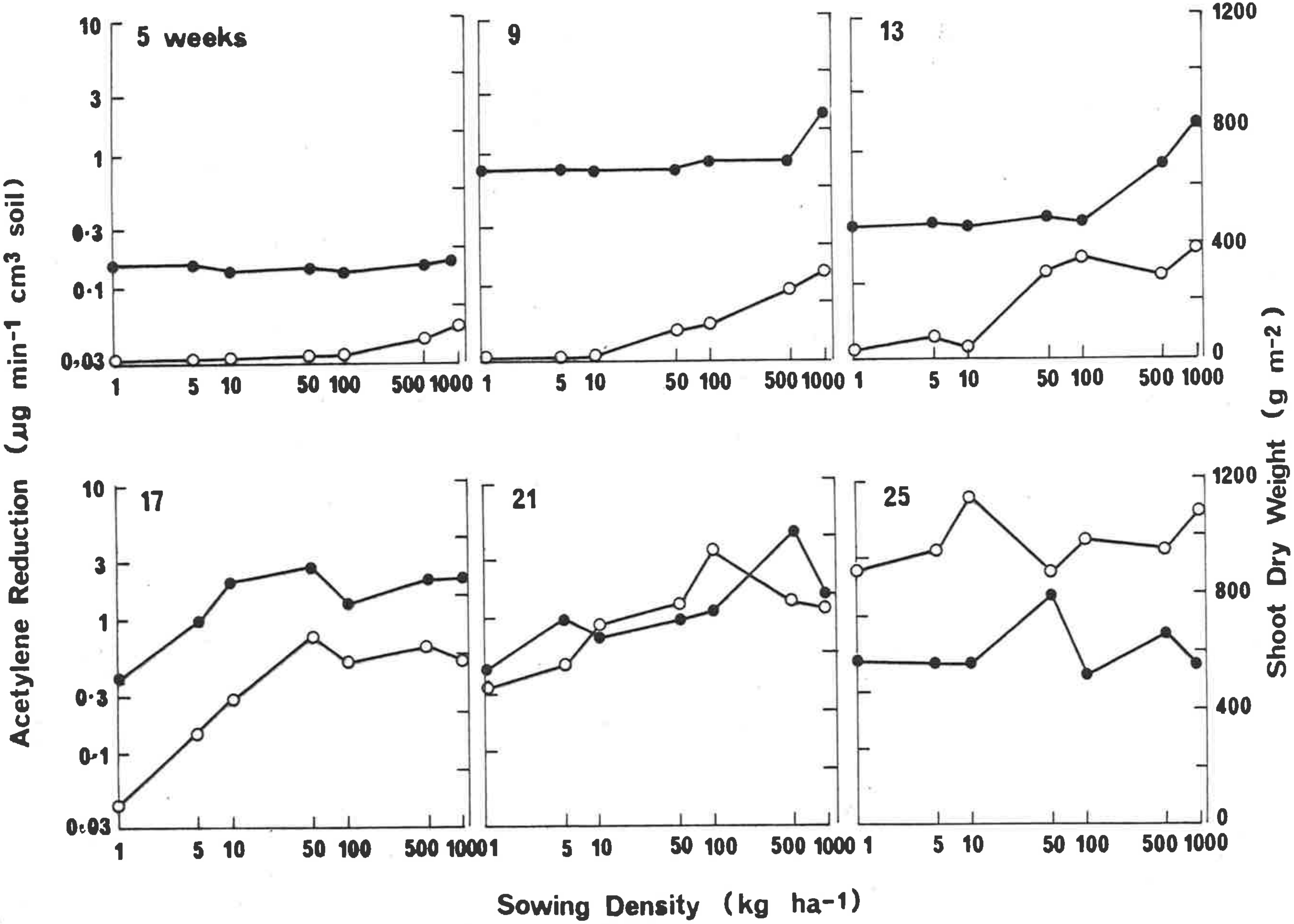

5 weeks
9
13
17
21
25
Acetylene Reduction (µg min-1 cm3 soil)
Shoot Dry Weight (g m-2)
Sowing Density (kg ha-1)

Table 4.3 . Coefficients of correlation between acetylene reduction rates of soil cores and plant growth parameters.

(a) Early sowing date

Age of plants in weeks	Shoot dry weight	Plant population	Relative growth rate
8	.64**	.75***	-.56**
12	.87***	.80***	-.23 NS
16	.46*	.58*	-.23 NS
20	.62**	.53*	-.02 NS
24	.47*	.31 NS	-.09 NS
28	.47*	.21 NS	.31 NS
32	.50*	.18 NS	.21 NS

(b) Late sowing date

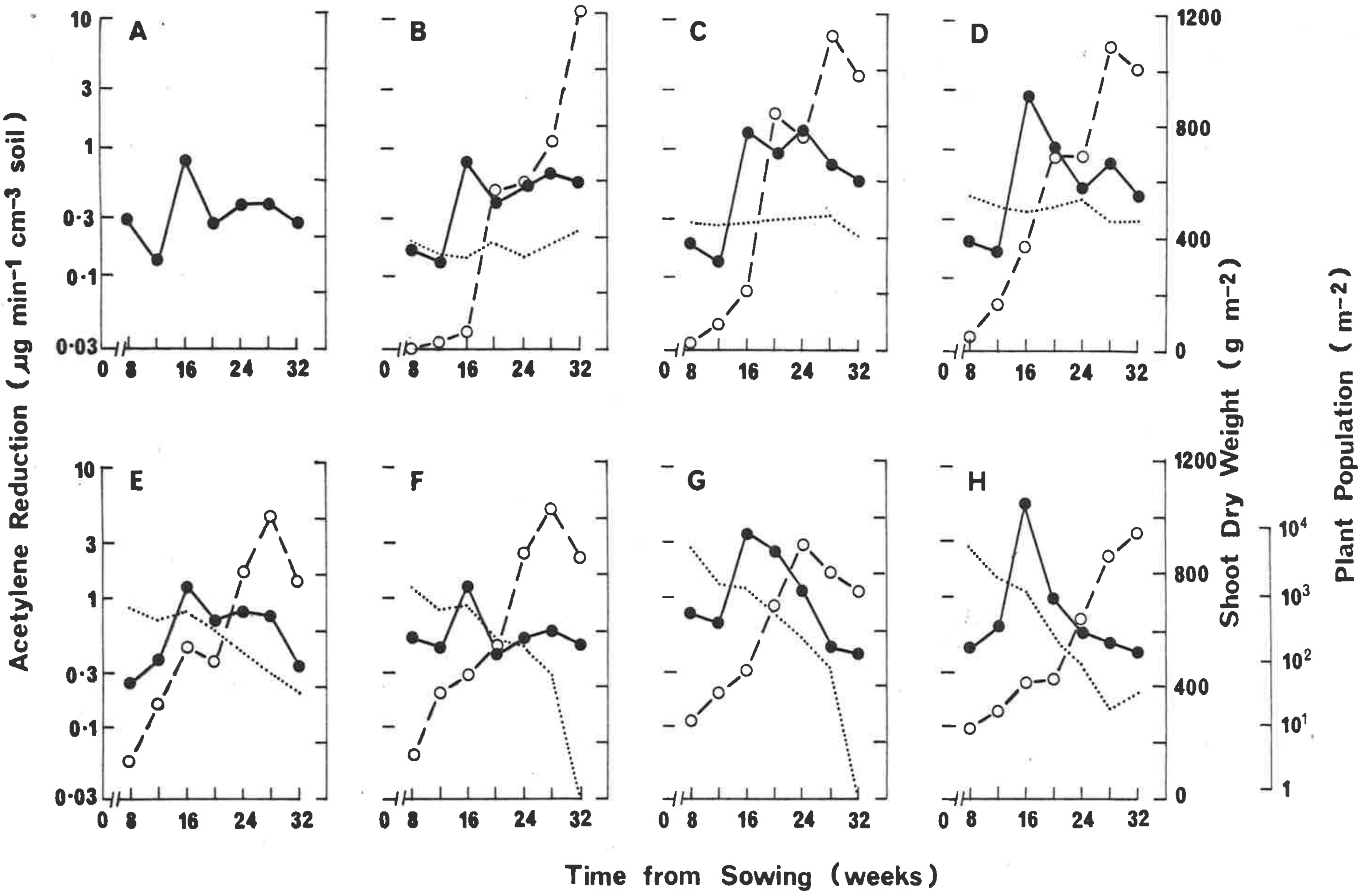
5	.42*	.25 NS	-
9	.65***	.58**	-.19 NS
13	.60**	.68*	-.56**
17	.74***	.59**	.11 NS
21	.50*	.46*	-.48 NS
25	.14 NS	.47*	-.16 NS

Level of significance:
* significant at P = 0.05
** significant at P = 0.01
*** significant at P = 0.001
NS non significant at P = 0.05

Figure 4.4 Dependence of acetylene reduction activity in soil cores on plant age.
(early sowing date)

●	Acetylene reduction ($\mu g\ min^{-1}\ cm^{-3}$ soil)					
O	Shoot dry matter ($g\ m^{-2}$)					
.....	Plant numbers (m^{-2})					
A	is	sowing	density	0	$kg\ ha^{-1}$	
B	"	"	"	1	"	
C	"	"	"	5	"	
D	"	"	"	10	"	
E	"	"	"	50	"	
F	"	"	"	100	"	
G	"	"	"	500	"	
H	"	"	"	1000	"	

0, 8, 16, 24, 32 - represent weeks from sowing.


A
B
C
D
E
F
G
H
Acetylene Reduction (µg min-1 cm-3 soil)
Shoot Dry Weight (g m-2)
Plant Population (m-2)
Time from Sowing (weeks)

densities than at the lower ones. The low densities (1, 5 and 10 kg ha^{-1}) had higher AR activity at the end than at the beginning of the growing season whereas the opposite was true of the high sowing densities.

The effect of plant age on the relationship between AR activity and sowing density is summarized in Figure 4.5 where the slopes of simple regressions of mean rates of AR activity on sowing densities for each harvest were plotted versus time. This figure emphasizes that AR activity of soil cores increased in relation to sowing densities from sowing to flowering. The relationship then decreased steeply to almost zero and it then reversed as the plants matured.

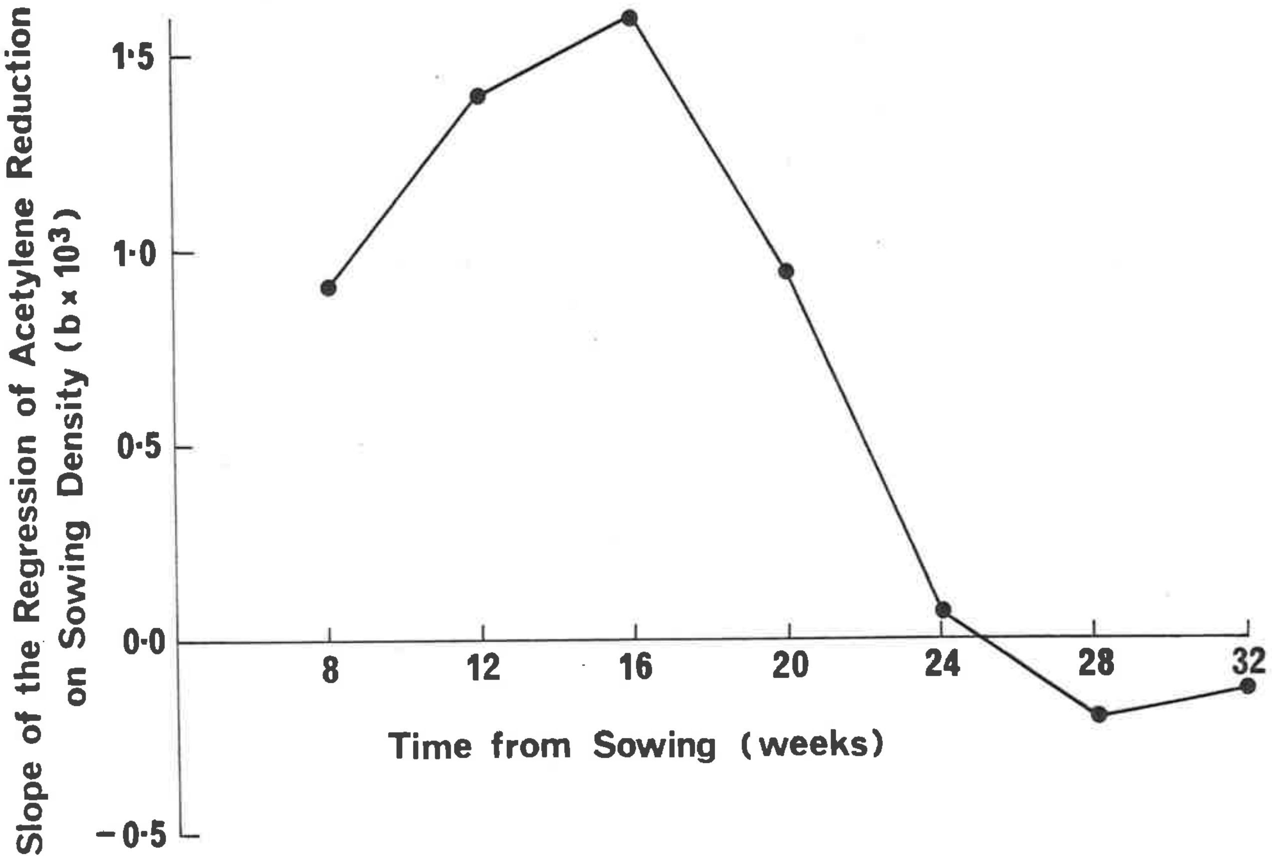
For the late sowing date the same pattern of variation of AR activity with plant age was observed (Figure 4.6). At full flowering (17 weeks from sowing) a sharp increase in AR activity of soil cores occurred at all sowing densities. At 21 weeks from sowing (podfilling stage) the mean rates of AR activity declined again with the exception of sowing density G (500 kg ha^{-1}) where one of the replicates had a much higher AR activity (47 μg min^{-1} cm^{-3} soil) than the other replicates.

Irrespective of sowing date, the post-flowering decline in AR activity coincided with a sharp decrease in plant population mainly at the medium and high sowing densities (Figures 4.4 and 4.6). In contrast there was only a small variation in plant population at the low densities.

The relations between AR activity, shoot dry weight and plant number per unit area in the pre-flowering and post-flowering periods were examined using simple regression analysis. For the early sowing date, the regression of AR activity on shoot dry weight for the pre-flowering period is described by the equation:

Figure 4.5 Slope of the regression of mean acetylene reduction activity on sowing density as a function of plant age (early sowing date).

Weeks for sowing	R^2 values
8	38 NS
12	59^{*}
16	68^{**}
20	24 NS
24	0.3 NS
28	6 NS
32	4 NS



Slope of the Regression of Acetylene Reduction on Sowing Density (b × 10³)
1·5
1·0
0·5
0·0
−0·5
8
12
16
20
24
28
32
Time from Sowing (weeks)

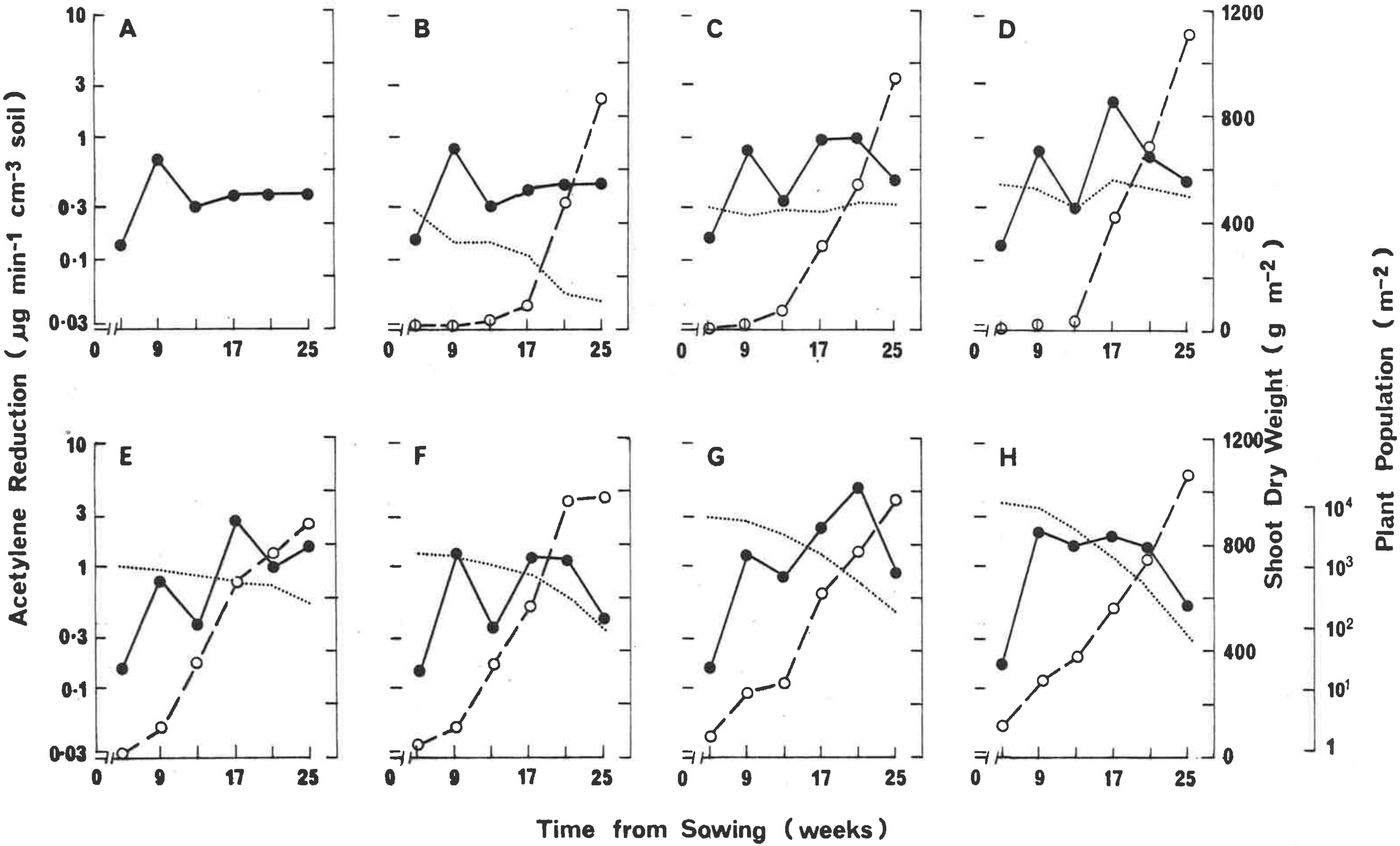
Figure 4.6 Dependence of acetylene reduction activity in soil cores on plant age (late sowing date).

● Acetylene reduction (μg min^{-1} cm^{-3} soil)

O Shoot dry matter (g m^{-2})

..... Plant population (m^{-2})

A	is sowing density	0	kg ha^{-1}
B	"	1	"
C	"	5	"
D	"	10	"
E	"	50	"
F	"	100	"
G	"	500	"
H	"	1000	"


A
B
C
D
E
F
G
H
10
3
1
0·3
0·1
0·03
0
9
17
25
1200
800
400
0
10^4
10^3
10^2
10^1
1
Acetylene Reduction (µg min-1 cm-3 soil)
Shoot Dry Weight (g m^{-2})
Plant Population (m^{-2})
Time from Sowing (weeks)

AR activity rate = -1.24 + 0.041 shoot dry weight (R^2 = 54%***)

AR activity rate = ($\mu g\ min^{-1}\ cm^{-3}$ soil)

shoot dry weight = ($g\ m^{-2}$)

For the post-flowering period the regression was not significant at $P = 0.05$ (R^2 = 0.11%). On the other hand, the association between AR activity and plant population per unit area was not significant for the pre-flowering period (R^2 = 2%), but was significant for the post-flowering period although the coefficient of determination was still low:

AR activity rate = -0.49 + 0.18 Plant population (R^2 = 30%***)

AR activity rate = ($\mu g\ min^{-1}\ cm^{-3}$ soil)

plant population = (m^{-2}).

The R^2 is low because one density (F = 100 kg ha^{-1}) seems anomalous.

Similar results were obtained for the late sown plants with the same R^2 (53%***) for the relation between AR activity and shoot dry weight at the vegetative period of plant growth (up to 17 weeks from sowing), and with a better R^2 (52%***) for the relation between AR activity and plant population at the post-flowering period.

4.5. DISCUSSION

The preliminary investigation showed that the rate of AR activity of soil cores was linear with time for incubation up to 30 min. for medics (cv. Jemalong) as for other species (Hardy *et al.*, 1968; Dalton and Naylor, 1975), after which presumably roots run out of their store of carbohydrates. Incubation of soil cores can give rise to errors stemming from the spatial variability of legume pasture growth (Sinclair, Hannagan and Risk 1976) and from soil sampling depth which should be adjusted to reach the zone of maximum nodule depth.

Although the present study deals merely with the instantaneous rate of AR rather than with nett seasonal inputs of nitrogen into the soil, the average rates of the activity by soil cores over the whole season and at all sowing densities (Appendix II. 3a,b) tended to support the hypothesis advanced, namely that higher inputs of N occur under high medic densities than under low medic densities. However, there was a superimposed effect of age of plants on the rate of AR activity by the various sowing densities which led towards the end of the season to a change in the relationship between sowing densities and the rates of AR activity of soil sores. But rates of AR activity of soil cores declined at all sowing densities after flowering, so that over all times, medium and high densities had higher average rates of AR activity than low densities. These responses must be viewed against a relatively high background AR activity due presumably to free-living microorganisms (zero medic density).

The increase in the rates of AR activity by soil cores at all sowing densities during the vegetative period of plant development (i.e., from sowing to flowering) probably reflects the increase in nodule number per unit area at each density and the increase in the supply of carbohydrates from developing shoots. The slight decrease in AR activity observed under all densities at 12 weeks from (early) sowing probably was due to water stress as a result of a dry spell between sampling 1 and sampling 2 (Table 4.2). This decrease in AR activity would seem to support the results of the soil fertility survey which indicated a lower soil N accumulation in a dry season.

The higher AR activity at the medium and high sowing densities observed during the vegetative period of plant growth is associated with increased leaf area index and probable increases in number of nodules per unit area at these densities. At the early stage of plant growth during which the rate of photosynthesis increased linearly with the leaf area index up to a ceiling value (7 for subterranean clover according to Silsbury and Fukai, 1977) the increased rate of AR activity can probably be explained by a greater supply of carbohydrates to the associated bacteria at the higher leaf area index at the medium and high sowing densities.

In the present study, the sharp decrease in AR activity recorded at 20 weeks from sowing in the early sown plots was perhaps accentuated by wet soil conditions and overcast weather at the time of sampling. This is supported by the same decrease observed under the control (0 kg ha^{-1} sowing density) and by a similar decrease in AR activity of soil cores from the late sown plots which had not reached flowering at that date (13 weeks from sowing). However, AR activity of the late sown plots increased again at the next sampling date whereas AR activity of the early sown plots continued to decrease confirming the general decline in AR activity which started after flowering.

The post-flowering decline in AR activity observed at all sowing densities of medic agrees with that reported for pea *(Pisum sativum)* (La Rue and Kurz, 1973) and for soybean (Lawn and Brun, 1974) although for soybean the decline started a little later (at the early podfilling stage). This post-flowering decline in AR activity of certain legumes has been attributed to several factors. Bond (1936) suggested that the efficiency of N_2-fixation (i.e., the ratio of N_2 fixed to the dry weight of nodules) decreased with age because of the supply of carbohydrates per bacterium decreased or the number of inactive cells increased. Pate (1958a,b) observed that plant flowering (*Pisum arvense* L. and *Vicia sativa* L.) occasioned heavy losses in nodule number and total nodule weight and promoted emptying of the nodules. Lawn and Brun (1974), using treatments designed to alter the photosynthate supply to the nodules concluded that there was a limitation to symbiotic N_2-fixation by competition for photosynthates from developing pods of soybeans. For medics, the post-flowering decline in AR activity of soil cores at all sowing densities was found to be slightly correlated with a decrease in plant population per unit area. The continual increase in shoot dry matter beyond flowering was dominantly due to filling of medic pods which competed with root nodules for the available carbohydrates.

Interpretation of the results of the experiment is clarified if AR activity is expressed on a per plant basis. At each harvest, AR activity per plant was higher where plant population was lower (Figures 4.7; 4.8). This negative association was found to be highly significant throughout the growing season (Tables 4.4a,b). During the vegetative period of plant growth and at both early and late sowing dates, AR activity per plant at all densities increased steadily, whereas beyond flowering the rate of AR per plant tended to decrease or remain constant depending on the sowing density. The reduction in plant population per unit area at each harvest tended to be associated with an increase in plant relative growth rate. Thus, the results suggest that during the pre-flowering period the decrease in plant population resulted in better growth of individual plants owing to less competition for light, water and nutrients, and led to a higher supply of carbohydrates to nodule bacteria which increased their rate of AR. After flowering, the decrease in plant population was not supported by a high supply of carbohydrates to the nodules because of the formation of new non-photosynthetic tissues which competed for available carbohydrates. A high demand for N at the pod-filling stage was reported for soybeans

Figure 4.7 Variation in acetylene reduction activity on a per plant basis with sowing density (early sowing date).

● Acetylene reduction per plant (ng min^{-1} $plant^{-1}$)

Δ Plant population (m^{-2})

.... Relative growth rate (g g^{-1} day^{-1})

8, 12, 16, 20, 24, 28, 32 - represent weeks from sowing.

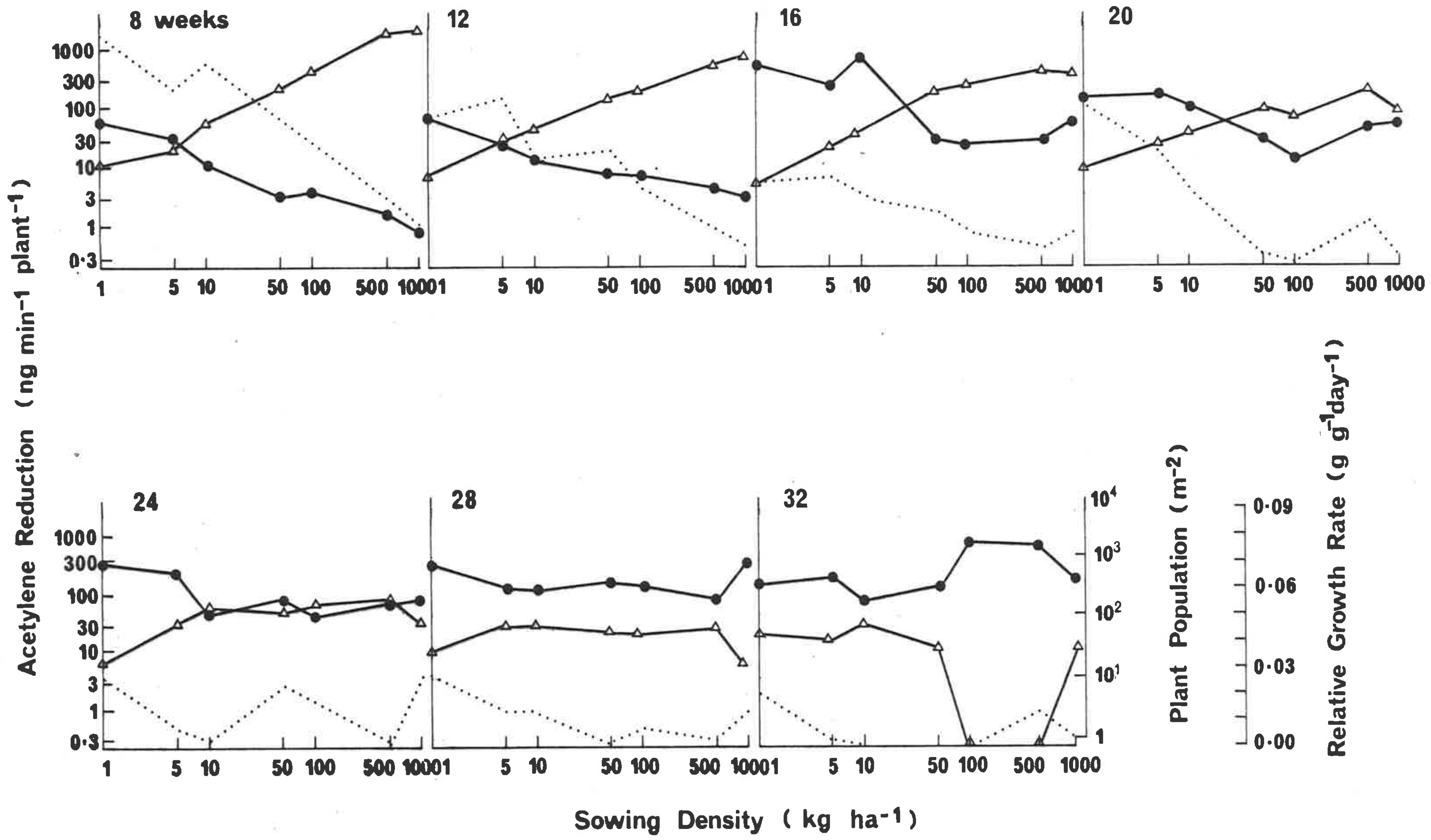

Acetylene Reduction (ng min-1 plant-1)
8 weeks
12
16
20
24
28
32
Sowing Density (kg ha-1)
Plant Population (m-2)
Relative Growth Rate (g g-1 day-1)
0·00
0·03
0·06
0·09

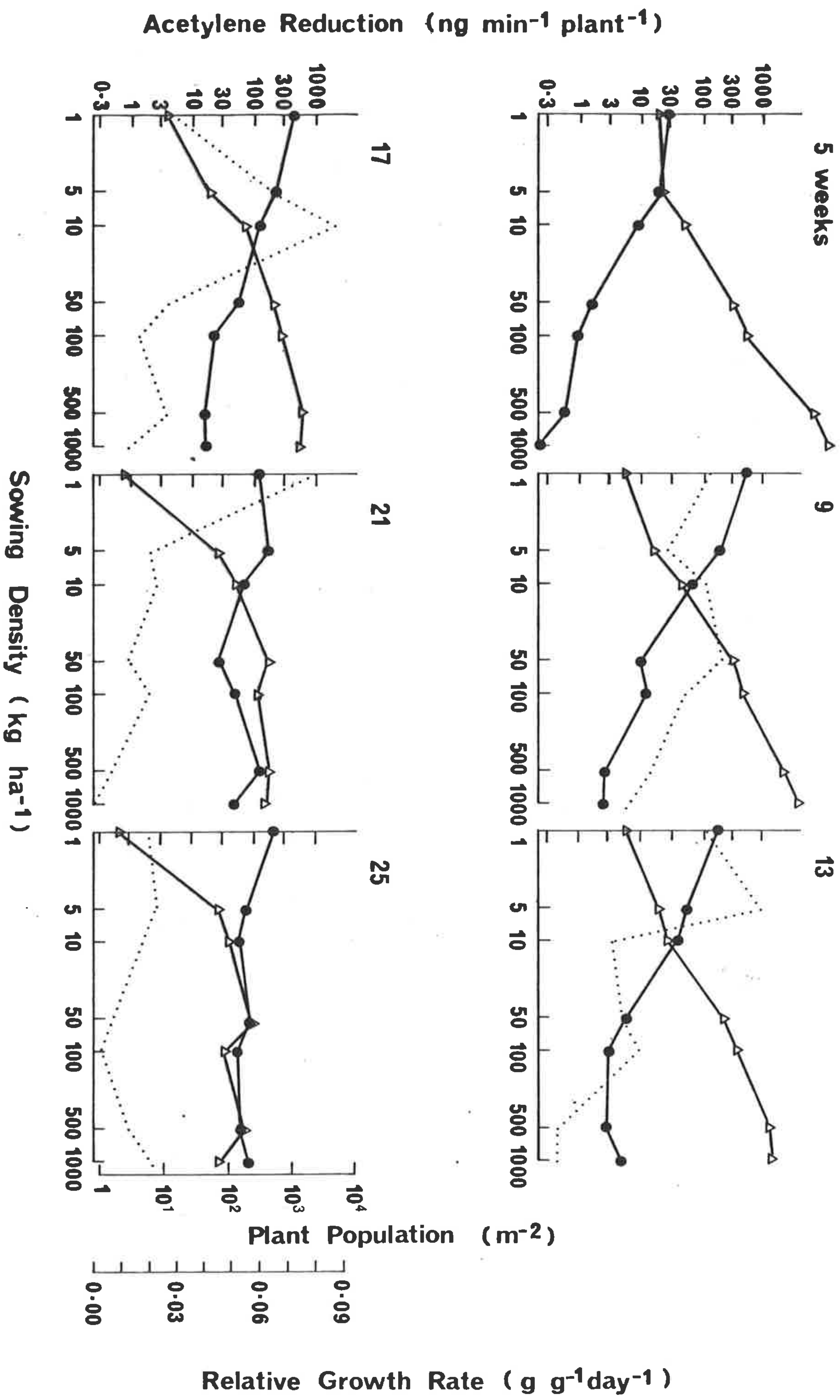
Figure 4.8 Variation in acetylene reduction activity on a per plant basis with sowing density (late sowing date).

● Acetylene reduction per plant (ng min^{-1} $plant^{-1}$)

Δ Plant population (m^{-2})

.... Relative growth rate (g g^{-1} day^{-1})

5, 9, 13, 17, 21, 25 - represent weeks from sowing.



Acetylene Reduction (ng min-1 plant-1)
1000
300
100
30
10
3
1
0·3
5 weeks
9
13
17
21
25
1
5
10
50
100
500
1000
Sowing Density (kg ha-1)
1
10¹
10²
10³
10⁴
Plant Population (m-2)
0·00
0·03
0·06
0·09
Relative Growth Rate (g g-1 day-1)

Table 4.4 Coefficients of correlations between acetylene reduction rate per plant (ng min^{-1} $plant^{-1}$) and plant population, and plant relative growth rate at different harvests.

(a) Early sowing date

Age of plants in weeks	Plant population	Relative growth rate
8	-.96***	.45*
12	-.89***	.32 NS
16	-.90***	.40 NS
20	-.58*	.60**
24	-.62**	-.10 NS
28	-.89***	.39 NS
32	-.76***	.01 NS

(b) Late sowing date

5	-.99***	-
9	-.98***	.14 NS
13	-.94***	.45*
17	-.85***	.31 NS
21	-.42 NS	.12 NS
25	-.60**	.03 NS

Level of significance
* significant at P = 0.05
** " " P = 0.01
*** " " P = 0.01
NS non significant at P 0.05

by Latimore, Giddens and Ashley (1977), but this did not stimulate N_2-fixation; on the contrary, they observed a decline in N_2-fixation at the pod-filling stage.

In the present experiment, medics were not grazed or defoliated. Light grazing of medic pasture at the early stages of plant growth can be expected to extend the period of vegetative growth by maintaining the rate of appearance of young leaves for longer periods (Donald 1963) and, presumably, it will enhance the inputs of nitrogen by medics to the soil.

4.6 CONCLUSION

The AR assay provides only an indirect estimate of nitrogen fixation over short intervals of time. These constraints limit the use of the technique to comparisons of specific treatments rather than to the determination of integrated seasonal accretions of nitrogen.

In the present study the effects of density, age and time of sowing were evaluated. The results support the hypothesis of higher AR activity under higher medic sowing density (i.e., very good medic pasture). AR activity increased with both plant age and sowing density during the vegetative phase. Data of this type for medics have not previously been reported. The implications for farming practice are: the higher the sowing density the higher the N inputs. However, economics would probably dictate that an intermediate sowing density e.g., 10 kg ha^{-1} or more should be used, which is very much on the high side of current farming practice.

Further investigations are needed to elucidate:

1) The effect of grazing pressure on the rate on N_2-fixation by swards of medics.

2) The optimum leaf area index for maximum rate of N_2-fixation. The variation in N_2-fixation with leaf area index is meaningful in relation to stocking rate and soil N improvement in the ley farming system. Heavy grazing at the period of optimum N_2-fixation may reduce the contribution to soil N of annual legume pastures.

3) Causes of the decline in the rate of AR activity after flowering, e.g., competition for carbohydrates, senescence or other hormonal effects.

4) The calibration ratio of the C_2H_2 reduced to N_2 fixed for medics. Quantification of the amounts of N fixed by medics at several times during the season and in different seasons is needed.

5.0 THE GLASSHOUSE EXPERIMENT

5.0 Effect of phosphate supply and competition from grasses on growth and nitrogen fixation by *Medicago truncatula* (cv.Jemalong)

5.1 INTRODUCTION

The nature of the dependence of soil total N on the amount of superphosphate applied has given rise to some controversy since studies under different environmental conditions have produced different results. Donald and Williams (1954) found that soil total N under permanent subterranean clover pastures increased linearly with the amounts of superphosphate applied over a period of 20 years. Watson (1969) reported that the effect of applied superphosphate on soil total N under subterranean clover pastures was slow and not significant during the first 4 years of a long-term field experiment (11 years). In a long-term study in South Australia at Kybybolite, Russell (1960) found that soil N accumulation was not dependent on the amount of superphosphate applied. In New Zealand, Brock (1973) found that applied superphosphate had no effect on soil N accumulation under white clover pastures. More recently, Kohn, Osborne, Batton, Smith and Lill (1977) reported that, in a field experiment at Wagga Wagga (New South Wales), nitrogen accumulation in red earth soil (Gn 2-12, Northcote 1971) increased irrespective of superphosphate level and that the rate of increase was reduced on pastures topdressed with superphosphate.

The results of the soil fertility survey (Part 3.0) revealed likewise that soil P extracted by 0.5M $NaHCO_3$ and soil total N over 95 farm sites were not correlated. This is surprising in view of the recognized demand of medics for P (Rudd 1972, French and Rudd 1967, French *et al.*, 1975). Furthermore, the range of available P in the surveyed sites varied from deficient for medics to sufficient (i.e., from 6 ppm to 89 ppm P in the 0-5cm depth interval) but still there was no correlation between soil total N and available P. Five possible hypothesis not mutually exclusive for the lack of association of soil total N and available P in this particular survey are:

I. Soils in the survey area were above the P requirement for medics because of farmers satisfying the higher superphosphate requirements of cereals (in cropping years). Presumably, medic growth was not affected by available P levels in these soils and therefore soil total N varied irrespective of soil available P levels.

Conceivably, cereals could have higher P requirements than medics, for superphosphate has been the major fertilizer continuously applied to cereals for more than 50 years and farmers still use much superphosphate for cereals (100-120 kg ha^{-1} per year) but generally do not topdress medic pastures. Admitedly, reports (French and Rudd 1967, Matz 1973) indicate that medics have a higher P requirement than cereals.

II. Medics do respond in yield to P over the range of levels in the survey area but not the N_2-fixation process itself. So far no studies have been made with medics concerning the effect of superphosphate application on N_2-fixation by medic root nodules.

III. N_2-fixation may increase with increasing P supply but the increased N is all translocated to the shoots and removed by grazing or cutting leaving the N remaining in the soil (roots) relatively independent of P supply.

IV. N_2-fixation may respond to P supply both in shoots and roots but also, subsequent cereal growth and yield may respond likewise to P supply with consequent higher N removal, the two effects tending to cancel out.

Data reported by Clarke and Russell (1977) for N and P removals in wheat grain in South Australia showed that exports of N and P increased together. These exports (in kg ha^{-1}) being approximately in the ratio of 10N to 1P exported.

V. Although P may enhance N_2-fixation, it also increases grass competition, the two effects on N_2-fixation tending to cancel out.

The increase in soil total N under legume-based pastures and the regular application of phosphate fertilizer have been found to enhance the development and proportion of associated grasses (Trumble and Donald 1938, Anderson and McLachlan 1951, Watson 1969, Kohn 1975). Higher P requirements of grasses compared to legumes have been reported (Rossiter 1966, Valentine and Barley 1975). The first named author found that brome grass and capeweed (*Cryptostemma calendula*) were the dominant species under high P supply where subterranean clover had been previously introduced, and Valentine and Barley (1976) pointed out that at 14-18°C brome grass showed a significant response to additional P whereas subterranean clover (cv. Mt.Barker) did not.

The present experiment aimed to test some of these hypotheses namely:

(i) P may increase medic growth but it may not increase the rate of N_2-fixation (hypothesis II);

(ii) P may enhance N_2-fixation but because of a feedback relationship between N_2-fixation and shoot growth most of the N fixed is translocated to the shoots and removed. The net effect of P on soil N (or root N) is nil (hypothesis III);

(iii) P may enhance N_2-fixation but it also increases grass competition, the two effects tending to cancel out (hypothesis V).

5.2 METHODS AND MATERIALS

5.2.1 Design and treatments

A factorial experiment with a complete randomized block design included 3 replications of 8 phosphate treatments applied to 3 plant communities.

Plant communities:

- Pure medic (*Medicago truncatula*, cv. Jemalong), 6 plants per pot.
- Pure Wimmera ryegrass (*Lolium rigidum* Gaud., cv. Wimmera) 6 plants per pot.
- Mixture of medic and ryegrass, 3 plants of each species per pot.

Jemalong will be referred to as medic and wimmera ryegrass as ryegrass.

Phosphate application rates:

- Calcium tetrahydrogen di-orthophosphate ($Ca(H_2PO_4)_2.H_2O$) was used to give the following rates (ppm P added to the soil):

  0, 10, 25, 63, 160, 400, 1000, 2500.

5.2.2 Cultural technique

The experiment was conducted in a glasshouse under natural illumination and at temperatures varying between a mean monthly minimum of 12-14^{o}C and a mean monthly maximum of 20-30^{o}C. A red-brown earth from the Mallala district was used. The soil characteristics in the 0-10cm depth interval were:

Total N (%)	Organic Carbon (%)	Mineral N (ppm)	Available P (ppm)	Total P (ppm)	pH	$CaCO_3$ (%)	Mineralizable N (ppm)
0.14	2.1	8.0	8.0	176	7.4	0.0	17

The field capacity of the soil was estimated to be 21% by the method of Bouyoucos cited by Leeper (1948).

Pots of 12cm diameter and 1 litre capacity were filled with 1kg of soil. The required amount of $Ca(H_2PO_4)_2.H_2O$ in solid form together with a basal application (250mg per pot) of $CaSO_4.2H_2O$ in solid form were thoroughly mixed into each 1kg of soil prior to potting. The basal application of $CaSO_4.2H_2O$ was used to mask the effect of increasing Ca when using $Ca(H_2PO_4)_2.H_2O$ for P treatments. Each pot received 15 mls of basal nutrient solution containing the following:

$MgSO_4.7H_2O$, 6.67g;	$MnSO_4.4H_2O$, 0.67g;	$ZnSO_4.7H_2O$, 0.40g;
$CuSO_4.5H_2O$, 0.27g;	H_3BO_3, 0.27g;	$CoSO_4.7H_2O$, 0.03g;
H_2MoO_4, 0.03g;	$FeSO_4.7H_2O$, 0.67g;	K_2SO_4, 11.14g;

in two litres of water.

Medic seeds of uniform size were inoculated with *Rhizobium meliloti* (strain U.45) prior to sowing. Medic seedlings were grown in sand culture in a glasshouse and when 10 days old selected seedlings were transplanted into the pots of soil. Ryegrass seeds (12 per pot) were sown directly in pots of soil 2 weeks before medic transplantation took place. The seedlings were thinned to six per pot for the monocultures, and three of each species per pot for the mixed culture. Pots were watered to field capacity and restored to the same water content (21%) by regular weighing followed by the addition of the required amount of water. The experiment lasted from July 11 to September 20 (i.e. 81 days).

5.2.3 Measurements

Nitrogen fixation AR assay was used to estimate the rate of N_2-fixation in each pot. Pots were brought to field capacity 24 h before AR measurements in order to eliminate the effects of variable soil water content on the measured rate. Plants in soil were incubated in the presence of 0.1 atmosphere C_2H_2 (in air) generated *in situ* by the reaction of CaC_2 with de-ionized water. Incubation chambers consisted of glass jars of 5 litres capacity (Plate 5.1). The gas sampling procedure and measurement of C_2H_4 were the same as described previously (Part 4.0). Measurements were made of the amount of C_2H_4 produced after 20, 40 and 60 minutes incubation. For each pot, the amounts of C_2H_4 produced were plotted against incubation time and the eye-fitted line to each set of 3 points was used to give the rate of AR per pot per hour.

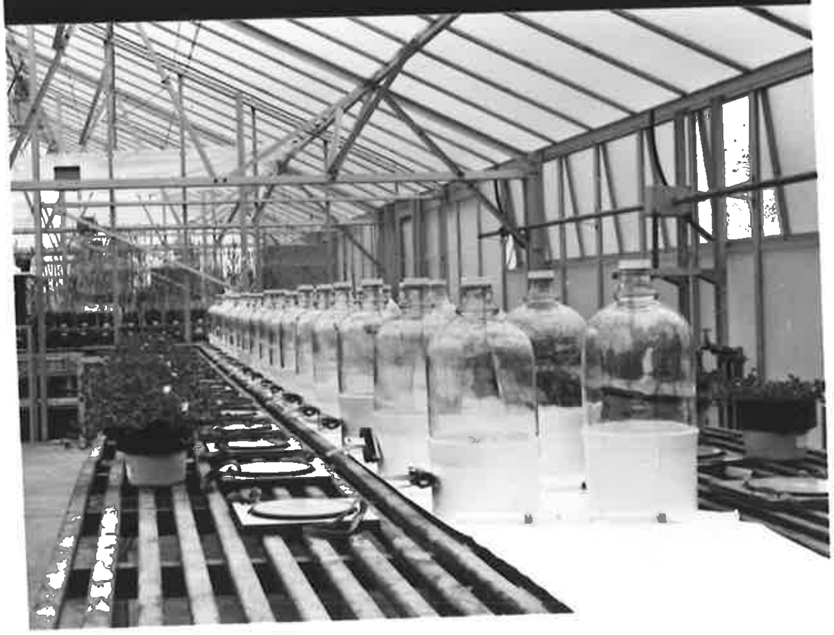
Dry matter production The yields of shoots and roots of each species grown in monoculture and in mixture were obtained after oven-drying the fresh plant material at 65°C to constant weight. The dry weight of roots of single species and species mixture was corrected for the weight of soil particles adhering to the roots. This was determined by combustion at 550°C of subsamples and treatment of the ash with dilute HCl (Piper 1950) to dissolve salts, leaving soil particles as an insoluble residue.

Total N of medic and ryegrass plants Total N of the shoots and roots of plants was determined by Kjeldahl method using 0.250g of oven-dried plant material. Corrections for the weight of soil particles adhering to the root material were made (Piper 1950). Total N in plant parts does not include nitrate and will be referred to as nitrogen content of shoots or roots.

Plate 5.1 Technique used for measuring acetylene reduction of plants in soil.

Above: Glass jar used for the incubation of intact plant in soil (pot). Small glass vials ($15cm^{-3}$ capacity) identical to that in the picture were used for gas sampling after 20, 40 and 60 minutes of incubation.

Below: Set up of the incubation jars and pots prior to incubation of intact plants in the glasshouse.



5.3 RESULTS

5.3.1 Growth response to applied phosphate

Yield of shoots and roots of medic Shoot yield increased with rate of added P to a maximum at 160 ppm. P and declined again beyond this rate (Figure 5.1A). In the absence of phosphate fertilizer medic plants were stunted and some of them died while at the two highest levels of fertilizer application, P toxicity appeared 20 days (2500 pppm P) and 30 days (1000 ppm P) after transplantation. At the end of the experiment, symptoms of toxicity were starting to show at the 400 ppm P application rate. The growth response of medics to applied P is illustrated in Plate 5.2.

In the species mixture, the response of medic to phosphate fertilizer was similar to that found in medic monoculture (Figure 5.1A) with a maximum at 160 ppm P and similar symptoms of P deficiency and toxicity at the extreme rates of supply. However, the yield of shoots per plant of medic was slightly lower than that obtained in monoculture.

Analysis of variance (Table 5.1a) showed highly significant effects of added P and plant community on the yield of medic shoots per pot and per plant. The interaction phosphate x community was significant on a pot basis but not on a per plant basis.

The yield of roots of medic in monoculture varied from 0.06 to 0.32g per plant. Maximum root production was obtained at P application rates of 63 and 160 ppm P while plants which received 0, 10 or 2500 pppm P produced the lowest yields (Figure 5.1B). Roots showed more tolerance of the extremes of P availability than did the shoots. The yield of roots of medic in mixture was not determined because the roots of the 2 species were so closely interlaced.

Plate 5.2 Response of species in monoculture and in mixture to applied phosphate. Plants six weeks old.

The numbers from 1 to 8 correspond to the following P rates:

1	=	0 ppm P
2	=	10 "
3	=	25 "
4	=	63 "
5	=	160 "
6	=	400 "
7	=	1000 "
8	=	2500 "

Top picture: medic in monoculture
Middle picture: ryegrass in monoculture
Bottom picture: medic + ryegrass mixture


5 6 7 8
1 2 3 4


5 6 7 8
1 2 3 4


5 6 7 8
1 2 3 4

Figure 5.1

A - Shoot yields (g $plant^{-1}$) of:

- ● Mp = pure medic
- o Mm = medic in mixture with ryegrass
- ■ Gp = pure ryegrass
- □ Gm = ryegrass in mixture with medic

B - Root yields (g $plant^{-1}$) of:

- ● Mp = pure medic
- ■ Gp = pure ryegrass
- x G+M = ryegrass + medic

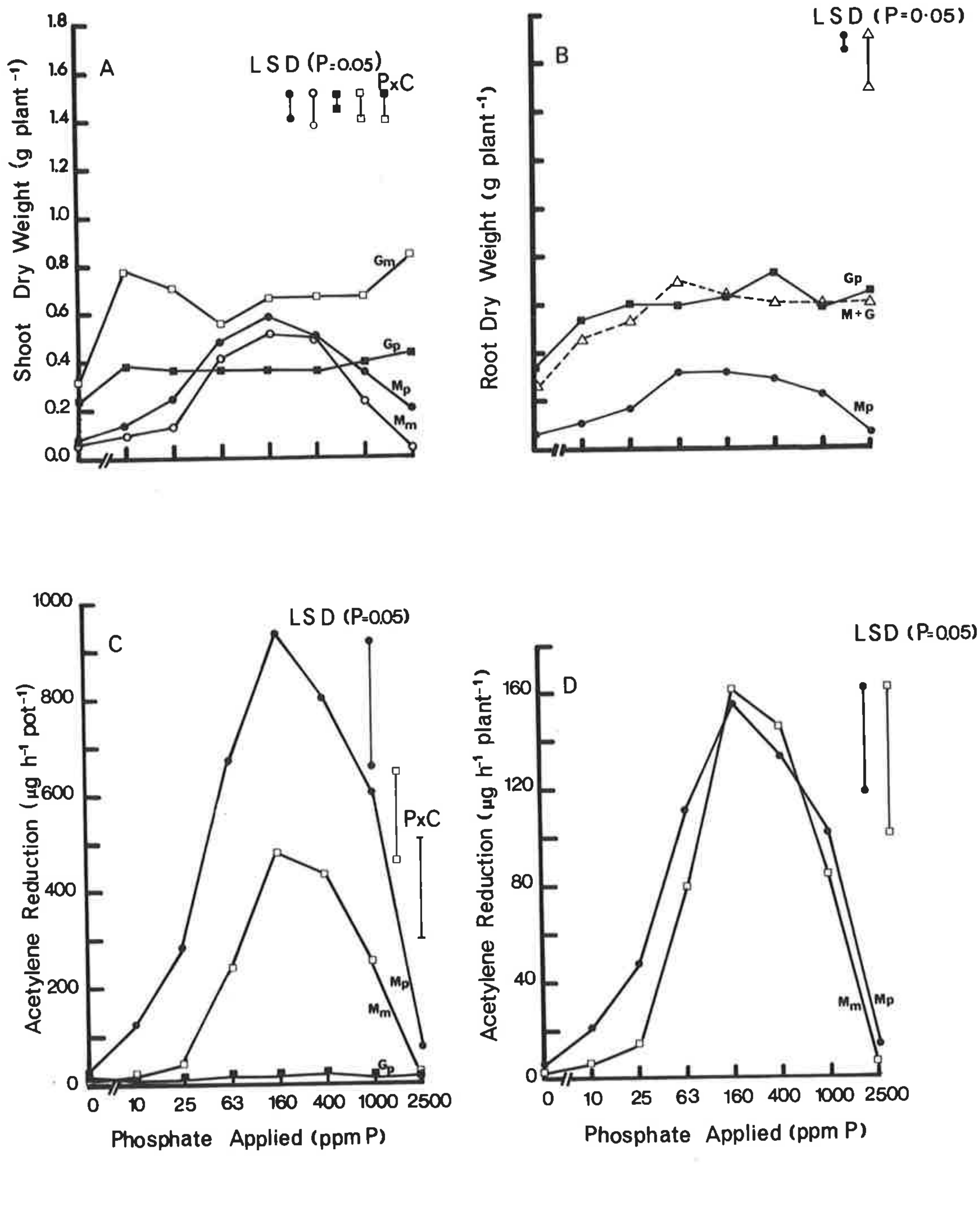
C - Acetylene reduction rate ($\mu g\ h^{-1}\ pot^{-1}$):

- ● Mp = pure medic
- □ Mm = medic in mixture
- ■ Gp = pure ryegrass

D - Acetylene reduction rate ($\mu g\ h^{-1}\ plant^{-1}$):

- ● Mp = pure medic
- □ Mm = medic in mixture

The LSD's refer to the main effects of phosphate and phosphate x community interactions.



A
B
C
D
Shoot Dry Weight (g plant -1)
Root Dry Weight (g plant -1)
Acetylene Reduction (µg h-1 pot-1)
Acetylene Reduction (µg h-1 plant-1)
LSD (P=0.05)
LSD (P=0·05)
PxC
Gm
Gp
Mp
Mm
M+G
Phosphate Applied (ppm P)
0
10
25
63
160
400
1000
2500

Yield of shoots and roots of ryegrass In monoculture the yield per plant of ryegrass shoots varied from 0.23 to 0.43g. Maximum shoot dry weight was obtained at a much lower rate than for medic (10 ppm P), with another unusual rise at the highest rate (2500 ppm P) (Figure 5.1A, Plate 5.2). The kick up in yield of grass at 2500 ppm P may be due to control of some disease organism.

Comparing ryegrass and medic in pure stands, ryegrass had greater yield of shoots at the low P rates (namely 0, 10 and 25 ppm P) whereas medic outyielded ryegrass at medium to high P rates (63, 160 and 400 ppm P). In contrast, ryegrass grown in association with medic yielded more dry weight of shoots per plant than either species in monoculture (Figure 5.1A). The yield of ryegrass shoots per plant in species mixture was twofold higher than that of ryegrass in pure stand. Yet again, maximum yield of shoots was reached at the second lowest rate of P application (10 ppm P). However, a significant decline in shoot dry weight of ryegrass was associated with medic yield increases as P rates were increased from 10 to 160 ppm P. Beyond the latter rate the yield of medic shoots decreased while that of ryegrass increased. At all P treatments, the association medic + ryegrass stimulated the growth of ryegrass plants. There was a highly significant phosphate x community interaction in relation to the yield of ryegrass shoots per plant (Table 5.1b).

The yield of ryegrass roots per plant was not significantly enhanced by increasing rates of P application (Figure 5.1B). The production of maximum root dry matter (0.73g) was obtained at 400 ppm P but almost the same maximum yield (0.60g) was reached at 25 ppm P rate. Although no attempt was made to separate the roots of ryegrass from those of medic in the species mixture, it was clear that there was a

Table 5.1a Analysis of variance for the yield+ of shoots of medic

	per pot	per plant
Treatments	F value	F value
Phosphate	55.3***	46.4***
Community	194.9***	15.6***
Phosphate x Community	6.3***	1.17 NS

NS = non significant at $P \leq 0.05$

+ = Only medic shoot yields were considered in monoculture and in mixture.

Table 5.1b Analysis of variance for the yield‡ of shoots of ryegrass

	per pot	per plant
Treatments	F value	F value
Phosphate	30.8***	32.5***
Community	16.9***	477.4***
Phosphate x community	2.0 NS	8.4***

‡ = Only ryegrass shoot yields were considered in monoculture and in mixture.

much greater quantity of ryegrass roots than medic roots in the mixture. Yields of root-mixture at the different P levels are presented in Figure 5.1B. Over the range of P treatments medic + ryegrass community yielded about the same amounts of roots as did pure ryegrass even though there were only half the number of ryegrass plants in the mixture (Figure 5.1B).

5.3.2 Acetylene reduction per pot of monoculture and mixture

AR activity of pure medic pots was enhanced to a maximum of 930 µg C_2H_2 h^{-1} pot^{-1} by increasing P application rates up to 160 ppm P per pot (Figure 5.1C), the same maximum as for shoot yield. Higher rates of P decreased significantly the rate of AR, ultimately to less than 10% of the maximum. The sharpest decline occurred between 1000 and 2500 ppm P. The variation in AR activity per pot between P treatments was comparable to the variations in shoot and root dry matter per pot. Indeed there were close positive correlations between AR activity, the yield of shoots and of roots of medic. Correlation coefficients were $R^2 = 0.97^{***}$ and 0.97^{***} for shoots and roots respectively (Figure 5.2A). Analysis of variance (Table 5.2) indicated that AR activity of medic pots was significantly affected by P treatments.

In contrast, pure ryegrass pots showed very low AR activity at all rates of P application (Figure 5.1C). AR readings rarely exceeded 20 µg C_2H_2 h^{-1} pot^{-1}. The AR activity found in ryegrass monoculture pots can represent either an activity associated with ryegrass roots or an activity of certain non-symbiotic bacteria and blue-green algae. A bare soil treatment might be seen here as a suitable control to measure the effect of blue-green algae, but algae or free-living organisms grow essentially on the soil surface under the canopy of plant cover where the surface is both moist and partly lit. A bare soil surface would be subject to regular drying out and therefore a poor control.

AR activity per pot of the species mixture varied from 8 to a maximum of 480 μg h^{-1} pot^{-1} and was affected by P treatments in the same manner as was that of pure medic (Figure 5.1C). The species mixture treatment reduced the rate of AR of medic in mixture to about 50% of that of medic in monoculture. AR activity per pot of mixture varied linearly with medic shoot yield ($R^2 = 0.92^{***}$) but there was a lack of correlation between the former and dry matter yield of root-mixture ($R^2 = 0.002$) owing to the dominance of the latter by the ryegrass roots. The interaction phosphate x community was significant at P = 0.05 (Table 5.2) but again was primarily due to the different number of medic plants involved.

5.3.3 Comparison of acetylene reduction of medic in monoculture and in mixture on a per plant basis

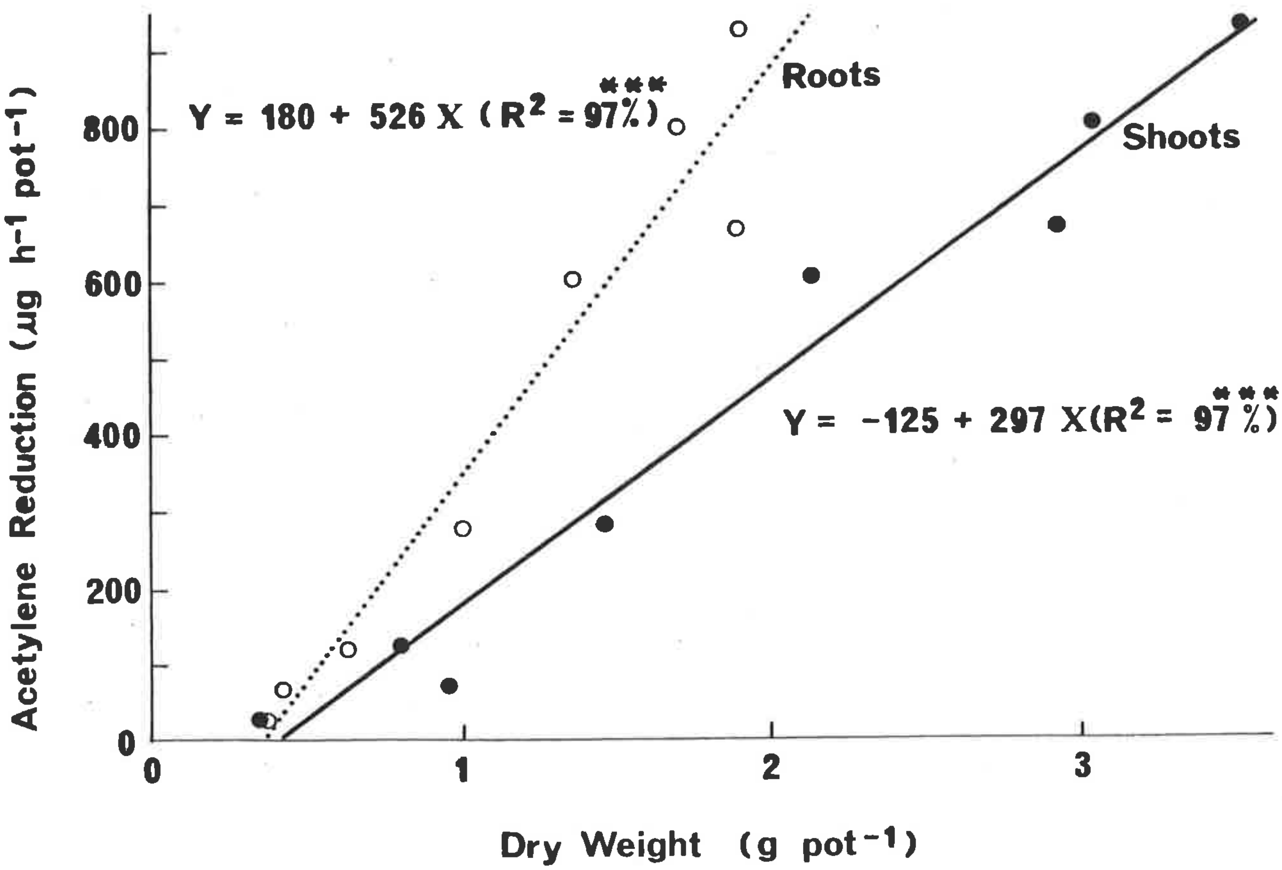
Since medic density was not the same in monoculture and in mixture, AR activity per medic plant was regarded as a better measure of the specific rate of N_2-fixation by medic in each community. On the assumption that the activity under ryegrass was primarily due to free-living soil organisms and therefore the same in all pots (Figure 5.1C), AR activity of pure medic pots and of medic + ryegrass pots was corrected for each P treatment by deduction of the mean of C_2H_2 reduced in the corresponding pure ryegrass pots before dividing by the number of medic plants involved.

In both monoculture and mixture, AR activity per medic plant followed the same response patterns to increasing rates of P (Figure 5.1D) with a similar optimum P rate (≃160 ppm P) and similar AR rates. However, the curve for the mixture was depressed relative to the pure stand at the lower P rates. The phosphate x community interaction was

Figure 5.2

A = Linear regression of acetylene reduction rate per pot on shoot dry weight (●) and on root dry weight (o) of medic in monoculture.

B = Effect of applied P on N content of medic roots in monoculture (□) in comparison with acetylene reduction rate per pot (●) of medic monoculture.


Acetylene Reduction (µg h-1 pot-1)
0
200
400
600
800
Y = 180 + 526 X (R2 = 97%)***
Roots
Shoots
Y = -125 + 297 X(R2 = 97%)***
0
1
2
3
Dry Weight (g pot-1)

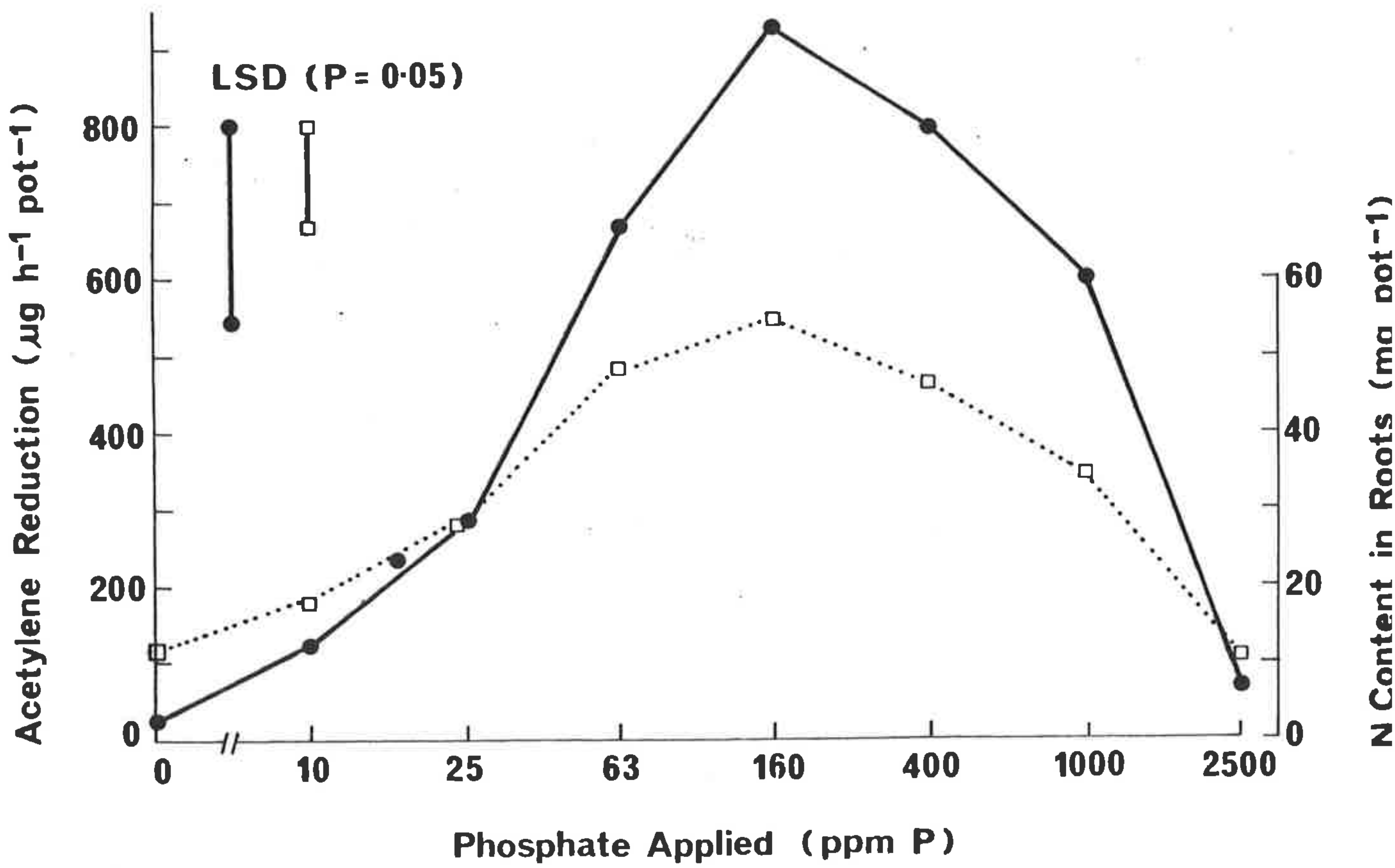

Acetylene Reduction (µg h-1 pot-1)
0
200
400
600
800
LSD (P = 0·05)
N Content in Roots (mg pot-1)
0
20
40
60
0
10
25
63
160
400
1000
2500
Phosphate Applied (ppm P)

Table 5.2 Analysis of variance for acetylene reduction by medics

	per pot	per plant
Treatments	F value	F value
Phosphate	28.7***	26.7***
Community	49.0***	1.6 NS
Phosphate x community	2.8*	0.5 NS

NS = non signficant at P $\leq$0.05

not statistically significant but is quite possibly real since these are the P rate treatments already above the optimum for ryegrass but below the optimum for medic where competition of the grass on the medics might be expected to be most severe.

5.3.4 Nitrogen in plants

- Nitrogen concentration

Shoots Analysis of variance (Table 5.3a) revealed a significant effect of plant community on N concentration in the tops of medic but the interaction phosphate x community was not significant. For medic in monoculture, the percentage N varied very little over the range of applied P (Figure 5.3A) and ranged from 2.76% to 3.36% with a mean of 3.11%.

For the mixture, the percentage N in medic shoots was lower than that of the shoots of medic in monoculture. The differences in N concentration were greater at the lower P levels (viz., 0, 10 and 25 ppm P). The main effects of P and plant community and the interaction were all significant (Table 5.3b).

For ryegrass monoculture, N concentration in the shoots was highest at the lowest P treatment (O ppm P), decreased markedly at the next P application rate (10 ppm P) and thereafter remained constant (Figure 5.3A).

The concentration of N in the tops of ryegrass grown in association with medics was higher than that of the ryegrass in monoculture (Figure 5.3A), especially at the lower levels of applied P.

Roots To obtain the dry matter yield of roots, it was necessary to correct for adhering soil particles by ashing a subsample. For the medic, soil contamination amounted to about 15% only (range 11-23%) but for ryegrass (and the mixture) was much higher at about 40% (25-60%).

Table 5.4 shows the N concentrations in the roots. Medic had the highest concentrations of N, ryegrass the lowest, and as expected, the mixture was intermediate but closer to the ryegrass. The effect of increasing P rates was to decrease N concentration in medic roots but in ryegrass there was no trend. The mixture was again intermediate but closer to ryegrass.

- Nitrogen content

Shoots N content of medic in monoculture was enhanced to maximum at a P application of 160 ppm (Figure 5.3B), the same optimum rate of P as for maximum AR activity (Figure 5.1C).

For ryegrass monoculture N content of the shoots was relatively constant over all P treatments (Figure 5.3B). In the mixture, N content of medic shoots (mg plant^{-1}) was lower than that of medic in monoculture (Figure 5.3B). The response curve to applied P of N content in medic shoots grown in association with ryegrass was similar to that of medic in monoculture. There was no significant interaction phosphate x community (Table 5.3a). N content in tops of ryegrass grown in mixture was about twice that of ryegrass shoots in monoculture (Figure 5.3B) and the curve shape reflected that of dry weight. For ryegrass shoots, there was a highly significant effect of plant community and a significant interaction phosphate x community (P = 0.05) (Table 5.3b).

At the lower levels of applied P (viz., 0 and 10 ppm P) N content in ryegrass shoots in the mixture was higher than that of the medic shoots in the mixture.

Roots N content of medic roots in monoculture reached a maximum at an application rate of 160 ppm P (Figure 5.3C). Within the range of applied P rates N content of roots per plant was highly correlated with

Table 5.3 Analysis of variance for N concentation and N content of shoots as affected by plant community and applied phosphate:

(a) Medic

	N concentration (%)	N content (mg plant^{-1})
Treatments	F value	F value
Phosphate	1.4 NS	25.3***
Community	4.5*	10.3**
Phosphate x community	1.0 NS	0.56 NS

(b) Ryegrass

	N concentration (%)	N content (mg plant^{-1})
Treatments	F value	F value
Phosphate	64.6***	7.5***
Community	30.3***	503.4***
Phosphate x community	4.0**	2.5*

Level of significance: *significant at P = 0.05
**significant at P - 0.01
***significant at P = 0.001
NS non significant at P = 0.05

Figure 5.3

A - Nitrogen concentration of shoots (%)

- ● Mp = pure medic
- o Mm = medic in mixture with ryegrass
- ■ Gp = pure ryegrass
- □ Gm = ryegrass in mixture with medic

B - Nitrogen content of shoots (mg plant^{-1})

- ● Mp = pure medic
- o Mm = medic in mixture with ryegrass
- ■ Gp = pure ryegrass
- □ Gm = ryegrass in mixture with medic

C - Nitrogen content of roots (mg plant^{-1})

- ● Mp = pure medic
- ■ Gp = pure ryegrass
- Δ M+G = medic + grass mixture

D - Nitrogen content (shoots + roots) per pot (mg)

- ● Mp = pure medic
- ■ Gp = pure ryegrass
- Δ M+G = medic + ryegrass mixture

The LSD's refer to the main effects of phosphate and phosphate x community interactions.

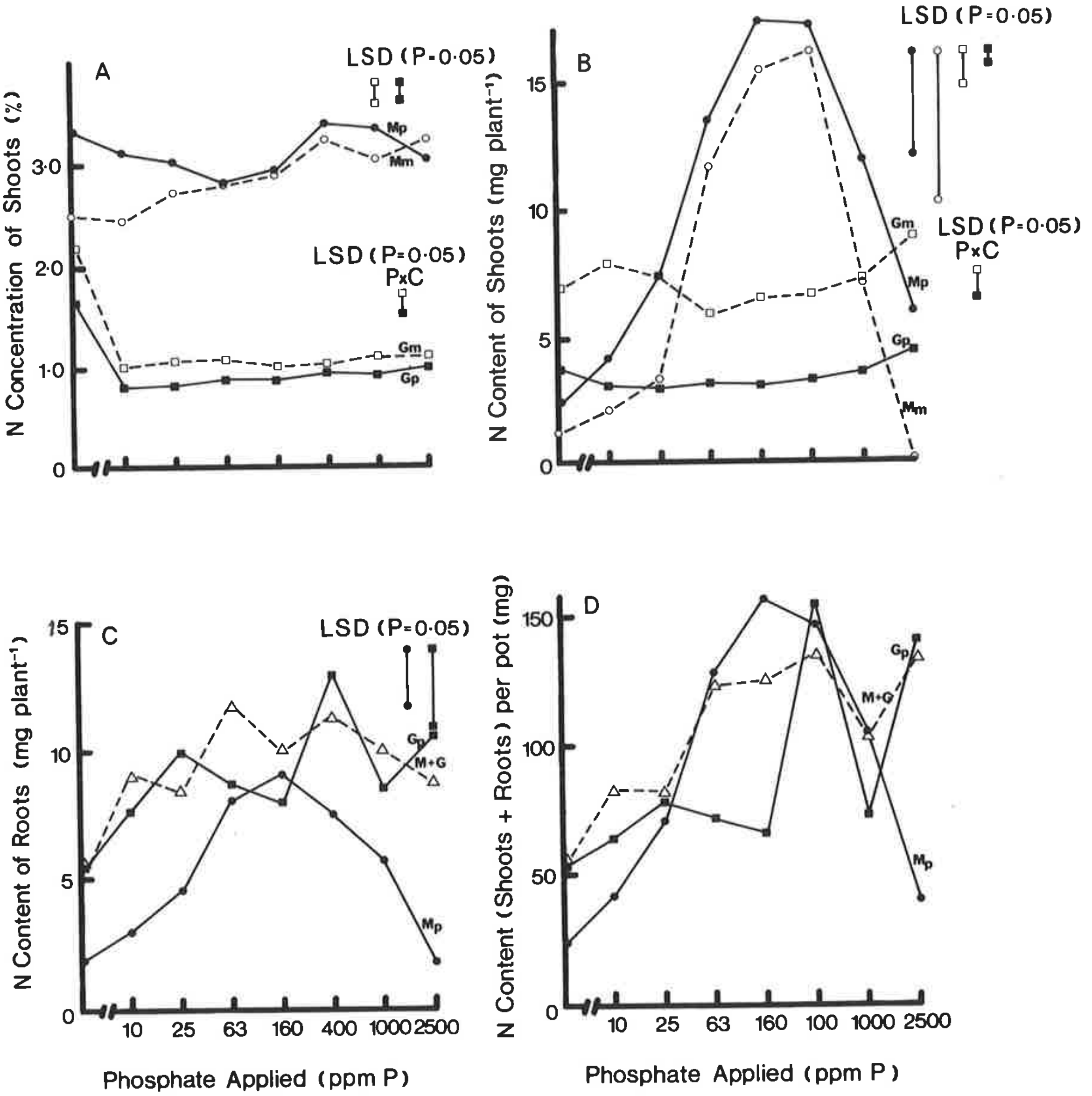

A
LSD (P=0·05)
LSD (P=0·05)
P×C
N Concentration of Shoots (%)
Mp
Mm
Gm
Gp
0
1·0
2·0
3·0
B
LSD (P=0·05)
LSD (P=0·05)
P×C
N Content of Shoots (mg plant⁻¹)
Gm
Mp
Gp
Mm
0
5
10
15
C
LSD (P=0·05)
N Content of Roots (mg plant⁻¹)
Gp
M+G
Mp
0
5
10
15
10 25 63 160 400 1000 2500
Phosphate Applied (ppm P)
D
N Content (Shoots + Roots) per pot (mg)
Gp
M+G
Mp
0
50
100
150
10 25 63 160 100 1000 2500
Phosphate Applied (ppm P)

Table 5.4 Nitrogen concentration (%) in the roots of medic, ryegrass and medic + ryegrass mixture at each level of applied phosphate.

	P rates (ppm P)								
	0	10	25	63	160	400	1000	2500	Mean
Pure medic	3.25	2.80	2.69	2.54	2.82	2.63	2.53	2.55	2.73
Pure ryegrass	1.58	1.45	1.71	1.51	1.28	1.76	1.47	1.66	1.55
Medic + ryegrass in mixture	1.98	1.99	1.62	1.68	1.57	1.88	1.67	1.45	1.73

Table 5.5 Root/shoot ratio of N content per pot of medic, ryegrass and medic + ryegrass mixture at each level of applied P.

	P rates (ppm P)								
	0	10	25	63	160	400	1000	2500	Mean
Pure medic[1]	1.09	0.72	0.65	0.60	0.53	0.45	0.49	0.36	0.61
Pure ryegrass	1.38	2.43	3.33	2.69	2.52	6.45	2.29	4.02	3.14
Medic + ryegrass in mixture	1.20	1.87	1.66	1.41	1.01	1.02	1.46	4.27	1.74

[1]LSD (P = 0.05) = 0.21 for comparison of phosphate rates.

shoot dry matter per plant ($R^2 = 0.93^{***}$), root dry matter per plant ($R^2 = 0.99^{***}$) and AR activity per plant ($R^2 = 0.95^{***}$).

For ryegrass monoculture, N content of the roots varied over the range of applied P from 5 to 13 mg plant^{-1}. This was generally higher than N content of the roots of medic in monoculture and about the same as the mixture (Figure 5.3C). The ryegrass showed large unexplained fluctuations as P rates increased.

The ratio of root N content to shoot N content (mg plant^{-1}) was higher for ryegrass in monoculture than for mèdic in monoculture or medic + grass mixture (Table 5.5).

- Nitrogen content (shoots + roots) per pot

The amount of N (shoots + roots) per pot of medic in monoculture also increased to a maximum at the P application rate of 160 ppm P (Figure 5.3D). Both in the mixture and ryegrass in monoculture, N content per pot was higher than that of medic in monoculture at the lower levels of applied P (viz. O, 10 and 25 ppm P) and at the highest (2500 ppm P). The yield of N per pot of medic + ryegrass mixture was higher than that of ryegrass in monoculture except in 2 occasions (400 and 2500 ppm P) (Figure 5.3D). Over all P treatments the mean N content of shoots + roots was higher for medic + grass mixture (106 mg pot^{-1}) than for medic in monoculture (90 mg pot^{-1}) or for ryegrass in monoculture (88 mg pot^{-1}).

5.4 DISCUSSION

5.4.1 Growth response of each species to applied phosphate in each plant community

The use of a soil naturally deficient in P revealed different response of the legume and grass species to added P. The ability of ryegrass to produce maximum shoot dry matter at the very low rate of P application (10 ppm P) contrasted with the considerably higher demand of medic for P. If the P requirement of cereals is similar to that of ryegrass in this experiment, then clearly the hypothesis that cereals have higher P requirement than medic cannot be sustained; but according to Ozanne *et al.*, (1976), wheat had a much higher P requirement (118 kg ha^{-1}) than wimmera ryegrass (56 kg ha^{-1}) when grown on a loamy sand soil. The need of medic for high P rates to produce maximum yield agrees with the report of Asher and Loneragan (1967) who found that of the species tested barrel medic and flatweed (*Hypochoeris glabra* L.) had the highest P requirement.

The difference in P requirement between medic and ryegrass is similar to that reported between subterranean clover (cv. Daliak) and wimmera ryegrass (Ozanne *et al.*, 1976). Ryegrass efficiency in the utilization of low availability of P supply is probably linked with its greater production of roots (Figure 5.1B) and perhaps also with differential mycorrhizal effects. McLachlan (1976) reported for several species that the efficiency of P utilization was associated with root morphology and root growth rate.

Although plant community did not alter the P requirement of each species for maximum shoot production, the yield per plant of ryegrass in plant mixture was greatly enhanced in comparison to that of ryegrass alone. The two-fold increase in yield of ryegrass in the mixture in comparison to that of pure culture may be associated with lower competition from medics than from ryegrass plants and/or transfer of fixed N from the medic to the associated grass. The results of N concentration and N content of the shoots of ryegrass (Figure 5.3A, 3B) strengthen the hypothesis of an additional supply of N from the medic in the medic + grass mixture.

The underground transfer of N compounds from legume to associated grasses within the growing season has been reported in the literature (Walker, Orchiston and Adams 1954, Simpson 1965, Chan 1970, Ozanne *et al.*, 1976). Walker *et al.*, (1954) reported that a small amount of shading of white clover induced by the associated ryegrass caused a considerable excretion of N from white clover, whereas under good conditions for clover growth N excretion may not take place. Simpson (1965) suggested that N transfer in pasture could be most important under conditions of N deficiency, which is true of the present investigation. A soil of moderately high available N was avoided for this experiment because of the risk of suppresion of nodulation and N_2-fixation by excessive mineralization following disturbance, drying and potting. The severity of N deficiency in this soil, however, turned out to be greater than desired.

Inter-species competition could be expected for P and N both of which were deficient in this soil. The significant decrease in ryegrass yields at the maximum yields of medic (Figure 5.1A) indicates that at moderately high P rates (63 and 160 ppm P) competition from medics is relatively greater near their own P optimum. Likewise grass competition in the non-significant interactions on AR activity per plant (Figure 5.1D) is closer to grass P optimum. The results (Figure 5.1A, Table 5.1a) reflect the need of medics for high availability of P supply presumably because of their small root mass (Figure 5.1B). The observed decrease in ryegrass shoot yields between P treatments 10 and 160 ppm P may also result from a greater competition from medic foı light and nutrients. The results show the competitive advantage to the legume of moderately high dressings of superphosphate in establishing a medic pasture on an infertile soil.

5.4.2 The response curves of acetylene reduction activity to phosphate application rates

Measurements of AR activity did not support hypothesis II that N_2-fixation of medic is independent of availability of soil P. It could be that the growth effect of added P enhanced nodulation and increased root nodule numbers (Anderson and Thomas 1946, Diener 1950, Robson 1969), leading to improved nitrogenase activity as measured by the AR assay. Differential effects on nodulation could not be measured in the time available, as medics produce large numbers of nodules of small yet highly variable size, and judging from haemoglobin content, highly variable activity.

The hypothesis V concerning the decrease in AR of medic due to grass competition can barely be sustained in view of the results from the addition of phosphate fertilizer to medic + grass mixture. Figure 5.1D suggests that the competition from ryegrass may have taken place at the low levels of applied P rather than at the high levels of P as was expected. However, analysis of variance (Table 5.2) showed that the phosphorus x community interaction was not significant which does not support the hypothesis, at least under the conditions of the present experiment. The lack of interaction could partly be attributed to high variability in the data for AR (Appendix III.1).

Transformation of AR values to logarithms brought no change with regard to the phosphate x community interaction. Donald (1954) reported that the increase in soil total N by legume-based pastures enhances the ability of annual grasses to compete with pasture plants. In the present experiment soil N was low, and the competition for light and nutrients from ryegrass was probably not high enough to induce a significant reduction in AR activity per plant of medic in mixture.

5.4.3 Nitrogen content of medic shoots and roots in relation to applied phosphate

The magnitude of the response of N content in medic roots and AR activity to P application rates does not lend support for hypothesis III. It is true that in the present experiment, N content of medic roots may represent both N fixed symbiotically and N taken up from the soil and the amount of "non-fixed" N in medic roots cannot be estimated in this experiment. However, the close association between N content of medic roots in monoculture and AR activity (Figure 5.2B) suggests that most of the N accumulated in medic roots may come from biological N_2-fixation. Thus, contrary to our hypothesis, phosphate fertilizer increased both N content of medic shoots and roots; it should be pointed out, however, that increasing P application rates enhanced more the N content of the shoots than that of the roots of medic in monoculture as indicated by the root/shoot ratio of N content (Table 5.5). This was primarily due to the differential effect of applied P on shoot and root growth of medic in monoculture (Figures 5.1A, 1B) since N concentrations in shoots and roots were similar (Figure 5.3A, Table 5.4). The ratio of root/shoot N content was highest in the pure ryegrass community (Table 5.5) where because of the N deficient soil and the absence of legume, N deficiency is most severe.

Nitrogen per pot reflect the root data. The mixture is generally as high in N as the pure medic but in the mixture N was derived both from N_2-fixation and from soil sources to an extent greater than for pure medic.

5.5. CONCLUSION

This experiment clearly demonstrated that increasing P application rates increased the rate of AR by barrel medic grown either in monoculture or in mixture with wimmera ryegrass, and enhanced the accumulation of N in medic roots. The results of AR and N analyses did not support the advanced hypotheses concerning the lack of interdependence between P supply and N_2-fixation and N accumulation in medic roots. The hypothesis that a reduction in medic growth and N_2-fixation can be caused by increasing rates of P supply on ryegrass growth and increased competition in mixtures was not supported by the results of this experiment. This shifts the interest to an alternative hypothesis (No.IV, page 130) that mining of soil N by cereal crops could be the key to the lack of association between soil total N and soil available P found in the survey study (Part 3.0).

In order to show whether the interaction phosphate x community is significant on AR activity per plant of medic, the experiment needs to be repeated with more replications and more control. As others have found (Ruegg and Alston 1978, A.D. Robson personal communication) variability in AR measurements can at times be extremely high.

The effect of applied P on medic growth, AR rate, and N accumulation in the roots suggest that the lack of association between soil total N and soil available P found in the survey is not a simple expression of the relation between the 2 factors but may reflect more complex inter-relations between several environmental factors including botanical composition of pasture, soil N supply and grazing intensity.

The two-fold increase in the yield of shoots of ryegrass in mixture compared to that of ryegrass monoculture may be an expression of lower interplant competition in the mixture for light and nutrients or may reflect an underground transfer of fixed N from medic to the ryegrass within the growing period. This underground transfer of N from medic needs further detailed study to clarify the N nutrition of annual grasses associated with annual legumes.

The results suggest that:

(i) Biological N_2-fixation by barrel medic is a function of soil P status and medic density per unit area:

(ii) Where soil P status is low, moderate to high applications of superphosphate are needed to maintain a medic dominance over wimmera ryegrass in pasture.

6.0 GENERAL CONCLUSION

6.0 General Conclusion

1. Quantification of the relationship between soil total nitrogen and the frequency of medic leys using the accumulated seasonal rainfall has been achieved using a farm soil survey technique. In the survey area the higher the frequency of medic-dominant pastures over the cropping history period (1965-1974) the higher the level of soil total nitrogen. The relationships were developed in the form of linear equations by correlation and regression analysis.

2. From these regressions, inputs of nitrogen (kg N ha^{-1} $year^{-1}$) by a medic pasture may be estimated. These estimates suggest that annual medics growing in drier parts of the cereal belt were able to fix relatively as much nitrogen as subterranean clover does in higher rainfall areas.

3. The survey results produced evidence that the accumulation of total nitrogen in the surface 10cm of soil was higher under medic-dominant pastures than under grass-dominant pastures, and that soil total N was greater in solonized brown soils than in the red-brown earths (lime-free soils).

4. The rate of nitrogen fixation (as estimated by acetylene reduction assays) by barrel medic was generally higher during the vegetative period of plant growth than after flowering, probably owing to post-flowering competition for substrate from developing pods.

5. The higher the medic sowing density the higher the rate of symbiotic nitrogen fixation during the vegetative phase of plant growth.

6. Medic growth, acetylene reduction and nitrogen content increased in a phosphate deficient soil as applied phosphate increased to rates well above the rate needed for maximum growth for ryegrass. In contrast the results of the survey indicated that total nitrogen of the surface 10cm of soil and available phosphorus of the same depth interval were not correlated. Several hypotheses have been advanced to explain these apparently contrasting results.

7. Evidence has been produced for underground transfer of nitrogen from medic to ryegrass in a mixed sward in pots within the same growing season, though proof is lacking.

8. Yields of shoot dry matter, root dry matter, acetylene reduction and nitrogen content of the roots and shoots of barrel medic had all the same optimum phosphate rate (160 ppm P in small pots).

To the future:

The lower levels of soil total nitrogen found under grass-dominant pastures suggest that most of the nitrogen accumulated under medic-dominant pastures came from symbiotic nitrogen fixation. Changes in soil total nitrogen associated with the frequency of medic leys and increases in the rate of symbiotic nitrogen fixation with increasing medic sowing density suggest that maintaining a high proportion of medics in the pasture phase and reducing the interval of time separating pasture leys by the adoption of short cereal-medic ley rotations (e.g., crop-pasture-crop-pasture) should lead to better fertility (in terms of nitrogen status) in these soils.

The evidence produced by the survey technique concerning a lower rate of soil nitrogen accumulation in a dry season needs to be supported by critical experimentation under limited water supply conditions. If substantiated this could lead to the use of more nitrogen fertilizer for cereal crops sown after drought-affected pastures.

In the field experiment, increasing the sowing density increased the rate of acetylene reduction by barrel medic. It is likely that under farm conditions the number of germinating and viable medic seeds per unit area surviving to form a pasture is often lower than the 10 kg/ha optimum indicated in this study, leading to suboptimal nitrogen fixation. A medic population density higher or equivalent to 10 kg/ha seed rate may serve both purposes of soil nitrogen accretion and adequate winter feed production. Field experiments of at least 5 years duration to allow for medic self-regeneration over 2 cycles are needed to provide a better assessment of medic sowing density in relation to symbiotic nitrogen fixation and winter feed production in different season.

The higher rates of nitrogen fixation of medic plants during the vegetative phase has consequence for pasture management. The effect of grazing in delaying flowering may be important in the overall seasonal nitrogen fixation by a pasture. This needs study and may lead to mixtures of early and late flowering cultivars of *Medicago truncatula* e.g., Early Cyprus and Jemalong (Mathison 1973), which could extend the vegetative period of growth of the mixed pasture and lead to higher nitrogen inputs per unit area.

Further studies are needed to determine whether soil total nitrogen in the survey area is increasing or decreasing with time. The association of high soil total nitrogen with high frequency of medic leys (Figure 3.5A) does not indicate trends in time for individual sites.

In short, critical experiments are needed to test the following hypotheses which emerge from the present work:

- The rate of nitrogen accumulation is lower in a dry season.
- Increasing levels of soil available phosphorus increase cereal yields and consequently the exports of soil nitrogen by cereal crops and, therefore, cancel out the additional soil nitrogen gains stemming from phosphorus-induced higher rates of symbiotic nitrogen fixation. (This would lead to the observed lack of correlation between soil total nitrogen and available phosphorus in the survey).
- Under semi-arid conditions, the accumulation of soil total nitrogen in field soils has an optimum phosphorus level.
- Heavy grazing during the vegetative phase of medic growth delays medic flowering and extends the period of significant symbiotic nitrogen fixation.
- Nitrogen is transferred from medics to grasses in mixed pastures during the same growing season. If valid, the mechanisms need to be understood.
- Because ryegrass produces much greater root mass than medics, mixed medic-grass pastures may lead to better soil structure than pure medic stands.

7.0 APPENDICES

APPENDIX I.1

PADDOCK HISTORY

FARMER'S NAME ....
TYPE OF SOIL ....

FARM LOCATION ....
SAMPLING DATE..........................

PADDOCK NUMBER...............
SAMPLE NUMBER ....

Years	Crops W* or B* Medic or Fal* or Grass	Fertilizer Applied		Time of Seeding approx.	Rainfall Average Annual in inches	Yields (Bushel/Acre			Remarks - Too Wet.... Poor ...... Diseases...
		Super (Phosphorus) lb/acre	Nitrogen lb/acre			Wheat	Barley	Others	
1974									
1973									
1972									
1971									
1970									
1969									
1968									
1967									
1966									
1965									
1964									
1963									
1962									
1961									

Remarks: W*= Wheat
B*= Barley
Fal* = Fallow
Grass - No medic
Medics (Clover

If Gypsum applied - lb/acre...............

No. of years since medics introduced ....

APPENDIX I.2

Appendix I.2 Basic data for the Soil Fertility Survey.
A. Farmers' fields

Key to symbols used:

Abbreviation	Variable name
M	Medic-dominant pasture in 1974
G	Grass-dominant pasture in 1974
ASRP	Accumulated seasonal rainfall on pasture
FYRC	Frequency of years of crop
PTNA	Total nitrogen (%) in the 0-5cm depth interval
PTNB	" " " " 5-10cm " "
AVPA	Available phosphorus (ppm) in the 0-5cm depth interval
AVPB	" " " " 5-10cm "
ORMA	Organic carbon (%) in the 0-5cm depth interval
ORMB	" " " " 5-10cm " "
MINA	Mineralizable nitrogen (ppm) in the 0-5cm depth interval
MINB	" " " " 5-10cm
PH A	pH in the 0-5cm depth interval
PH B	" " 5-10cm " "
LIMA	Calcium carbonate (%) in the 0-5cm depth interval
LIMB	" " " " 5-10cm "
PCLA	Clay content (%) in the 0-10cm depth interval
TOTP	Total phosphorus (ppm) in the 0-10cm depth interval
RFL	Mean annual rainfall (mm)
BDA	Bulk density (g cm^{-3}) in the 0-5cm depth interval
BDB	" " " " 5-10cm " "
SUP	Superphosphate (kg ha^{-1}) applied (1965-1974)

SITE No.	MQ	ASRP	FYRC	PTHA	PTHB	AVPA	AVPB	ORMA	ORMB	MINA	MINB	PHA	PHB	LIMA	LIMB	PCLA	TOTP	RFL	BDA	BDB	SUP
1	M	1575	3	.210	.180	15.5	9.5	2.99	2.25	69.7	5.3	7.7	7.9	8.92	13.26	24	336	390	1.07	1.02	439
2	G	805	5	.140	.121	14.0	1.2	2.10	1.90	51.5	17.4	7.5	7.8	2.74	4.66	17	175	386	1.47	1.24	404
3	G	1225	4	.160	.180	23.0	41.5	2.99	2.03	49.2	23.5	7.8	7.7	15.27	16.64	18	395	392	.94	1.14	323
4	G	945	5	.180	150	18.5	10.5	2.41	1.80	68.1	6.1	7.5	8.0	16.28	16.55	22	247	389	1.23	1.09	215
5	G	1645	3	.140	.140	18.7	13.0	2.32	1.67	56.0	2.3	7.5	7.8	12.16	9.92	23	281	395	1.24	1.14	–
6	G	980	5	.0660	.0588	20.0	1.0	.81	.55	26.5	18.2	7.0	7.8	0	0	8	135	350	1.62	1.49	605
7	M	1225	4	.170	.107	9.5	5.0	2.43	1.44	70.4	21.2	7.5	7.5	6.95	7.32	24	277	363	1.37	1.21	286
8	M	700	5	.0730	.0498	40.0	25.0	.77	.29	22.7	4.5	6.9	7.5	0	0	5	147	359	1.51	1.55	561
9	G	875	6	.0790	.0432	28.5	14.0	.98	.38	22.7	6.8	7.5	7.5	0	0	4	167	357	1.79	1.52	–
10	M	700	7	.0800	.0560	40.5	24.5	.87	.50	36.3	10.6	6.9	7.7	0	0	10	195	358	1.45	1.43	605
11	G	700	4	.150	.117	10.0	2.0	2.06	1.31	56.0	18.2	7.8	7.2	2.99	4.75	22	247	391	1.39	1.35	509
12	G	805	5	.0846	.0513	23.0	13.0	1.15	.59	27.3	10.6	7.1	6.7	0	0	6	186	352	1.62	1.49	–
13	G	735	4	.0885	.0661	19.0	14.0	1.73	.80	9.1	1.5	6.7	6.9	0	0	20	155	368	1.24	1.35	404
14	G	1190	4	.0902	.0701	17.5	5.0	1.19	.83	18.2	12.9	7.1	7.4	0	0	13	139	352	1.63	1.61	–
15	G	910	6	.0952	.104	39.0	8.6	1.79	1.17	56.0	2.3	7.2	7.3	1.00	3.29	32	236	365	1.21	1.21	–
16	G	665	6	.0952	.0493	32.5	9.3	1.31	.65	21.2	1.5	6.9	7.4	0	0	6	127	358	1.56	1.57	–
17	G	1295	4	.0952	.0560	48.5	31.2	1.29	.68	19.7	6.8	6.6	7.0	0	0	2	195	367	1.44	1.28	1009
18	G	1120	5	.0986	.0672	34.0	13.5	1.43	.83	11.4	6.8	6.8	7.1	0	0	18	147	351	1.52	1.36	605
19	G	770	5	.0986	.0807	18.0	8.7	1.14	.98	18.9	10.6	7.5	7.3	2.54	4.34	34	137	371	1.26	1.36	578
20	G	770	5	.0991	.0869	23.0	11.0	1.59	.98	18.9	10.6	7.2	6.7	0	0	30	194	368	1.39	1.22	–
21	M	1085	4	.190	.117	19.0	12.0	2.82	2.31	59.1	17.4	7.8	7.9	12.02	13.81	27	223	393	1.28	.94	346
22	M	525	5	.160	.0913	25.7	12.2	2.25	1.44	62.1	17.4	7.8	7.5	1.17	1.42	14	207	365	1.33	1.43	406
23	M	1400	5	.180	.139	8.0	1.0	2.53	1.67	65.9	22.0	7.9	7.8	7.06	7.86	10	247	381	1.23	1.11	168
24	G	735	6	.106	.0639	19.5	15.0	1.38	.56	22.0	9.1	7.2	7.4	0	0	14	205	369	1.34	1.43	–
25	G	1155	4	.107	.170	35.5	29.0	2.65	2.21	34.1	18.2	7.6	7.7	13.30	14.72	19	347	393	.97	1.08	215
26	G	805	5	.108	.0773	27.5	10.0	1.56	.73	25.0	11.4	6.9	6.9	0	0	15	219	382	1.49	1.27	504
27	G	1050	4	.109	.0510	17.7	7.2	1.32	.56	51.5	15.1	7.3	7.5	0	0	10	171	364	1.49	1.76	475
28	G	875	7	.170	.180	89.0	54.5	2.84	2.34	18.9	24.2	7.5	7.7	12.39	13.72	19	417	394	1.17	1.10	646
29	G	1050	6	.116	.0695	36.5	26.5	1.47	1.04	19.7	9.1	7.0	7.6	0	0	11	195	350	1.48	1.57	428
30	G	1155	3	.116	.0953	36.1	21.2	1.67	1.17	12.9	3.8	7.4	6.8	0	0	26	200	369	1.34	1.43	565
31	G	700	7	.117	.0852	41.0	12.4	1.50	.80	28.0	10.4	7.1	7.0	0	0	23	212	356	1.47	1.24	807
32	G	700	6	.118	.0757	61.5	19.5	1.48	.92	15.1	9.1	7.2	7.1	0	0	29	220	357	1.39	1.37	404
33	G	945	5	.119	.0538	17.5	5.5	1.60	.90	39.4	18.2	7.1	7.3	0	0	12	162	401	1.65	1.44	504
34	M	560	5	.121	.0869	22.0	7.0	1.63	1.01	27.3	15.1	7.3	7.7	0	0	23	189	355	1.48	1.21	553
35	G	1365	4	.121	.0846	13.5	8.5	1.54	1.04	45.4	9.8	7.5	7.4	0	0	14	177	398	1.42	1.27	327
36	G	700	6	.180	.143	18.5	11.3	2.58	2.08	66.6	26.5	7.9	7.6	14.26	17.01	13	287	383	1.36	1.11	639
37	M	805	5	.126	.0919	47.5	12.5	1.43	.95	53.0	21.2	7.0	7.6	2.26	1.17	13	252	360	1.08	1.54	605
38	M	700	5	.129	.0773	26.2	12.0	1.83	.95	20.4	9.8	6.9	7.2	0	0	15	206	380	1.38	1.31	–
39	M	525	6	.129	.109	54.0	27.5	1.83	1.45	35.6	18.9	7.1	7.2	0	0	30	262	375	1.14	1.41	755
40	M	735	5	.130	.0745	39.0	15.0	1.76	.86	28.8	15.9	6.6	7.2	0	0	10	242	367	1.34	1.23	1009
41	M	770	4	.130	.0656	18.0	8.2	1.69	.70	52.2	13.6	6.8	6.8	0	0	16	178	378	1.33	1.24	–
42	G	945	5	.131	.109	48.5	20.0	2.00	1.42	11.4	.8	7.3	7.7	0	0	20	235	393	1.33	1.27	630
43	M	840	5	.132	.0860	28.5	14.0	1.54	.71	22.7	15.1	6.9	7.8	0	0	25	227	359	1.44	1.18	656
44	M	875	4	.132	.0835	10.5	1.0	1.58	1.01	44.7	14.4	7.0	7.4	0	0	11	150	345	1.46	1.25	–
45	G	945	5	.134	.160	27.0	17.0	2.92	1.90	18.9	17.4	8.0	7.8	15.50	4.66	29	281	390	1.14	1.02	507
46	G	1085	4	.136	.0925	54.0	12.4	1.86	1.19	46.9	10.6	6.9	7.3	0	0	33	265	353	1.35	1.14	–
47	M	630	6	.138	.0874	5.5	10.5	1.87	.80	62.8	4.5	7.0	6.8	0	0	28	195	350	1.33	1.21	538
48	M	805	5	.140	.153	13.5	2.5	2.84	2.09	11.4	9.1	7.8	7.6	5.83	7.73	18	252	365	1.28	1.09	–
49	M	875	5	.142	.106	17.0	5.7	1.89	1.22	42.4	9.8	7.8	8.0	6.56	7.27	14	252	385	1.44	1.04	428
50	M	735	5	.145	.0841	22.5	10.5	1.83	1.08	37.1	12.1	6.7	6.5	0	0	33	207	368	1.26	1.24	527
51	M	840	5	.146	.115	39.5	7.7	1.71	1.03	43.9	14.4	7.5	8.2	1.90	5.90	16	280	362	1.42	1.53	607
52	M	350	6	.148	.139	15.5	6.0	2.49	1.96	56.0	23.5	7.9	7.5	6.56	10.10	23	280	392	1.40	1.23	554
53	G	770	5	.149	.125	30.5	12.5	2.48	1.81	46.9	24.2	7.9	7.9	11.70	12.76	24	265	390	1.13	1.21	404
54	M	875	5	.149	.111	33.7	21.5	2.13	1.35	40.1	8.3	7.0	7.3	0	0	15	220	382	1.34	1.11	–
55	M	735	5	.150	.106	17.0	1.0	1.94	1.31	57.5	14.4	7.0	7.5	0	0	21	172	370	1.29	1.48	–
56	M	735	5	.151	.117	12.5	4.0	2.15	1.49	39.4	7.6	7.8	7.8	4.78	5.90	14	251	390	1.31	1.32	404
57	M	735	4	.152	.100	10.5	4.0	1.92	1.08	11.4	15.1	7.5	7.4	0	0	25	181	371	1.12	1.12	314
58	G	1540	4	.152	.0672	17.2	2.5	1.82	.86	86.3	17.4	7.4	7.7	0	0	19	210	369	1.39	1.27	–
59	M	910	7	.152	.0992	21.5	14.0	1.91	1.28	56.8	15.9	7.3	7.6	0	0	17	175	388	1.35	1.28	404
60	M	735	5	.152	.0857	20.0	2.2	1.91	1.01	45.4	6.1	7.0	7.6	0	0	21	162	370	1.46	1.47	–
61	M	945	4	.152	.0734	10.0	2.7	1.81	1.24	46.2	14.4	7.6	7.4	0	0	20	174	370	1.55	1.39	574
62	M	875	4	.153	.0986	12.5	2.1	1.86	1.28	56.8	11.4	7.1	7.3	1.00	1.42	15	201	350	1.41	1.25	–
63	M	1155	4	.153	.0939	52.5	8.5	1.77	1.12	59.8	12.9	7.1	7.3	0	0	9	223	356	1.38	1.17	695
64	M	875	4	.154	.115	16.5	2.0	2.04	1.31	49.2	46.9	7.4	8.0	1.05	2.93	11	161	362	1.19	1.35	364
65	M	1050	4	.154	.0933	8.0	5.0	1.79	1.17	124.9	26.5	7.7	7.4	.53	1.33	19	191	402	1.16	1.09	–
66	M	805	4	.154	.110	20.0	11.5	1.77	1.08	63.6	21.2	7.6	7.6	13.17	15.32	17	275	355	1.40	1.34	453
67	M	1050	5	.155	.120	20.7	1.0	2.17	1.12	60.6	9.8	7.6	7.3	5.88	.32	21	300	363	1.29	1.05	–
68	M	1050	4	.155	.107	24.5	7.7	2.10	1.53	70.4	22.0	8.0	7.8	10.74	14.58	15	252	372	1.29	1.32	415
69	M	980	4	.156	.0925	12.5	4.2	2.07	1.37	62.8	15.1	7.3	7.4	0	0	21	181	372	1.40	1.49	280
70	M	945	4	.159	.104	17.5	1.0	2.08	1.04	77.2	22.7	7.2	7.1	0	0	33	167	372	1.32	1.18	258
71	M	1050	4	.160	.110	10.5	2.0	1.79	1.15	71.2	33.3	7.4	7.6	3.84	10.15	13	219	350	1.52	1.17	–
72	M	805	5	.162	.118	39.5	12.3	2.36	1.49	19.7	18.2	7.3	7.3	0	0	29	237	370	1.20	1.16	516
73	M	1050	3	.169	.107	16.2	1.0	2.06	1.26	39.4	6.8	7.5	7.5	0	0	20	152	387	1.32	1.50	202
74	M	910	5	.170	.139	30.0	5.5	2.34	1.60	40.1	18.9	7.8	7.7	10.06	10.20	14	247	363	1.19	1.05	–
75	G	1295	4	.172	.107	62.0	13.0	2.13	1.17	38.6	14.4	7.0	7.4	0	0	24	257	366	1.23	1.31	1009
76	M	1155	4	.175	.140	49.2	22.7	1.93	1.44	124.9	49.2	7.7	8.0	9.05	11.43	23	272	400	1.30	1.28	504
77	G	630	5	.178	.112	44.5	22.5	2.00	1.24	39.4	15.1	7.8	7.7	9.33	10.38	20	252	401	1.17	1.17	355
78	M	1120	4	.179	.0953	10.0	10.0	1.89	1.49	47.7	19.7	7.8	7.3	4.57	6.04	16	232	391	1.41	1.43	755
79	M	1155	4	.184	.122	23.0	7.2	2.65	1.62	93.9	13.6	7.7	7.8	7.64	7.91	13	241	361	1.25	1.28	406
80	M	1155	6	.188	.115	30.0	10.0	2.32	1.53	21.2	11.4	7.7	7.4	1.00	1.00	14	294	383	1.17	1.03	226
81	M	875	5	.189	.120	11.5	1.0	2.65	1.60	45.4	4.5	7.5	7.5	2.51	2.65	22	294	399	1.16	1.16	–
82	M	1085	4	.190	.120	16.6	1.0	2.53	1.44	51.5	22.0	6.6	7.6	3.25	4.66	15	230	375	1.38	1.20	504
83	M	1120	4	.192	.110	18.5	7.7	2.36	1.63	71.2	18.2	7.5	8.0	0	0	22	169	372	1.14	1.25	325
84	M	1085	4	.192	.137	10.0	1.2	2.63	1.90	45.4	18.2	7.7	7.8	8.57	10.84	21	265	364	1.14	1.32	364
85	M	1120	4	.195	.135	41.0	1.0	2.34	1.42	62.1	22.0	7.7	7.8	6.17	9.14	15	223	396	1.25	1.28	426
86	M	945	5	.196	.154	7.5	4.1	2.39	1.90	51.5	15.1	7.8	7.7	7.98	9.05	18	234	366	1.40	1.17	426
87	M	875	4	.197	.170	29.5	1.0	2.59	1.98	54.5	37.9	7.6	8.1	3.11	3.93	14	228	389	1.24	1.13	404
88	M	1050	4	.199	.122	12.1	1.0	1.84	1.44	71.9	27.3	7.8	7.8	2.36	.87	21	178	362	1.22	1.25	510
89	M	980	4	.205	.133	30.0	15.5	2.79	1.96	75.7	28.8	7.6	7.7	3.77	5.58	16	210	386	1.45	1.25	426
90	M	945	5	.175	.130	30.0	1.0	2.61	1.76	48.5	15.9	7.5	7.6	7.48	5.97	11	260	375	1.29	1.26	–
91	M	1015	4	.218	.130	8.0	10.5	2.52	1.76	101.5	27.3	7.6	7.4	1.26	3.66	11	277	369	1.22	1.21	475
92	M	1190	3	.226	.126	17.0	4.5	2.96	2.13	45.4	14.4	7.8	7.8	1.63	22.31	8	280	378	1.16	1.11	347
93	M	1190	4	.229	.111	22.5	5.5	2.78	1.53	99.2	16.7	7.1	8.1	12.25	11.34	14	262	376	1.11	1.30	437
94	M	1085	5	.233	.134	80.7	13.0	2.85	1.86	71.2	22.0	7.2	7.9	7.00	11.29	11	280	383	1.11	1.29	908
95	M	1155	4	.266	.173	43.5	11.5	3.35	2.36	53.0	17.4	7.9	7.7	10.20	11.84	5	297	377	1.17	1.25	404
MIN		350	3	.0660	.0432	5.5	1.0	.77	29	9.1	.8	6.6	6.5	0	0	2	127	345	.94	.94	168
MEAN		944	5	.149	.105	26.3	10.3	2.02	1.31	46.8	15.4	7.4	7.5	3.55	4.33	18	226	373	1.33	1.27	394
MAX		1645	7	.266	.180	89.0	54.5	3.35	2.34	124.9	49.2	8.0	8.2	16.28	22.31	34	417	402	1.79	1.76	1009

Appendix I.2 Basic data for Soil Fertility Survey

B. Reference sites

Key to symbols used : the same as for Appendix I.2A.

Site No.	PTNA	PTNB	AVPA	AVPB	ORMA	ORMB	MINA	MINB	PHA	PHB	LIMA	LIMB	PCLA	TOTP	BDA	DBDB
1	0.1098	0.0710	5.00	15.50	3.50	1.15	14.40	9.10	7.95	7.60	9.00	1.20	-	265	1.17	1.22
2	0.1311	0.1479	42.00	34.50	1.86	1.90	33.31	44.70	8.00	7.70	3.30	8.10	-	288	1.32	1.13
3	0.1412	0.1065	10.00	8.75	3.73	1.91	56.03	21.96	8.05	8.00	5.90	12.50	-	185	1.31	1.26
4	0.1445	0.1345	13.50	10.25	2.72	2.41	43.16	40.89	8.00	8.00	3.34	10.19	-	223	1.32	1.15
5	0.1800	0.1900	13.50	23.00	3.44	3.45	44.00	40.13	7.90	7.80	13.70	15.50	-	251	0.96	0.99
6	0.1826	0.1500	2.00	1.00	2.92	2.32	59.06	27.26	7.90	7.70	11.20	13.03	-	208	1.15	1.17
7	0.1967	0.1500	6.25	1.00	2.68	1.53	28.77	5.30	7.60	7.25	0.00	0.00	-	176	0.98	1.35
8	0.2174	0.1300	29.50	6.00	2.87	1.69	50.00	24.23	7.50	7.50	0.14	0.50	-	243	1.19	1.26
9	0.2359	0.1429	13.75	3.00	3.35	2.60	61.33	21.20	7.65	7.90	0.27	0.96	-	160	1.07	1.14
10	0.2415	0.1603	29.00	16.00	2.75	3.20	66.63	32.56	7.80	7.60	2.24	2.24	-	213	1.01	1.03
11	0.2432	0.1950	45.00	31.00	3.94	3.26	153.00	84.00	7.40	7.70	7.80	11.60	-	425	1.20	1.06

APPENDIX I.3

Appendix I.3 Mean values and standard deviations of soil properties for "solonized brown soils" and "red-brown earths".

Variable	Solonized brown soils : with lime (50 sites) 0-5cm	Solonized brown soils : with lime (50 sites) 5-10cm	Red-brown earths : with no lime (45 sites) 0-5cm	Red-brown earths : with no lime (45 sites) 5-10cm
Total N	0.171 ± 0.003	0.128 ± 0.025	0.126 ± 0.029	0.081 ± 0.021
Ln. Available P	3.07 ± 0.57	1.92 ± 1.00	3.25 ± 0.52	2.18 ± 0.98
pH	7.64 ± 0.23	7.70 ± 0.25	7.08 ± 0.26	7.30 ± 0.32
Bulk density	1.25 ± 0.13	1.21 ± 0.13	1.40 ± 0.14	1.34 ± 0.15
Total P[‡]	257 ± 51		191 ± 34	
% Clay[‡]	18.4 ± 6.1		19.0 ± 8.6	

[‡]Measured in the 0-10cm depth only.

APPENDIX I.4

Appendix I.4 Seasonal May-October rainfall for the period 1925-1974 in the Mallala district.

Year	May-October rainfall (mm)	Year	May-October rainfall (mm)
1925	251	1950	278
1926	334	1951	378
1927	210	1952	319
1928	229	1953	268
1929	178	1954	166
1930	265	1955	312
1931	306	1956	323
1932	327	1957	174
1933	237	1958	331
1934	221	1959	115
1935	240	1960	300
1936	204	1961	144
1937	251	1962	231
1938	140	1963	294
1939	274	1964	231
1940	127	1965	199
1941	304	1966	223
1942	311	1967	124
1943	181	1968	353
1944	135	1969	225
1945	235	1970	228
1946	208	1971	266
1947	286	1972	173
1948	234	1973	294
1949	280	1974	352

APPENDIX II.1

Appendix II.1 Analysis of variance for acetylene reduction activity : F values for the different variables (Early sowing date).

For each harvest:

Age from sowing in weeks	AR ($\mu g\ min^{-1}\ cm^{-3}$ soil)	AR per plant‡ ($\mu g\ min^{-1}\ plant^{-1}$)	Shoot dry-weight ($g\ m^{-2}$)	Plant Population‡
8	7.4**	18.6***	35.4***	54.6***
12	4.3*	10.0***	6.1**	25.0***
16	3.9*	11.7***	7.7**	56.4***
20	1.4	2.4	0.5	19.2***
24	0.4	1.9	1.4	2.8
28	0.8	1.0	0.7	1.0
32	0.8	1.3	4.1*	5.6**

For all harvests together:

Sowing density	3.2*	25.4***	1.8	8.4***
Age of plants	14.2***	38.5***	57.2***	40.6***
Interaction (density x age)	1.2	1.6*	1.5	8.1***

‡ Because of the heterogeneity of the original data and the skewness in the distribution of these data, both AR values and plant population numbers were replaced by their logarithms(e) for statistical analysis.

APPENDIX II.2

Appendix II.2 Analysis of variance for acetylene reduction activity : F values for the different variables (Late sowing date)

For each harvest:

Age from sowing in weeks	AR (μg min^{-1} cm^{-3} soil)	AR per plant‡ (μg min^{-1} $plant^{-1}$)	Shoot dry-weight (g m^{-2})	Plant Population‡ (m^{-2})
5	0.5	157.1***	85.6***	132.4***
9	(2.7)‡	16.7***	86.5***	60.8***
13	8.1**	13.2***	20.3***	26.1***
17	1.7	6.3**	4.2*	50.8***
21	3.2*	1.3	(2.9)‡	3.0*
25	(2.9)‡	1.3	0.5	2.0

Over-all significance

For all harvests together:

Sowing density	11.9***	7.25***	23.3***	51.5***
Age of plants	40.9***	50.2***	141.3***	31.1***
Interreaction (density x age)	2.1**	5.7***	5.0***	5.8***

(2.7)‡ P = 0.065; (2.9)‡ P = 0.054

‡ Data for AR values and plant population numbers were transformed to logarithm (e) for statistical analysis.

APPENDIX II.3

Appendix II.3 Mean values of acetylene reduction rate ($\mu g\ min^{-1}\ cm^{-3}$ soil) for the field experiment.

Age of plants in weeks	Sowing densities (kg ha^{-1}) 0	1	5	10	50	100	500	1000	Mean
(a) Early sowing date:									
8	3.33	1.94	2.10	2.24	2.56	6.02	10.17	4.78	4.14
12	1.58	1.50	1.50	1.92	4.00	7.58	8.08	7.67	4.23
16	9.00	9.33	15.67	11.17	14.00	14.00	34.67	96.00	23.25
20	3.00	4.67	15.50	11.75	9.33	4.00	28.00	19.83	12.79
24	4.00	7.00	32.17	6.00	13.83	5.67	33.33	6.67	13.58
28	4.17	8.00	12.33	10.17	8.83	6.75	4.67	5.00	7.52
32	3.17	10.25	6.93	5.17	3.50	5.00	4.33	4.33	5.34
Mean	4.04	6.10	12.31	6.44	8.01	6.96	17.61	16.84	9.86
(b) Late sowing date:									
5	1.50	1.67	1.67	1.58	1.67	1.58	1.67	1.83	1.65
9	9.00	9.00	9.00	9.00	9.00	22.00	16.53	22.50	13.48
13	3.17	3.17	3.33	3.17	4.00	3.50	11.17	22.67	6.77
17	4.00	4.17	12.00	30.83	41.17	14.83	31.67	30.00	21.08
21	4.00	4.50	15.33	11.67	22.50	13.25	70.00	19.83	20.34
25	4.00	5.58	5.17	5.42	19.17	4.25	10.73	6.08	7.55
Mean	4.28	4.68	7.68	10.28	15.88	9.71	23.68	17.15	11.68

APPENDIX III.1

Appendix III.1 Results of the glasshouse experiment : effects of applied phosphate on the acetylene reduction of species in monoculture and in mixture.

Key to symbols used:

Abbreviation	Variable name
REP	Replication
COM	Community: 1 = Medic monoculture 2 = Medic + grass 3 = Ryegrass monoculture
PHO	Phosphate application rate: (ppm per pot) 1 = 0 2 = 10 3 = 25 4 = 63 5 = 160 6 = 400 7 = 1000 8 = 2500
NPT	Acetylene reduction rate per pot
NPL	Acetylene reduction rate per plant
SPT	Shoot dry weight per pot
RPT	Root dry weight per plant
SPL	Shoot dry weight per plant
CNS	Concentration of nitrogen in shoots
CNR	Concentration of nitrogen in roots
Mm	Medic in mixture
Gm	Grass in mixture

REP	COM	PHO	NPT	NPL	SPT	RPT	SPL	CNS	CNR
1	1 Mp	1	73.00	14.60	.50	.44	.10	33.90	30.20
1	1	2	140.00	23.33	.71	.64	.12	32.50	27.30
1	1	3	147.00	24.50	1.35	.95	.23	25.50	23.40
1	1	4	705.00	117.50	3.00	2.09	.50	30.20	28.40
1	1	5	1304.00	217.33	4.00	2.19	.67	30.70	28.70
1	1	6	924.00	154.00	3.35	1.88	.56	36.20	28.50
1	1	7	673.00	112.17	2.25	1.52	.38	35.50	27.70
1	1	8	59.00	11.80	1.00	.49	.20	32.60	23.40
2	1	1	3.00	.50	.30	.35	.050	33.60	35.90
2	1	2	168.00	28.00	.90	.75	.15	29.70	29.80
2	1	3	252.00	42.00	1.50	1.03	.25	29.70	26.60
2	1	4	535.00	89.17	2.90	1.72	.48	27.50	24.50
2	1	5	774.00	129.00	2.68	1.54	.45	27.00	26.10
2	1	6	677.00	112.83	2.40	1.37	.40	29.50	23.60
2	1	7	636.00	106.00	2.10	1.38	.35	29.50	25.50
2	1	8	125.00	25.00	1.22	.46	.24	29.00	27.30
3	1	1	20.00	5.00	.25	.32	.060	32.40	31.50
3	1	2	68.00	11.33	.80	.53	.13	31.20	26.80
3	1	3	452.00	75.33	1.52	1.16	.25	34.40	30.70
3	1	4	766.00	127.66	2.90	1.94	.48	25.20	23.20
3	1	5	713.00	118.83	3.85	2.04	.64	29.90	29.70
3	1	6	798.00	133.00	3.30	1.90	.55	35.20	26.80
3	1	7	495.00	82.50	2.05	1.21	.34	34.50	22.60
3	1	8	30.00	6.00	.75	.32	.15	29.90	25.90
1	2 Mm	1	18.00	6.00	.30	1.53	.10	23.30	20.60
1	2	2	4.00	2.00	.20	2.85	.10	24.50	19.30
1	2	3	37.00	12.33	.40	3.10	.13	23.50	17.50
1	2	4	324.00	108.00	1.60	3.45	.53	28.20	15.20
1	2	5	636.00	212.00	1.80	4.09	.60	32.50	18.20
1	2	6	422.00	140.66	1.80	3.84	.60	31.10	15.30
1	2	7	154.00	51.33	.70	4.60	.23	30.80	16.80
1	2	8	12.00	12.00	0	14.96	0	34.60	16.60
2	2	1	14.00	4.67	.10	1.41	.030	26.60	19.40
2	2	2	39.00	13.00	.30	3.32	.10	25.10	20.80
2	2	3	40.00	13.33	.20	3.12	.070	25.90	18.00
2	2	4	243.00	81.00	1.35	5.14	.45	30.20	18.90
2	2	5	566.00	188.67	1.80	3.60	.60	33.50	15.70
2	2	6	539.00	179.67	1.45	3.71	.48	35.90	18.80
2	2	7	203.00	67.67	.50	3.61	.17	31.00	18.90
2	2	8	7.00	3.50	.050	2.30	.030	21.00	16.50
3	2	1	0	0	.11	1.43	.040	25.30	19.40
3	2	2	20.00	6.67	.20	2.09	.070	23.50	19.50
3	2	3	58.00	19.33	.50	3.23	.17	31.80	13.00
3	2	4	150.00	50.00	.75	3.93	.25	26.00	16.30
3	2	5	247.00	82.33	.98	3.80	.33	19.70	13.10
3	2	6	353.00	117.67	1.30	3.39	.43	29.10	22.30
3	2	7	402.00	134.00	.90	2.50	.30	30.80	14.50
3	2	8	5.00	5.00	0	3.70	0	41.20	10.40
		MIN	0	0	0	.32	0	19.70	10.40
		MEAN	313.12	68.30	1.31	2.44	.28	29.80	22.27
		MAX	1304.00	217.33	4.00	14.96	.67	41.20	35.90
1	2 Gm	1	18.00	6.00	1.27	1.53	.42	20.50	20.60
1	2	2	4.00	2.00	2.35	2.85	.78	10.30	19.30
1	2	3	37.00	12.33	2.02	3.10	.67	11.90	17.50
1	2	4	324.00	108.00	1.70	3.45	.57	11.30	15.20
1	2	5	636.00	212.00	2.00	4.09	.67	10.30	18.20
1	2	6	422.00	140.66	2.11	3.84	.70	9.60	15.30
1	2	7	154.00	51.33	2.10	4.60	.70	10.60	16.80
1	2	8	12.00	12.00	2.35	14.96	.78	10.50	16.60
2	2	1	14.00	4.67	.80	1.41	.27	23.00	19.40
2	2	2	39.00	13.00	2.50	3.32	.83	9.60	20.80
2	2	3	40.00	13.33	2.00	3.12	.67	10.00	10.00
2	2	4	243.00	81.00	1.50	5.14	.50	10.80	18.90
2	2	5	566.00	188.67	2.05	3.60	.68	10.40	15.70
2	2	6	539.00	179.67	2.00	3.71	.67	10.70	18.80
2	2	7	203.00	67.67	2.10	3.61	.70	9.70	18.90
2	2	8	7.00	3.50	2.48	2.30	.83	12.20	16.50
3	2	1	0	0	.75	1.43	.25	23.80	19.40
3	2	2	20.00	6.67	2.18	2.09	.73	10.20	19.50
3	2	3	58.00	19.33	2.30	3.23	.77	9.50	13.00
3	2	4	150.00	50.00	1.80	3.93	.60	9.80	16.30
3	2	5	247.00	82.33	1.91	3.80	.64	8.70	13.10
3	2	6	353.00	117.67	1.90	3.39	.63	9.70	22.30
3	2	7	402.00	134.00	1.80	2.50	.60	12.30	14.50
3	2	8	5.00	5.00	2.70	3.70	.90	9.20	10.40
1	3 Gp	1	24.00	4.00	1.52	2.21	.25	16.30	14.40
1	3	2	19.00	3.17	2.45	3.17	.41	7.50	11.80
1	3	3	11.00	1.83	2.20	2.98	.37	9.00	18.30
1	3	4	16.00	2.67	2.15	3.20	.36	9.70	17.30
1	3	5	20.00	3.33	1.90	3.90	.32	9.70	14.20
1	3	6	28.00	4.67	2.25	15.13	.38	9.80	17.30
1	3	7	16.00	2.67	2.27	3.23	.38	9.50	16.60
1	3	8	19.00	3.17	2.70	15.51	.45	11.30	15.30
2	3	1	15.00	2.50	1.50	2.44	.25	15.50	16.40
2	3	2	18.00	3.00	2.30	4.00	.38	8.80	15.20
2	3	3	15.00	2.50	2.30	3.67	.38	8.80	19.20
2	3	4	28.00	4.67	2.20	3.71	.37	8.80	16.50
2	3	5	16.00	2.67	2.48	3.55	.41	8.60	12.10
2	3	6	20.00	3.33	1.87	4.60	.31	10.10	19.90
2	3	7	12.00	2.00	2.20	4.00	.37	11.10	15.90
2	3	8	16.00	2.67	2.50	3.25	.42	10.20	22.70
3	3	1	21.00	3.50	1.20	1.45	.20	18.40	16.70
3	3	2	15.00	2.50	2.08	2.53	.35	8.90	16.40
3	3	3	23.00	3.83	2.00	4.16	.33	7.60	13.70
3	3	4	20.00	3.33	2.18	3.71	.36	8.70	11.50
3	3	5	24.00	4.00	2.18	3.82	.36	8.40	12.20
3	3	6	28.00	4.67	2.20	3.43	.37	9.30	15.70
3	3	7	15.00	2.50	2.50	3.13	.42	8.30	11.70
3	3	8	24.00	4.00	2.60	2.95	.43	9.70	11.90
		MIN	0	0	.75	1.41	.20	7.50	10.40
		MEAN	103.25	33.08	2.05	4.01	.50	11.01	16.41
		MAX	636.00	212.00	2.70	15.51	.90	23.80	22.70

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