Nitrogen Fixation and Cycling in Natal Valley Bushveld *Acacia* Species.

by

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ABSTRACT

Five species, *Acacia karroo*, *A. robusta*, *A. nilotica*, *A. sieberana* and *A. tortilis*, were inoculated with *Rhizobium* and grown in potted sand in a temperature controlled greenhouse. After six months, results showed a higher percentage plant nitrogen for all five species when inoculated plants were compared to uninoculated controls. Inoculated treatments of *A. karroo* and *A. sieberana* had the greatest growth in shoot length and biomass. *Acacia robusta* showed the highest nitrogenase activity when nodules were tested using acetylene reduction methods. Inoculants of *A. tortilis* showed the poorest growth for all parameters measured.

A. karroo and *A. nilotica* were studied at a field site at Ashburton, 15km east of Pietermaritzburg. *Acacia karroo* and *A. nilotica* had similar mean percentage leaf nitrogen, but *A. karroo* had a significantly higher mean percentage stem nitrogen than *A. nilotica*. Rainfall, canopy throughfall and stemflow from *A. karroo* and *A. nilotica* were collected in late spring and examined for inorganic nitrogen content. *Acacia nilotica* yielded the highest nitrate levels in both throughfall and stemflow samples. *Acacia karroo* produced lower nitrate concentrations in samples of both throughfall and stemflow, than was found in rainfall. Both *A. nilotica* and *A. karroo* exhibited higher concentrations of ammonium in samples of throughfall and stemflow as compared to levels found in rainfall.

Soil analyses yielded highest levels of organic nitrogen at the surface (0 - 5 cm) but this decreased significantly at 20 cm deep. Surface organic nitrogen was highest under *A. karroo* canopies and lowest in open grassland. At 20 cm, there was little difference in organic nitrogen content between soils sampled from open patches and those under canopies of *A. nilotica* or *A. karroo*. Nitrate showed little variation with species, but highest levels were found in the top five centimetres and levels were higher under grasslands than under canopies. Ammonium showed no significant differences between different depths but was higher in open grassland sites than under canopies. No pattern could be found to relate tree size to soil organic nitrogen content.

PREFACE

The experimental work described in this dissertation was carried out in the Department of Botany, University of Natal, Pietermaritzburg, from January 1993 to December 1994, under the supervision of Prof R.I. Yeaton and Dr. J.E. Granger.

These studies represent original work by the author and have not been submitted in any other form for any degree or diploma to any University. Where use has been made of the work of others it is duly acknowledged in the text.

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Chapter 1 INTRODUCTION

The province of KwaZulu-Natal lies on the east side of the southern African subcontinent, below the Drakensberg escarpment, between c. 31°10′ and 26°50′ S and 29°00′ and 33°55′ E. In the east, the province borders the Indian Ocean; hence its climate is strongly influenced by the warm tropical Mozambique and Agullas ocean currents. The climate is sub-tropical at the coast, becoming more temperate with altitude.

The region has experienced two completely different forms of agriculture, namely subsistence and commercial agriculture, which still co-exist today. For much of its early history, from c. 2000 BP to early 1840's, agricultural practice revolved around a low impact, semi-nomadic subsistence culture (Guest and Sellers, 1985). These so-called Iron Age subsistence farmers will be called rural agriculturalists. Once colonialism fully established itself, private ownership and economically productive methods became the dominant agricultural system in Natal. Rural agriculturalists were pushed off the more productive land into the arid valleys and steep mountainous uplands. These valleys are hot and dry and support the distinctive flora identified by Acocks (1953) as the Northern variation of Valley Bushveld (Veld type 23a). Acocks separated VT 23a from his Southern variation on the basis that it included more grass, less succulents and more species of a tropical nature. Acocks felt that a climax state in this vegetation was characterised by scrub forest or dense

savanna.

The changes in land use since the mid 1800's have produced many environmental management problems. In rural areas, the local population has increased rapidly causing serious overgrazing by cattle and goats. This has resulted in species of early woody succession dominating the floral composition. These areas usually show varying degrees of stress in the form of soil erosion and reduced species richness. A different set of problems has evolved where commercial agriculture is the dominant landuse. Intensive agricultural practices have resulted in an invasion of pioneer woody species out of the valleys and into these important grasslands. This invasion of woody species has only recently been documented (Le Roux, pers. comm.). In both cases the genus *Acacia* Wild. of the family Fabaceae, subfamily Mimosoideae plays a prominent role. The genus *Acacia* includes in excess of 900 species, has a tropical to subtropical distribution and is found in Australia, Asia, Africa and North and South America (New, 1984).

One general aim of this study was to examine specific aspects of the nitrogen cycle in the immediate vicinity of *Acacia* trees. This included investigating whether all or any of the species of *Acacia*, characteristic of Valley Bushveld, fixed nitrogen symbiotically. The study focused on five species: *Acacia karroo* Hayne, *A. nilotica* (L.) Willd. ex Del. var. *kraussiana* (Benth.) Brenan, *A. robusta* Burch. var. *clavigera*

(E. Meyer) Brenan, *A. sieberana* DC var. *woodii* (Burtt Davy) Keay and Brenan, and *A. tortilis* (Forsk.) Hayne (Coates-Palgrave, 1977). Apart from *A. sieberana* and *A. robusta*, the other three species play an important role as pioneers in the succession from grassland to woody bushveld. Furthermore in the areas of KwaZulu-Natal Valley Bushveld exposed to excessive overgrazing, soil erosion and population pressure, these *Acacia* species comprise some of the few indigenous species left. *Acacia robusta* and *A. sieberana* are of uncertain ecological status, with *A. robusta* possibly associated with later woody successional stages and *A. sieberana* associated with high elevation open grassland savanna where wildfires are frequent.

What are *Acacia* trees? Where do they come from and what ecological pressures operate on them? African *Acacia* species are woody shrubs, trees or less frequently vines with thorns which may arise as modified stipules or as superficial prickles (Coates-Palgraves, 1977). These thorns are probably a defence mechanism against herbivory. The extreme elaborations of these thorns exhibited by this genus in Africa support this concept. Observing an African elephant (*Loxodonta africana*) delicately destroying an Acacia tree, the need for these defences can be appreciated. Acacia's are found throughout sub-saharan Africa. They have ecological limits along moisture, temperature and altitudinal gradients. They can withstand arid conditions. *A. sieberana* is often found as the only woody tree in the upland and midland

grasslands and has the highest altitudinal niche of the KwaZulu-Natal species. It shows greater frost tolerance. All species, except possibly *A. robusta*, show some form of fire tolerance.

Taxonomically the genus is rather complex and I have followed that of Coates-Palgrave (1977). Of the five species studied, morphological distinctions are strong and hybridisation is probably rare. The five species range in shape from flat-topped umbrellas to ball-shaped canopies. The life strategies of these species seems to be somewhere between r- and K-selected attributes. This is supported by their strong seed dormancy but quick germination time, and their position as pioneers in woody successional patterns (Le Roux, pers. comm.). They show swarming tendencies in response to positive environmental fluctuations. Furthermore, they tend to show strong coppice reaction to shoot death. Under African conditions, the species are relatively robust and flexible.

It has been estimated that 10^8 tons of N_2 gas are fixed each year by natural and biological means with the bulk coming from the latter (Nutman, 1965). The above information leads to the basic question of this research - do southern African *Acacia* species have this ability to form effective symbioses and fix atmospheric nitrogen? The addition of nitrogen has been shown to be a major factor modifying successional dynamics in a number of ecosystems following disturbance (McLendon and Redente, 1992; Charley and West, 1972). New (1984) notes that

acacias are valuable in nitrogen replenishment of soils and land restoration. An example is *A. holoserica* which was used to restore nutrient-depleted backfill soils after surface mining activities in the Northern Territory of Australia (Langkamp *et al*, 1979, 1982) Acacias are often useful as forage with both foliage and pods eaten. In Africa, *A. tortilis* and *A. albida* provide an important nutrient supplement for livestock in the dry season (New, 1984).

This study composed the following major components:

1.1 Green house experiments

The five species studied in this component of the study were *Acacia karroo*, *A nilotica*, *A. robusta*, *A. sieberana* and *A. tortilis*. To determine if Acacia trees can fix nitrogen, seeds were inoculated with the rhizobium bacteria and grown in the greenhouse in acid-washed quartz sand. A modified Hoaglands solution, lacking in nitrogen, was fed to the seedlings once a week. The plants were then harvested and total plant dry mass, root dry mass and length, shoot dry mass and length, nodule dry mass, nodule number and level of acetylene reduction by nodules determined. Total plant tissue nitrogen was determined by Kjeldahl analysis. Chapter 2 discusses this experiment in more detail.

1.2 Field experiments on specific aspects of the nitrogen cycle

The nitrogen cycle in the vicinity of Acacia trees is a much more difficult problem to quantify accurately. My approach has been to focus on specific aspects of the nitrogen cycle. *Acacia karroo* and *A. nilotica* were the two species studied in the field as they were the dominant *Acacia* species on the field site. Total nitrogen and available inorganic nitrogen were determined for soil samples collected under *Acacia* canopies and in the open. Shoot nitrogen levels in *Acacia* trees were determined. Rainfall was collected and inorganic nitrogen levels quantified to examine inputs and movement of nitrogen into the soil. Rainfall was partitioned into openfall, canopy throughfall and stem flow. Total nitrogen was determined by Kjeldahl analysis while steam distillation was used to determine inorganic forms of nitrogen. These experiments and their results are discussed in more detail in chapters 2, 3 and 4. Chapter 5 is a discussion and summary of the results and conclusions of the research.

1.3 The study site

The study site is located at c. 29°40′ S and 30°25′ E, on private property in Ashburton, approximately 15 km southeast of Pietermaritzburg. The site is approximately 750 m above sea level and the climate subtropical to temperate. The following climatic data were obtained from the Computer Centre

for Water Research, University of Natal, Pietermaritzburg (Figure 1.3.1). These data come from the nearest representative weather station to the study site at Camperdown, 29° 43′ S and 30° 33′ E at an altitude of 762 m.

The study site is surrounded on three sides by private dwellings. The fourth side is bordered by a stream covered with thick riverine bush. The site is situated on a west-facing, gentle slope. Mammals seen at the site include common duiker (*Sylvicapra grimmia*) and scrub hare (*Lepus saxatilis*). There was also much evidence of small unidentified rodent activity with many tunnels through the grass and a few well-used holes in the ground. The site was visited by many bushveld bird species but none of these were identified or counted. Insect fauna were also not studied.

The vegetation forms a typical savanna with the woody component dominated by *Acacia karroo* and *A. nilotica* and a grassland component dominated by *Panicum maximum* Jacq. Other woody species include *Clerodendrum glabrum* E. Meyer, *Ehretia rigida* (Thunb.) Druce, *Acacia tortilis*, *A. sieberana*, *Dichrostachys cinerea* (L.) Wight and Arn. and *Ziziphus mucronata* Willd. Judging from the number of juvenile acacias in the grassland matrix, the successional sequence seems to be moving from an open savanna grassland with isolated bush clusters to a closed canopy woodland. Soil type is a Glenrosa form of the Dumisa family (Granger, pers. comm.) following the South African binomial system of soil classification (Soil

a) 762 m b) 15 c)53 d) 23.64 °C e)684.5 mm

f) 37.5 °C

g) 25.7 °C

h) 9.43 °C

i) 8.2 °C

j) 2.3 °C

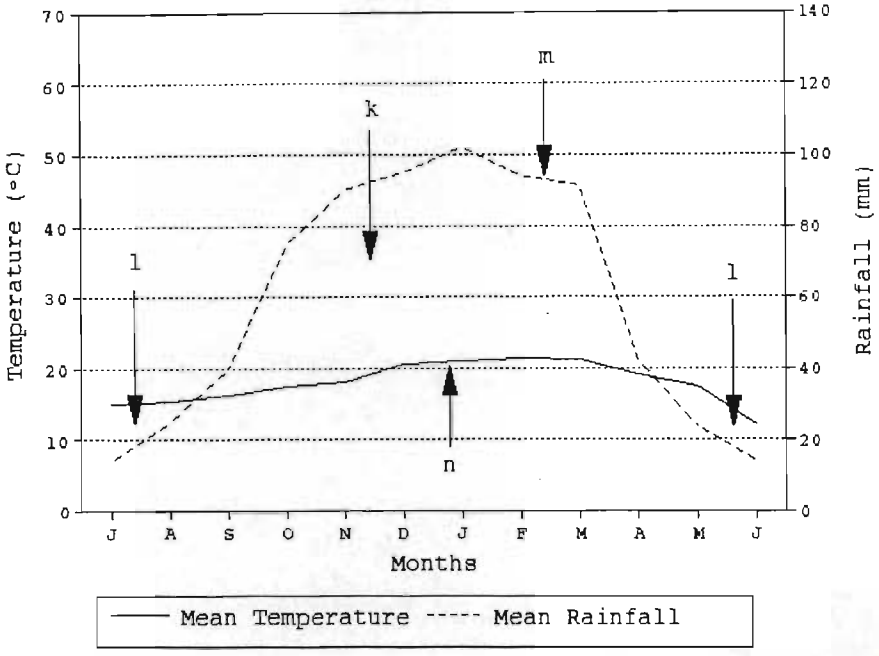


Figure 1.3.1 Climate diagram for the nearest representative field station to the field site. This weather station was located at Camperdown (29°43′ S, 30°33′ E). The data was obtained from the Computer Centre for Water Research (CCWR), University of Natal, Pietermaritzburg. The diagram format is after Walter (1985). Letters refer to: station altitude (a); number of years recording temperatures (b); number of years recording rainfall (c); mean annual temperature (d); mean annual rainfall (e); highest temperature recorded (f); maximum mean daily temperature of hottest month (g); mean daily temperature fluctuation (h); minimum mean daily temperature of coldest month (i); lowest temperature recorded (j); period of relative moisture availability (k); period of relative drought (l); curve of mean monthly rainfall (m) and curve of mean monthly temperature (n).

Classification Working Group, 1977). The profile is shallow (< 15 cm) to moderately developed (c. 25 cm) and had a relatively high clay content making it particularly difficult to find root nodules.

1.4 The nitrogen cycle

The nitrogen cycle is extremely complex because of the many natural compounds that nitrogen can form, the number of transformations between these compounds, and the influence of complex environmental and biological factors on their formation. Nitrogen occurs both as a gas in the atmosphere and bound in the earth in sedimentary and primary rocks (Postgate, 1978). Nitrogen locked up in rocks in the earth's crust is generally not available to biological processes. Nitrogen occurring in the atmosphere is dinitrogen gas (N_2) and forms 79.08% of the atmosphere (Stevenson, 1965). The atmosphere is the most available source of nitrogen for assimilation by organisms. Atmospheric nitrogen must be converted to inorganic nitrogen, either ammonia (NH_3) or nitrate (NO_3). Only then can most living organisms assimilate it. To compound the problem, N_2 is an inert gas and as such does not readily form compounds in nature. Only a few organisms (some prokaryotes) can use elemental nitrogen (by nitrogen fixation). All other organisms must obtain their nitrogen either directly or indirectly as combined nitrogen.

For clarity, it is important to define some terms and

processes. The process of converting N_2 gas to inorganic nitrogen compounds is called nitrogen fixation (Postgate, 1978). Assimilation is the term applied to the biological conversion of inorganic nitrogen to organic nitrogen such as amino acids and protein (Postgate, 1978). Uptake refers to the gathering of nitrates and ammonium by plant roots and must occur before assimilation can occur.

1.4.1 What is the nitrogen cycle

Nitrogen is cycled between the atmosphere, soil, soil microfauna, plants and animals (Figure 1.4.1.1). Switzer and Nelson (1972) defined the cycling processes in forest ecosystems in terms of (a) the biogeochemical cycle between the plant and the soil, (b) the biochemical cycle of internal transfer within the plant and (c) the geochemical cycle of import-export. Woodmansee (1978) separated the cycling process into biological cycles, geological cycles and meteorological cycles. He described three linking vectors between these cycles - water, atmosphere (gases, aerosols, and particulates), and animals. Much of the early research was focused on forests and the hydrological aspect of cycling. As a result, it was considered that water was the most important of these vectors (Duvigneaud and Denaeyer-DeSmet, 1975) and animals or consumers played an insignificant part in nitrogen cycling in forests (Bormann and Likens, 1967; Burton and Likens, 1975; Sturges, Holmes and Likens, 1974). Woodmansee (1978) studying North American grasslands disputes both these

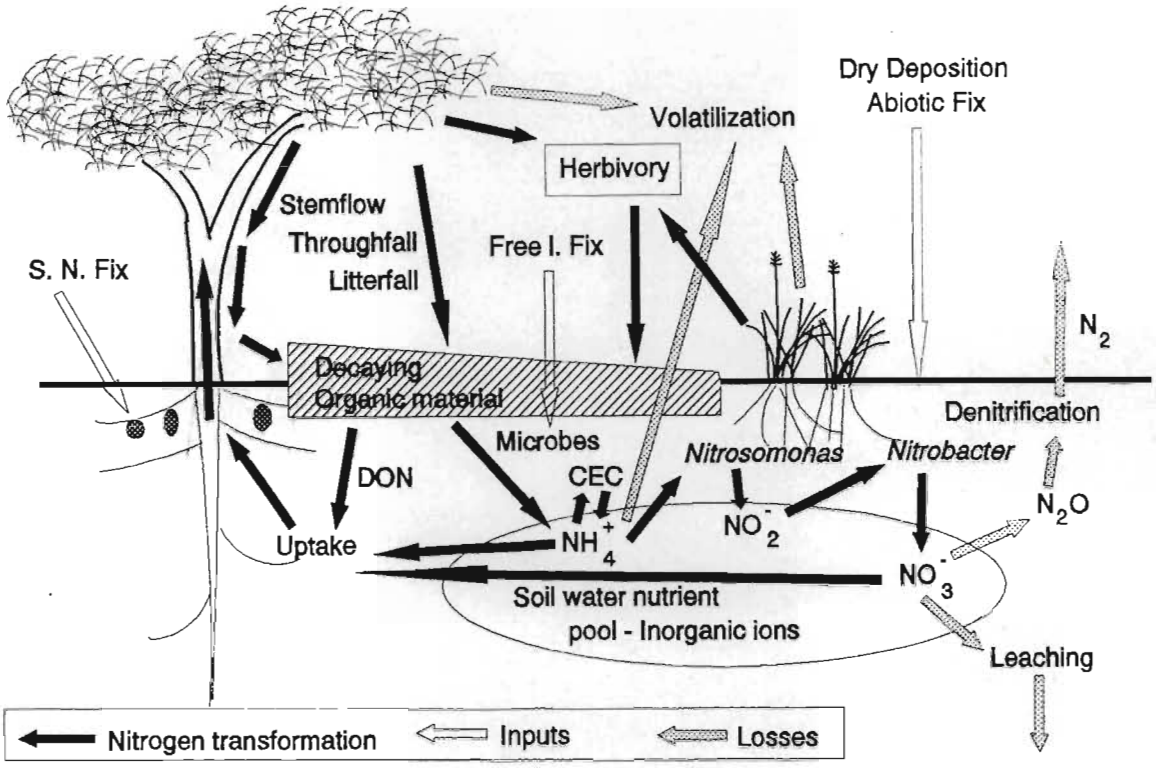


Figure 1.4.1.1 The nitrogen cycle: A stylized diagram of the flow of nitrogen around a single hypothetical *Acacia* tree. Abbreviations and symbols stand for: symbiotic nitrogen fixation (S.N.Fix); free-living fixation (Free l. Fix), dissolved organic nitrogen (DON), cation exchange capacity (CEC), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O) and dinitrogen gas (N_2).

ideas as they may be relevant in forests, but not in grasslands. Woodmansee (1978) believes consumers play a critical role in grasslands, and the hydrological cycle is of lesser importance in grasslands than in forests.

Most approaches to nutrient cycling deal with inputs and outputs in relation to succession and stability. It has been proposed that as ecosystems progress towards a climax condition, they tend to become more conservative of nutrients, and finally reach an input-output ratio equal to one. This implies that losses exceed inputs until the ecosystem reaches maturity (Bormann and Likens, 1967; Odum, 1969; Pomeroy, 1970; Jordan and Kline, 1972). Vitousek and Reiners (1975) suggested that ecosystems accrue nutrients in the middle stages of succession. At maturity, losses increase to equal inputs. Initially, nutrients may be lost from recently disturbed ecosystems because insufficient biomass is present to retard losses from leaching, runoff and volatilization (Woodmansee, 1978). Woodmansee (1978) suggests that vegetation and microorganisms of all successional stages have the capacity to take up all the NH_4^+ and NO_3 mineralized.

Under natural conditions, gains in nitrogen occur through fixation of elemental nitrogen by microorganisms, and from the accession of ammonia and nitrate in rain water (Eriksson, 1952; Stock and Lewis, 1986). Losses occur through crop removal, leaching, volatilization, fire and denitrification (Stevenson, 1965; Woodmansee, 1978; Hayes and Seastedt, 1989).

Nitrogen may be transformed by micro-organisms from organic forms to ammonium. This process is called ammonification and is the first step in nitrogen mineralisation. Specific bacteria may then convert this ammonium to nitrite and then nitrate. This process is called nitrification and is the final step in the mineralisation process.

1.5 Conclusion

The aspects of the nitrogen cycle discussed above illustrate clearly the intense complexity of nitrogen cycling in ecosystems. As a result, my approach has been to examine specific aspects as a foundation for further research. I examined inputs in the form of wet deposition and canopy leaching (Chapter 3). Attempts at quantifying inputs from litterfall were unsuccessful due to an inability to trap sufficient quantities of litter in short enough time frames. Soil nitrogen levels, inorganic and organic were quantified (Chapter 4) and plant nitrogen in stems and leaves was measured (Chapter 2). The quantification of soil organic nitrogen includes the soil microfauna as these are digested together with other organic material in the Kjeldahl analysis. Levels of symbiotic nitrogen fixation could not be quantified in the field as virtually no nodules were found. No attempt was made to quantify free living nitrogen fixation. Mechanisms of nitrogen loss were not examined.

Chapter 2 PLANT NITROGEN AND NITROGEN FIXATION

2.1 Introduction

Nitrogen is one of 15 elements known to be required by higher plants (Viets, 1965). It is a basic component of proteins, enzymes, DNA, RNA, and many other important compounds involved in the metabolism of plants. Plants suffering from nitrogen deficiency may exhibit abnormal growth as a result of reduced cell division and cell elongation, reduced photosynthate production and the reduced ability to produce important enzymes, hormones and other proteins. Other common symptoms of deficiency are delayed maturity in some species, a more acute angle between leaf and stem, frequent accumulation of anthocyanins, prolonged dormancy of flower and leaf buds in trees, premature death of lower leaves and possibly accelerated rates of root growth (Viets, 1965). An excess of nitrogen may cause lodging, a reduction in yields and quality, and forage which may be toxic to animals.

The plant root can absorb ammonium (NH_4^+), nitrate (NO_3^-), urea and some simple organic molecules like amino acids, dipeptides, and betaines from the soil or culture solution (Viets, 1965; Scarsbrook, 1965). In nature most nitrogen available to plants is in the nitrate form (Viets, 1965). Ammonium and nitrate are absorbed rapidly like small ions such as K^+ and Cl^-. Ions such as Ca^{2+}, Mg^{2+} and SO_4^{2-} are absorbed at much slower rates (Viets, 1965). Some plants may grow better

on NH_4^+, but generally plants growing in well aerated soil grow better on NO_3^- at all stages of growth (Scarsbrook, 1965).

Some plants, such as legumes, can obtain nitrogen directly from the atmosphere by symbiotic nitrogen fixation. Nitrogen fixation requires the breaking of the triple bond holding the N_2 molecule together. This bond is a strong bond resulting in N_2 gas being relatively inert and requiring 226.2 kcal to break 1 mole of nitrogen gas (Petrucci, 1972). In nature this bond is broken using an enzyme called nitrogenase which is found only in some prokaryotes (Postgate, 1978). The enzyme requires ATP, the magnesium ion Mg^{2+} and a reducing agent to function (Postgate, 1978). The efficiency of the process of reducing nitrogen to ammonia appears to be only about 80 % and results in a minimum energy cost of 15 ATP molecules per N_2 molecule fixed.

The bacterial genus of interest in this research is the *Rhizobium* bacteria. Infection of a N_2-fixing plant by a *Rhizobium* bacteria is a complex process. The first visual symptom of infection is deformation of the root hair (Sprent, 1979). This may be branching or curling of the root hair. The most effective symbioses often occur from the most extreme curling of the root hair (Sprent, 1979). The root then forms a nodule where the infection occurred and the bacteria proliferate within the nodule.

Two types of nodules occur characterized by how they develop.

Observations of *Acacia* nodules in this study suggest they have multi-lobed, spherically elongated nodules, with indeterminate growth. Infection of nodules of the *Acacia*-type have been extensively researched in *Pisum sativum* (Libbenga and Harkes, 1973). There is some evidence that certain plant proteins called lectins can interact with specific rhizobia thus allowing the plant to recognize and admit the correct strain of bacteria (Sprent, 1979). Once a plant has been colonized by a specific bacteria, others are excluded for that growing season. If the soil is reasonably rich in inorganic nitrogen, legumes will not admit rhizobia (Postgate, 1978). The establishment of a viable effective symbioses may be influenced by environmental factors during all or any one of three stages in the infection process (Vincent, 1965). They may (1) affect the occurrence, growth, and survival of the root-nodule bacteria; (2) modify nodule formation; (3) affect the functioning of the formed nodule.

Nearly all soils contain rhizobia. Compatible strains of *Rhizobium* colonize plants at about the stage in the plant's growth season when leaves begin to appear (Postgate, 1978). Nitrogen fixation usually only begins when the plant can safely divert a portion of photosynthate to nodules (Sprent, 1979). In *Acacia holoserica* Langkamp *et al* (1982) found that phyllode growth commenced just before or at the onset of the wet season and nodule growth followed shortly thereafter. They further found that nodule specific activity increased as the wet season progressed and presumed that this was due to an

increase in assimilates available for nodule functioning.

This chapter examines two aspects of Natal Valley Bushveld *Acacia* species. The first aspect is an examination of the effects of inoculation of five species of *Acacia* with pure cultures of *Rhizobium* bacteria under controlled conditions of nutrition and environment. The five species under study in this experiment were *Acacia karroo*, *A. nilotica*, *A. robusta*, *A. sieberana* and *A. tortilis*. The emphasis of this experiment was aimed at growth performance and nitrogen status as a result of inoculation with symbiotic nitrogen-fixing bacteria. The second part of this chapter examines the nitrogen status of leaves and stems, under field conditions, in *Acacia karroo* and *A. nilotica*.

2.2 Green house experiments on five species of *Acacia* inoculated with cultured *Rhizobium* species

2.2.1 Introduction

Symbiotic fixation in legumes may provide the main source of biologically available nitrogen in areas with low total soil nitrogen (Barnet, Catt and Hearne, 1985). In African savannas N_2 fixation by *Acacia* species may be important (Schulze, Gebauer, Ziegler and Lange, 1991) for nitrogen input to nutrient pools.

A number of studies around the world have examined symbiotic fixation in selected *Acacia* species (Umali-garcia, Libuit and

Baggayan, 1988; Galiana, Tibok and Duhoux, 1991; Barnet and Catt, 1991; Dela Cruz, Manalo, Aggangan and Tambolo, 1988; van Kessel, Roskoski, Wood and Montano, 1983; Högberg, 1986). Various species of *Acacia* inoculated with *Bradyrhizobium* had significantly higher total plant mass and total plant nitrogen compared to non-nodulating legumes (*Parkia biglobosa* and *Tamarindus indica*)(Ndoye, Gueye, Danso and Dreyfus, 1995). *Acacia albida* inoculated with *Rhizobium* produced about two times more shoot dry weight than uninoculated controls (Sanginga, Bowen and Danso, 1990). *Acacia mangium* inoculated with *Bradyrhizobium* exhibited significantly enhanced growth parameters such as shoot length and shoot dry weight (Galiana, Tibok and Duhoux, 1991). In another study, inoculation of *Acacia mangium* did not significantly affect dry matter production and nodulation compared to plants treated with nitrogen but was higher than untreated uninoculated controls (Umali-Garcia, Libuit and Baggayan, 1988). Fifty percent of the nitrogen in a *Prosopis glandulosa* stand, a genus closely related to *Acacia*, was estimated to be symbiotically fixed (Rundel *et al*, 1982).

The influence of symbiotic nitrogen fixation on *Acacia* species may vary with age and other factors. *Acacia alata* had a high degree of dependence on symbiotic N_2 fixation during the first growing season but this dependence declined with subsequent growing seasons (Hansen and Pate, 1987b; Hansen, Pate, Hansen and Bell, 1987). *Acacia pulchella* and *A. extensa* fixed substantially more nitrogen in their third to fifth year than

in their first, second or sixth years (Hansen *et al*, 1987). Other factors affecting the quantities of nitrogen fixed by a species are plant habitat, nodule abundance and nodule activity, which in turn are influenced by environmental parameters such as light, temperature and rainfall, both seasonally and diurnally (Lawrie, 1981).
For example, *Acacia pulchella* exhibited strong seasonal nodulation, with virtually no nodules to be found during the hot, dry summer (Monk, Pate and Loneragan, 1981).

Optimum rates of acetylene reduction in *Acacia dealbata* were achieved when average soil moisture content was between 25 - 50 % moisture content at field capacity (Hopmans, Douglas and Chalk, 1983). These authors also showed that addition of nitrate caused a rapid and significant decrease in acetylene reduction by *A. dealbata*. The supply of inorganic nitrogen in the field could have a significant influence over nitrogen fixation and nodulation (Hopmans *et al*, 1983). *Acacia alata* had a high sensitivity to nitrate which decreased symbiotic performance (Hansen and Pate, 1987b). Acetylene reduction appears to be strongly influenced by photosynthate availability since nodule activity in *Acacia pellita* dropped quickly with a fall in photosynthetically active radiation (Langkamp, Swinden and Dalling, 1979).

There is a great deal of variation in reaction to inoculation within a genus and a species. Plants of *Acacia albida* from different localities reacted differently to inoculation and

fertilizer treatment (Sanginga *et al*, 1990). Felker and Clark (1980) recorded a tenfold range in acetylene reduction between 12 species of *Prosopis*. In this greenhouse experiment, five species of *Acacia* were inoculated with pure cultures of *Rhizobium* bacteria. These species were *A. karroo*, *A. tortilis*, *A. nilotica*, *A. robusta* and *A. sieberana*. The aims of this experiment were twofold:

(1) Could Acacia's be successfully inoculated using pure *Rhizobium* cultures.

(2) Would inoculation improve the nitrogen status of inoculated plants compared to uninoculated controls.

2.2.2 Methods and materials

Accurate measurement of root development in soils are difficult because of the difficulty of separating the roots from the soil. Hence much of the work on root systems has used sand or solution cultures and such results may not reflect accurately what happens in soil (Viets, 1965). In this experiment all five species of *Acacia* were grown in acid washed quartz sand. Quartz sand was soaked in a 0.2 M H_2SO_4 solution for 48 hours and then rinsed with tap water for 24 hours. Seeds were hand-scarified using a metal file. After scarification, seeds were coated in a pure solution of their respective *Rhizobium* strains (Vincent, 1970) and these planted out. Seeds were planted 10 mm below the surface. Germination rates were not documented but seedlings were weeded down to one plant per pot. Each plant was fed once a week with 20 ml

of a modified Hoaglands solution which lacked nitrogen (Epstein, 1972)(see Appendix 1). Controls were not inoculated, but seeds were scarified and planted in the same way as the inoculated seeds and fed with the same nutrient solution. Control and inoculated treatments were grown in adjacent greenhouses to avoid contamination. Each treatment consisted of fifteen replicates, each replicate being one pot with one plant.

Seedlings were grown for six months from the time of seedling emergence to harvest. Emergence was defined as occurring when the shoot broke through the surface of the sand medium. After harvest, root length and shoot length were measured. The dry weights of nodules, roots and shoots were measured. The number of nodules per plant was also counted. The nitrogen fixing activity of nodules was measured using acetylene-reduction assays (Hardy, Holsten, Jackson and Burns, 1968; Hansen, Pate and Atkins, 1987) immediately after plants were harvested. There are difficulties in using acetylene reduction to quantify nitrogen fixation rates as the method is indirect and requires integration of nitrogenase activity (Grove and Malajczuk, 1987). Usually a theoretical value of three parts ethylene to one part nitrogen is employed but it has been shown that there can be large departures from this theoretical value (Lawrie, 1981; Hopmans, Chalk and Douglas, 1983; Hansen, Pate and Atkins, 1987). In essence the acetylene reduction assay gives a measure of enzyme activity rather than absolute nitrogen fixing potential. Thus results are given for

micromoles of ethylene produced from acetylene reduction as well as a conversion to nitrogen fixed in milligrams using the 3:1 ratio.

To measure the nitrogen content of roots, shoots and nodules, the plant parts for each species had to be pooled so that only one or two replicates per species could be analyzed. This problem occurred as there was insufficient biomass, especially in the controls, to analyze each plant individually for nitrogen content. Nitrogen content was determined by Kjeldahl analysis (Bremmer, 1965) as a percentage of dry mass.

As the data did not fit the normal distribution, it was transformed to get a better fit (Siegel and Castellan, 1988). It was found that a log (x+1) transformation gave the closest approximation of a normal distribution. The sample size for analysing differences within the various growth parameters was 15 individual plants for each treatment. As nodulation varied, the sample size for the acetylene reduction assay varied from 9 to 13 individual plants per species. This did not include controls as these recorded no nodulation or only a few plants exhibited some nodulation.

Cultures for inoculation of experimental plants were obtained by inoculating seedlings grown in pots with a mixed soil sample from the field. These plants grew in a standard potting soil to maximise their health. A mixed soil sample was made up by collecting individual samples from various

sites in the valleys outside Pietermaritzburg where *Acacia* bushveld was growing and combining them into one soil. This mixed soil sample was used in a dry state to inoculate all five species of *Acacia*. Inoculation was achieved by adding the soil inoculum to the surface of the potting medium. After harvesting, nodules were collected and the bacteria extracted by aseptically crushing the nodules and using the nodule sap to inoculate culture plates (Vincent, 1970). The bacteria were cultured on yeast-mannitol-agar plates (Vincent, 1970).

2.2.3 Results

Inoculating *Acacia* seeds with *Rhizobium* bacteria had a profound effect on all five species. Table 2.2.3.1 and 2.2.3.2 show that in all five species, inoculated plants performed better than did their respective uninoculated controls for the different growth parameters. Mean shoot length and mass (Table 2.2.3.1) were significantly greater ($P \leq 0.05$) for inoculated plants of all five species. Inoculated treatments of *A. karroo* showed the largest mean shoot length (209 ± 36.75 mm) and its control had the smallest mean shoot length (48.3 ± 4.5 mm). *Acacia sieberana* inoculated with *Rhizobium* had the largest mean shoot dry mass (1.347 ± 0.458 g) for any treatment while controls of *A. tortilis* exhibited the smallest mean shoot mass (0.098 ± 0.01 g).

Measurements of root length and root mass (Table 2.2.3.1)

Table 2.2.3.1 Growth parameters measured after six months growth for five species of Acacia inoculated with Rhizobium bacteria and grown in greenhouses. Symbols stand for comparisons between controls (c) and inoculated (i) plants for each species respectively: not significantly different (ns); significantly different, $P \leq 0.05$ (*) or $P \leq 0.01$ (**). n = 15 replicates for each treatment. The statistical test used was an ANOVA.

		A. karroo		A. nilotica		A. robusta		A. sieberana		A. tortilis	
		c	i	c	i	c	i	c	i	c	i
Shoot length (mm)											
	mean	48.33	209	75	138.8	87	164.9	75.53	197.7	60.53	144
	SE	4.516	36.75	4.655	23.48	3.999	25.9	8.177	40.19	3.928	26.8
			**		*		*		**		**
Shoot mass (g)											
	mean	0.154	1.218	0.186	0.613	0.358	0.897	0.236	1.347	0.098	0.428
	SE	0.012	0.279	0.024	0.157	0.025	0.2	0.028	0.458	0.01	0.104
			**		**		*		**		**
Root length (mm)											
	mean	306	247.3	332	309	346.3	311.33	287.7	265.3	317.3	286.7
	SE	16.14	19.09	30.57	17.13	16.38	15.62	9.842	9.455	23.51	16.99
			*		ns		ns		ns		ns
Root mass (g)											
	mean	0.314	1.199	0.181	0.34	0.480	0.547	0.511	2.019	0.184	0.319
	SE	0.038	0.272	0.016	0.072	0.055	0.105	0.066	0.442	0.041	0.081
			**		*		ns		**		ns

produced some interesting results. In all species, controls had longer mean root lengths than did their respective inoculated treatments, but only *A. karroo* showed this difference to be significant ($P \leq 0.05$). *Acacia karroo* inoculated with bacteria had the shortest root length (247.3 ± 19.09 mm). Control treatments of *A. robusta* had the longest root length (346.3 ± 16.38 mm) for all treatments but this was not significantly different from its respective inoculated treatment. Root mass, on the other hand, was greatest in the inoculated treatments of all species compared to the respective controls. This difference in root mass was non-significant in *A. robusta* and *A. tortilis*. Both *A karroo* and *A. sieberana* were significantly different ($P \leq 0.01$) from their respective controls. Inoculated *A. sieberana* had the heaviest mean root mass (2.019 ± 0.442 g).

All five species reacted to inoculation by producing nodules (Table 2.2.3.2). Of the controls, only *A. karroo* and *A. robusta* produced no nodules at all. Controls of the other three species produced a few nodules on one or a couple of the 15 plants in each treatment respectively. It is believed that bacteria responsible for these nodules in the control were a result of contaminantion, possibly from airborne sources. Comparison of nodules on inoculated plants versus controls showed that results were significantly different ($P \leq 0.01$) for all five species (Table 2.2.3.2). Inoculated treatments of *Acacia sieberana* had the highest mean number of nodules (34.1 ± 5.0) while inoculated *A. tortilis*

Table 2.2.3.2 Growth parameters measured after six months growth for five species of *Acacia* inoculated with *Rhizobium* bacteria and grown in greenhouses. Symbols stand for comparisons between controls and inoculated plants for each species respectively: not significantly different (ns); significantly different, $P \leq 0.05$ (*) or $P \leq 0.01$ (**). n = 15 replicates in each treatment. The statistical test used was an ANOVA.

		A. karroo		*A. nilotica*		*A. robusta*		*A. sieberana*		*A. tortilis*	
		c	i	c	i	c	i	c	i	c	i
Number of Nodules (per plant)	mean	0	27.53	2.87	18	0	17.6	6.8	34.13	0.067	16.87
	SE	0	5.329	2.79	3.93	0	4.75	3.842	4.979	0.067	4.691
			**		**		**		**		**
Nodule mass (per plant) (g)	mean	0	0.070	0.001	0.051	0	0.060	0.006	0.079	0.002	0.050
	SE	0	0.017	0.001	0.013	0	0.015	0.003	0.019	0.002	0.014
			**		**		**		**		**
Plant mass (g)	mean	0.468	2.488	0.362	1.003	0.838	1.504	0.732	3.445	0.286	0.799
	SE	0.049	0.563	0.041	0.241	0.078	0.308	0.091	0.852	0.043	0.183
			**		**		ns		**		**

had the lowest mean number of nodules per plant (16.9 ± 4.7) for the inoculated treatments.

Mean nodule mass per plant (Table 2.2.3.2) showed a similar pattern to that for the number of nodules per plant. In all species, inoculated treatments had mean nodule masses per plant significantly greater ($P \leq 0.01$) then their respective controls. Like nodule number, *A. sieberana* had the highest mean nodule mass (0.079 ± 0.019 g) out of the inoculated treatments, while *A.tortilis* had the lowest mean nodule mass (0.05 ± 0.014 g).

Mean total plant mass (Table 2.2.3.2) showed that inoculated treatments of all species except *A. robusta* had significantly greater plant masses ($P \leq 0.01$) then their respective controls had. *Acacia robusta* showed no significant difference from its control. The species with the greatest mean total plant mass (3.445 ± 0.852 g) was *A. sieberana*. *Acacia tortilis* had the lowest mean plant mass for both inoculated treatments (0.799 ± 0.183 g) and control treatments (0.286 ± 0.043 g).

The acetylene reduction assay (ARA) gives a measure of the activity of the nitrogen fixing enzyme in the nodules. Mean rates of acetylene reduction, or rather ethylene production, are given in Table 2.2.3.3. These values are only for inoculated treatments and do not include control data. *Acacia robusta* had the highest rate of ethylene production with a

Table 2.2.3.3 A. Ethylene produced during one hour by the acetylene reduction assay of nodules from five species of *Acacia* inoculated with *Rhizobium* bacteria.
B. Estimated nitrogen fixed by one gram of nodules from five species of *Acacia* inoculated with *Rhizobium* bacteria.
Species with the same letter are not significantly different at $P \leq 0.05$. The statistical test used was an ANOVA.

A Units are umols $C_2H_4.h^{-1}.plant^{-1}$

	A. tortilis	A. robusta	A. nilotica	A. karroo	A. sieberana
n	11	9	12	11	13
mean	1.895^{a}	8.988^{c}	3.089^{ab}	4.957^{bc}	3.516^{ab}
SE	0.671	2.076	0.665	0.813	1.654

B Units are mg nitrogen.h^{-1}.g^{-1} nodules

n	11	9	12	11	13
mean	0.154^{a}	0.467^{b}	0.261^{ab}	0.300^{ab}	0.258^{ab}
SE	0.025	0.101	0.042	0.059	0.071

mean of 8.9 ± 2.1 μmols ethylene.h^{-1}.$plant^{-1}$. This was only significantly greater ($P \leq 0.01$) than values for *A. tortilis*, which was the species with the lowest mean acetylene reduction value (1.9 ± 0.7 μmols ethylene.h^{-1}.$plant^{-1}$).

Estimates of nitrogen fixed from ARA data can be achieved by using a suitable conversion factor. Usually this is in the order of 3 parts ethylene to 1 part nitrogen (Lawrie, 1981; Hopmans, Chalk and Douglas, 1983; Hansen, Pate and Atkins, 1987). Results of this calculation from ARA data are shown in Table 2.2.3.3. In this case data is calculated per gram of nodules rather then on a per plant basis as was done for the ethylene data above. Values for *Acacia robusta* with the highest mean value (0.467 ± 0.101 mg nitrogen.h^{-1}.g^{-1} nodules), were only significantly greater ($P \leq 0.05$) than those of *A. tortilis* which had the lowest mean value (0.154 ± 0.025 mg nitrogen.h^{-1}.g^{-1} nodules). There was no significant differences between either of the other three species or *A. robusta* or *A. tortilis*.

Although *A. robusta* had the highest rates of acetylene reduction or nitrogen fixation, this pattern did not follow through in the nitrogen content of the respective species. Figure 2.2.3.1 illustrates the percentage nitrogen in shoots (A), roots (B) and nodules (C). No statistics are included as the yield of individual plants, especially for the controls, was too small to allow macro-Kjeldahl analysis for total

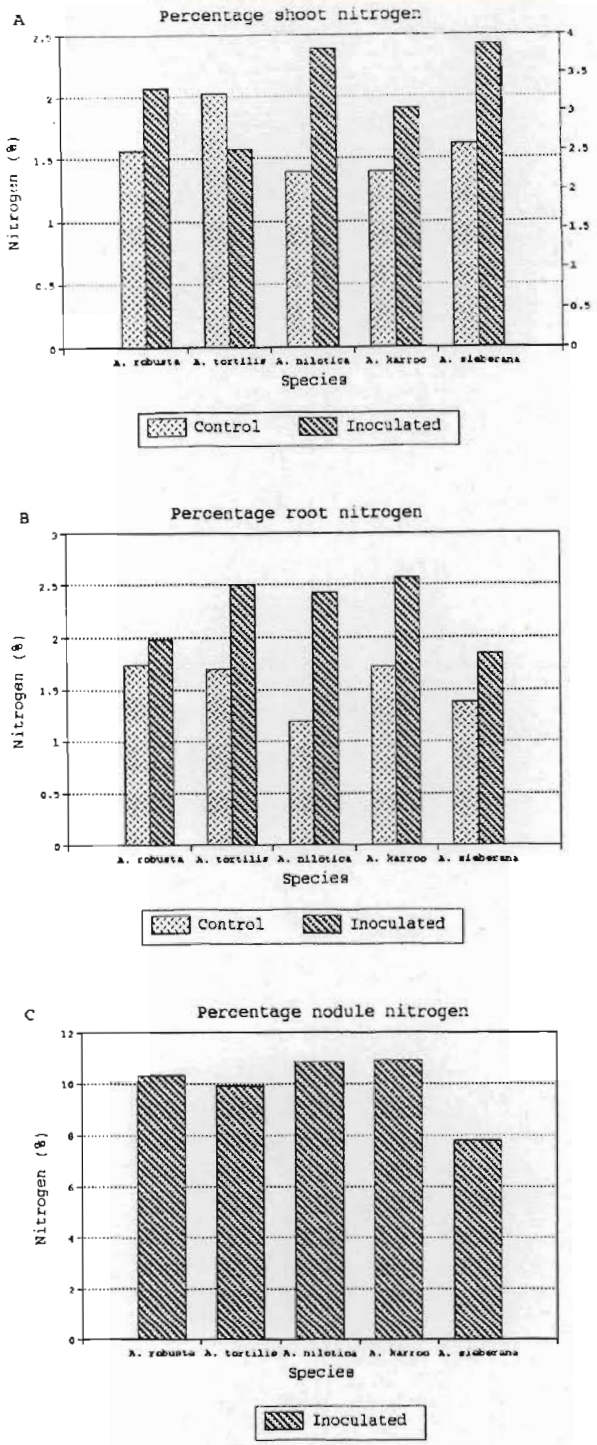


Figure 2.2.3.1 (A) Percentage shoot nitrogen, (B) percentage root nitrogen and (C) percentage nodule nitrogen for five species of *Acacia* inoculated with *Rhizobium* bacteria and grown for six months in a greenhouse.

nitrogen content of individual plants. As a result, there is no replication within a species. Figures 2.2.3.1A and B show clearly that for all five species inoculation resulted in a higher percentage plant nitrogen than uninoculated control plants. In shoots (Figure 2.2.3.1A), the percentage nitrogen content in inoculated plants was twice that of their respective controls. The exception is *Acacia tortilis* where the percentage shoot nitrogen in the inoculated plants was only slightly higher than control plants. Inoculated plants of *Acacia sieberana* had the highest shoot nitrogen content (3.88 %), closely followed by *A. nilotica* (3.82 %). *Acacia tortilis* had the lowest percentage shoot nitrogen for the inoculated treatments (2.51 %), but the highest percentage shoot nitrogen (2.02 %) in uninoculated control plants. Uninoculated control plants of *A. nilotica* and *A. karroo* had the lowest percentage shoot nitrogen (1.4 % each).

Percentage root nitrogen (Figure 2.2.3.1B) followed a different pattern to that of shoot nitrogen. Inoculated plants of all five species still had higher percentage nitrogen values than did their respective uninoculated controls but the difference between inoculated plants and controls was less than that in shoots. Inoculated plants of *A. karroo* had the highest percentage root nitrogen (2.57 %) closely followed by inoculated *A. nilotica* plants (2.51 %). Inoculated plants of *A. sieberana* had the lowest percentage root nitrogen (1.8 %). *Acacia robusta* was only slightly higher with a percentage root nitrogen of 1.98 % for

inoculated treatments. *Acacia robusta* had the highest percentage root nitrogen (1.74 %) in uninoculated control treatments. *Acacia nilotica* had the lowest percentage root nitrogen (1.4 %) for uninoculated control treatments.

Percentage nodule nitrogen (Figure 2.2.3.1C) in all species was much higher than either percentage root or shoot nitrogen with *A. karroo* exhibiting the highest value (10.9 %). This was closely followed by *A. nilotica* (10.8 %), *A. robusta* (10.3 %) and *A. tortilis* (9.9 %). Percentage nodule nitrogen in *A. sieberana* was lower (7.8 %) than the other four species. Only the percentage nitrogen of nodules from inoculated treatments is shown as uninoculated controls produced no nodules or insufficient nodules to allow nitrogen analysis.

2.2.4 Discussion

The results of inoculating acacias with nitrogen-fixing symbiotic bacteria clearly show that inoculated plants of all five species grow larger under the experimental conditions than their respective uninoculated control plants. Generally, of the five species and two treatments, inoculated plants of *A. sieberana* and *A. karroo* performed better than controls or inoculated plants of the other three species in terms of total plant mass, root mass, shoot mass, shoot length, nodule mass and number of nodules per plant. Root lengths support the hypothesis that inoculation with *Rhizobium* is beneficial to the nutrient status of *Acacia* species. Species that were

inoculated had greater root masses than their corresponding controls, but the uninoculated controls had longer root lengths than corresponding inoculated plants. This is probably a result of nitrogen stressed, uninoculated plants searching a greater volume of soil to find nitrogen sources than N_2-fixing inoculated plants do. Measurements of acetylene reduction ability of nodules did not fit the pattern of *A. sieberana* and *A. karroo* as the best performing plants. *Acacia robusta* had by far the largest values for acetylene reduction. It is difficult to separate a species natural growth patterns from effects of inoculation and improved nutrition. Controls without nitrogen amendments were chlorotic and stunted and so provide a poor baseline of natural growth patterns.

The nitrogen status of plants in the experiment supports the hypothesis of improved nutrient status in plants with symbiotic N_2-fixing associations. All plants inoculated with *Rhizobium* had higher nitrogen percentages (almost double in shoots) compared to their respective uninoculated controls. Data from acetylene reduction assays (ARA) did not reflect the percentage nitrogen found in plant tissues. *A. robusta* had the highest ARA value but its percentage shoot and nodule nitrogen for inoculated treatments is third highest. In contrast, *A. tortilis* had the lowest ARA values but also the lowest percentage nitrogen in shoots and nodules.

Relationships between plant nitrogen content and data of other growth parameters such as biomass and length are not clearcut.

Species which had high values with respect to growth parameters such as biomass and length sometimes exhibited high shoot or root nitrogen concentrations but not high values in both root and shoot. The explanation for this is not clear but may be related to a difference in the allocation of nitrogen in different species. It may also be due to the maturity of the symbiosis. Observations during the experiment showed that some inoculated plants remained stunted for a long period before rapidly increasing growth rates compared to their controls. Other inoculated individuals had a much shorter period prior to showing improved growth rates. As a result the length of the experiment may have been to short to allow the symbioses to properly stabilise.

These data compare favourably with some of the similar experiments performed by other researchers on the same genus but not with all of the previously published work. Ndoye *et al* (1995) found plant biomass after five months of 47.2 g, 63.6 g, 184,4 g and 60.2 g per plant for *A. raddiana*, *A. senegal*, *A. seyal* and *F. albida* respectively. Inoculated plants of *Acacia saligna* produced an average plant dry mass of 35 g after four months whereas controls could only produce less than 0.5 g (Stock, Wienand and Baker, 1995). These results are far higher than the results of this study even though this experiment ran for one month longer in the first case and two months longer in the second case.

Umali-Garcia *et al* (1988) found that the height of *Acacia*

mangium inoculants ranged from 18.9 to 23.33 cm and controls averaged 17.02 cm. In my experiment shoot length for inoculants ranged from 13.8 to 20.9 cm while controls ranged from 4.83 to 8.7 cm. Nodule weight for inoculants ranged from 0.082 - 0.240 g and that for controls was 0.112 g (Umali-Garcia *et al*, 1988), while in my experiment inoculants had nodule dry masses from 0.050 to 0.079 g. Shoot biomass for inoculants ranged from 0.88 - 1.33 g and for controls was 0.73 g (Umali-Garcia *et al*, 1988) and root biomass ranged from 0.2664 - 0.4538 g and that for controls was 0.2359 g (Umali-Garcia *et al*, 1988). My study recorded shoot masses for inoculated plants of 0.42 to 1.346 g and root masses of 0.31 to 2.01 g. Total biomass for inoculated plants ranged from 1.15 - 1.77 g while controls weighed 0.96 g (Umali-Garcia *et al*, 1988). In this study total plant biomass ranged from 0.79 to 3.44 g in inoculated treatments, while controls had a range of 0.28 - 0.84 g. Another study also on *Acacia mangium* recorded shoot dry weights between 47.9 - 27.1 $mg.plant^{-1}$ for inoculated plants compared to uninoculated controls which averaged 5.4 $mg.plant^{-1}$ (Galiana, Tibok and Duhoux, 1991). These results are considerably lower than results in my study or the previous study (Umali-Garcia *et al*, 1988).

Comparisons of acetylene reduction activity and nitrogen fixed with other studies are confounded by the units in which results are quoted such as nmol mg^{-1} fresh $wt.h^{-1}$ (Langkamp, Swinden and Dalling, 1979). In my study dry mass, not fresh mass, was measured. Most studies quote figures in kilograms

per hectare per year (Langkamp *et al*, 1979; Lawrie, 1981; Monk *et al*, 1981; Rundel *et al*, 1982; Sanginga, Bowen and Danso, 1990). To calculate this, one requires a measure of nodule number versus plant age as well as a measure plant age - density distributions which is not possible with pot experiments. Only a few studies of inoculation and nitrogen fixation in species of *Acacia* have quoted results on a per plant basis.

My research using the theoretical conversion factor of 3:1 calculated that *A. tortilis* averaged 0.507 mg N plant.h^{-1} which was the lowest value for the five species. *A. robusta* had the highest measurements of nitrogen fixed with a mean value of 2.54 mg.$plant^{-1}$.h^{-1}. Felker and Clark (1980) recorded a tenfold range in acetylene reduction between 12 species of *Prosopis*. The highest value they recorded was 1.05 mg N $plant^{-1}$.h^{-1} or 35 μmoles ethylene.h^{-1} for *Prosopis pallida* (Felker and Clark, 1980). This compares favourably with our results where acetylene reduction for *A. tortilis* was lowest (54.75 ± 18.976 μmoles ethylene.h^{-1}) and *A. robusta* which was highest (274.5 ± 63.41 μmoles ethylene.h^{-1}). The difference in mass of nitrogen fixed and ethylene produced between Felker *et al* (1980) and these results reflects a different ethylene to nitrogen conversion ratio.

2.2.5 Conclusion

The basic aims of this experiment were both met. The

technique of using pure cultures of *Rhizobium* bacteria to inoculate the five species of *Acacia* resulted in significant increases in nodulation for all five species compared to untreated controls. Furthermore some species, notably *A. robusta* and *A. karroo* formed no nodules at all in untreated controls. Uninoculated controls for the other three species had very poor nodulation with very few nodules on only a few individuals. This shows that intentional inoculation with pure cultures of bacteria under greenhouse conditions is a successful technique to increase the prevalence of symbioses in species of *Acacia*.

The second aim of this experiment was to determine the success of inoculation in improving the nitrogen status and growth of *Acacia* species. Visual evidence alone was conclusive as inoculated plants were green and vigourous whereas uninoculated controls were very pale with few leaves and short stunted stems. Measurements of growth parameters such as shoot length, shoot mass, root mass and nodule mass supported these observations. The most conclusive evidence for improved nitrogen nutrition as a result of inoculation is the data on the nitrogen content of the plants. In all species, inoculated plants had nearly double the amount of nitrogen that uninoculated control plants had.

In summary, inoculation of five species of *Acacia* under controlled conditions significantly enhanced nitrogen status and overall growth of plants while uninoculated controls were

nitrogen stressed and displayed low vigour generally.

2.3 Plant nitrogen in stems and leaves collected from two species of *Acacia* growing in the field

2.3.1 Introduction

Garcia-Moya and McKell (1970) examined nitrogen concentrations of *Acacia greggii* in the field and found that leaf tissue had the highest percentage tissue nitrogen on a dry weight basis. Roots had the lowest and stems had a slightly higher percentage nitrogen content. In *Prosopis glandulosa*, a nitrogen-fixing woody legume, it was found that initially leaves had in excess of 5 % nitrogen content but this dropped rapidly to less than 3 % in two months (Rundel *et al*, 1982). Young stems showed a similar pattern with initial values of 3 % which dropped to about 1.5 %. The two species sampled in the field were *Acacia karroo* and *A. nilotica*. The aims of this experiment were to determine whether:
(1) there was a difference in nitrogen content of leaves and stems between the two species growing in the field and
(2) there was any difference in nitrogen content of field plants from those grown in pots in a greenhouse.

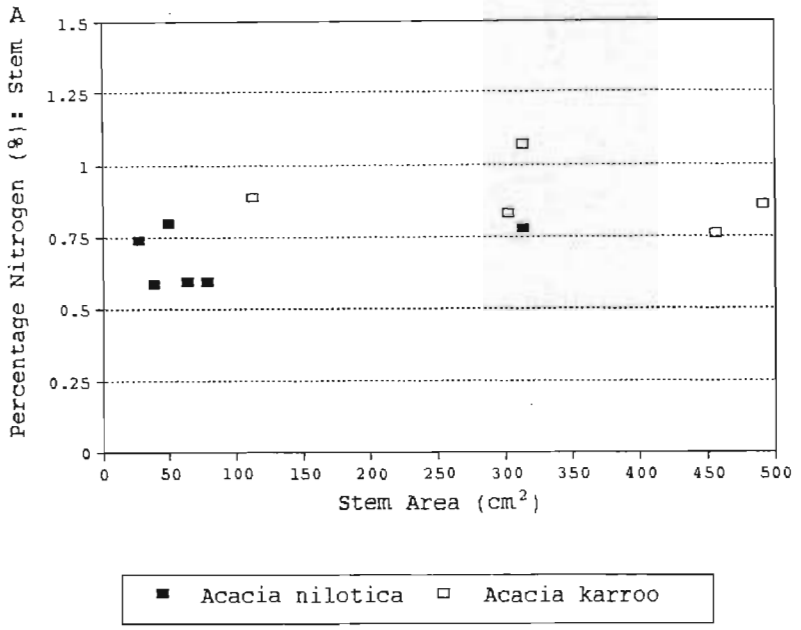
2.3.2 Methods and materials

Samples were collected in late spring (October 1994) from the study site in Ashburton, east of Pietermaritzburg. That

season's stems were identified by the fresh reddish tinge of the bark and harvested for nitrogen analysis. The leaves were removed and the stems cut into smaller pieces and stored in brown paper bags. Leaves were harvested from all parts of the canopy. Only fully open, mature leaves were harvested. They were also stored in brown paper bags. Both stem and leaf samples were dried in an oven at 80°C for 48 hrs. Stem samples were milled in a hippo mill before both stem and leaf samples were finely ground in a Wileys mill. The samples were then analyzed for total nitrogen content using macro-Kjeldahl analysis (Bremmer, 1965). Results were statistically analyzed using an analysis of variance (Siegel and Castellan, 1988).

2.3.3 Results

No pattern emerged that related percentage nitrogen content of either stems or leaves to tree size (Figure 2.3.3.1A and B). In fact, nitrogen content was remarkably constant regardless of tree size. The mean percentage leaf nitrogen for *A. karroo* (2.21 ± 0.3) was slightly higher than that from *A. nilotica* (2.14 ± 0.05)(Table 2.3.3.1). An analysis of variance (ANOVA) showed no statistically significant difference ($P \leq 0.05$) between leaf nitrogen from *A. karroo* and leaf nitrogen from *A. nilotica*. The mean percentage stem nitrogen in *A. karroo* (0.88 ± 0.047) was also higher than that for *A. nilotica* (0.685 ± 0.040). An ANOVA showed that the difference in stem nitrogen between the two species was significant ($P \leq 0.05$).



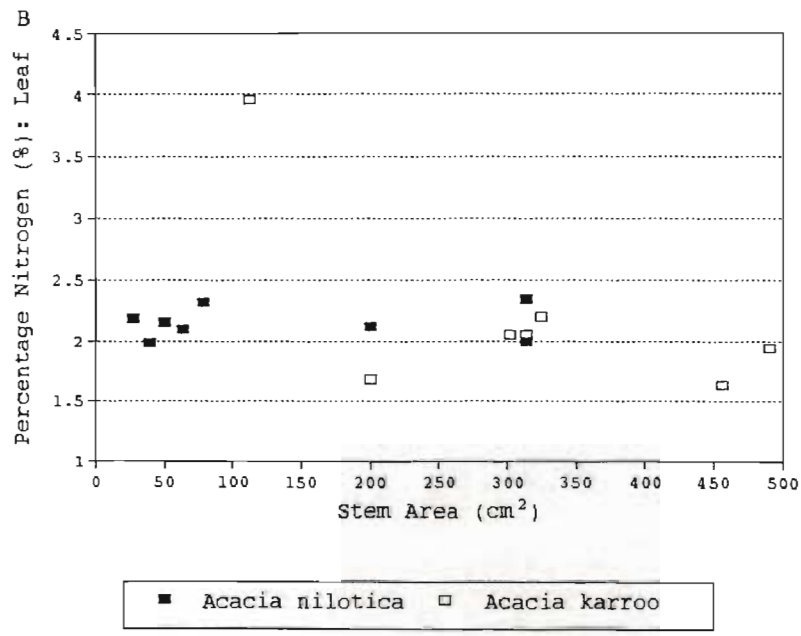


Figure 2.3.3.1 Percent total nitrogen for two species of *Acacia* growing at the Ashburton study site, as a function of basal stem area. (A) Stem total nitrogen. (B) Leaf total nitrogen.

Table 2.3.3.1 Summary of results for the total percentage nitrogen in stems and leaves. Two species of *Acacia* growing at the Ashburton study site were compared. Symbols in superscript refer to statistical results: $P \leq 0.05$ (*), not significant (ns).

Stem nitrogen (%)	*Acacia nilotica*	*Acacia karroo*
mean	0.685	0.882^{*}
SE	0.040	0.047
n	6	5

Leaf nitrogen (%)	*Acacia nilotica*	*Acacia karroo*
mean	2.145	2.214^{ns}
SE	0.045	0.301
n	8	7

2.3.4 Discussion

Acacia gregii in a desert wash in the Mojave Desert had percentage nitrogen in stems and leaves of 0.8 and 1.4 percent respectively (Garcia-Moya and McKell, 1970). This compares favourably with data for stems in this study in which results of 0.88 % and 0.68 % for *A. karroo* and *A. nilotica* respectively were obtained. Leaf nitrogen in the two southern African species was considerably higher than 1.4 % recorded for *A. gregii*. It is tempting to compare the results of tissue nitrogen from field plants with those from the greenhouse experiment. The greenhouse figures were obtained by pooling the whole shoot for analysis rather than separating the parts into stems and leaves. It is interesting though that for both *A. nilotica* and *A. karroo*, percentage shoot nitrogen from inoculated, greenhouse grown plants are higher (3.8 % and 3.04 respectively) than percentage nitrogen of

leaves from plants growing in the field.

2.3.5 Conclusion

There was no significant difference in percentage plant nitrogen of leaves between *A. karroo* and *A. nilotica* although *A. karroo* had slightly higher leaf nitrogen. This pattern was also found in stems with *A. karroo* having higher nitrogen levels than *A. nilotica*. This difference in stem nitrogen was found to be significantly different statistically. It was noted that values of nitrogen content in the field were higher than values for uninoculated controls from the green house experiment but lower than inoculated treatments from the same experiment.

2.4 Conclusions on plant nitrogen and nitrogen fixation

The results from the greenhouse experiment supports the conclusion of other researchers that inoculation with symbiotic nitrogen fixing bacteria enhances growth and nitrogen self-sufficiency in *Acacia* species (van Kessel *et al*, 1983; Hopmans *et al*, 1983; Hansen and Pate, 1987; Umali-Garcia *et al*, 1988; Galiana *et al*, 1991; Ndoye *et al*, 1995). Growth parameters such as total plant mass, shoot mass, root mass, nodule mass and number of nodules per plant were significantly enhanced by inoculation with *Rhizobium* bacteria. When compared with field data, the nature of the symbioses is shown to be complex. The quantity of nitrogen in shoot tissue in

the field is lower than that for inoculated greenhouse grown plants but higher than uninoculated control plants which lacked any source of external inorganic nitrogen. This suggests that field plants may not rely on symbiotic fixation to the extent that green house plants were forced to. This is supported by the low prevalence of nodules in soil samples from the field sites. Nodules were found, but so irregularly that no quantification of their influence could be made. Levels of inorganic soil nitrogen (discussed in chapter four) could have a major influence on nodulation in the field (Hopmans, Douglas and Chalk, 1983, Hansen and Pate, 1987b). Other factors that could influence symbioses in the field include mineral nutrition (O'Hara, Boonkerd and Dilworth; 1988), plant age (Hansen and Pate, 1987b; Hansen, Pate, Hansen and Bell, 1987) and bacterial competition in soil resulting in strains of bacteria which nodulate poorly.

Chapter 3 NITROGEN MOVEMENT FROM THE CANOPY TO THE SOIL SURFACE

3.1 Introduction

Nitrogen may move from the canopy of an *Acacia* tree to the soil nutrient pool either by litter fall or by leaching from rain water. Nitrogen in litter is mostly in the organic form, whereas that in water passing through the canopy has both inorganic and organic nitrogen present. Most nutrients transferred from the canopy to the forest floor in precipitation are added directly to the available nutrient pool without having to first pass through decomposition (Eaton, Likens and Bormann, 1973). That such contributions may be considerable are suggested by Sollins (1980) who found that precipitation supplied 30 % of the nitrogen input to a Douglas-fir ecosystem. In contrast, nutrients in litter fall must first be decomposed before nutrients are added to the available nutrient pool. Litter fall is a major pathway for nutrient transfer in woody species (Gorham and Bray, 1964). Carlisle, Brown and White, (1966) found that the dry weight of leaves in autumn litter fall was 2202-2476 $kg.ha^{-1}$ which contained 21-27 $kg.ha^{-1}$ of nitrogen. This is low compared to figures from Hubbard Brook experimental forest where litter fall averaged 5,702 $kg.ha^{-1}.y^{-1}$ (Gosz, Likens and Bormann, 1972). This litter contributed 40.9 $kg.ha^{-1}.y^{-1}$ to the soil nitrogen pool. This clearly illustrates the importance of litter fall in nutrient cycling of natural ecosystems.

At the study site, litter fall data were difficult to collect. The problem lay in a number of areas. Firstly, for any litter fall measurements to be effective, one requires a measurement of nitrogen translocated out of senescing tissues. Langkamp *et al* (1982) found this nitrogen translocated back into the plant prior to senescence to be 73 % of the total leaf nitrogen. This could be done if freshly senesced leaves could be gathered. The difference in nitrogen content between mature fully functioning leaves and freshly senesced leaves would give an accurate measure of nitrogen translocation back into shoots. The problem lies in how *Acacia* species senesce their leaves and the type of leaves they have.

The leaves are compound with small leaflets measurable in millimetres. Leaves are senesced as entire units or as leaflets. Senescence does not rigidly follow a set seasonal pattern but is a direct response to environmental stresses such as water and frost stress. This means that leaves may senesce during any extended period of water stress with the biggest senescence event occurring early in the winter dry period. Leaves also senesce when their age or other environmental factors reduce their efficiency. Gosz *et al* (1972) found that a progressive natural mortality of leaves in the Hubbard Brook experimental forest occurred from leaf initiation to senescence. In either case the small size of *Acacia* leaflets and the continuous irregular pattern of senescence means leaf litter has a tendency not to collect on the ground in any great quantity except at the beginning of

winter. Even during this period it was difficult to find enough suitable material to conduct any worthwhile mineralisation experiments. Considering the small size of the leaflets, one would expect mineralisation rates to be rapid.

Two types of trap were tested in an attempt to sample senesced leaves directly from the trees. Both traps were set for periods of one month over successive months but results were poor. Litter collected by the traps was insufficient to allow macro-Kjeldahl analysis for nitrogen content. It has since been decided that a more effective approach would be to lay large sheets under the canopy and shake the tree. This approach has not been tested yet.

3.2 Nitrogen leached from *Acacia* canopies by rainfall as throughfall and stemflow

The pattern of rainfall falling on the canopy of a tree or moving through it, is one of the primary factors regulating nutrient movement from the canopy to the soil nutrient pool. Rainfall can be partitioned into that falling over the canopy and openfall. Openfall is defined as that rainfall falling between adjacent canopies without intercepting any plant part before reaching the ground layer vegetation (Langkamp, Farnell and Dalling; 1982). In other words, openfall is normal rainfall as would be collected by a standard rain gauge. That rainfall falling over the canopy may be separated into interception (Rutter, 1963), throughfall and stemflow (Eaton

et al, 1973). Interception is defined as that rainfall collecting in the canopy and not reaching the ground (Rutter, 1963). This water may be absorbed by the plant or lost as evaporation. Rutter (1963) described interception as determined by (a) the amount of water required to saturate the canopy surface, (b) the size of individual showers, especially those small enough not to allow canopy saturation and (c) evaporation between showers. It is also influenced by the intensity of rainfall, the species of tree and its size and form (Carlisle, Brown and White, 1966; Eaton *et al*, 1973)

A tree canopy must first be saturated before stemflow or throughfall will occur. Throughfall is defined as that rainfall passing through the tree canopy (Rutter, 1963; Eaton *et al*, 1973). It is impossible to separate rainfall which drips off the canopy and that which passes directly through the canopy without striking any surface so measurements of throughfall include both these forms. Stemflow is rainfall which collects in the tree canopy and reaches the ground by flowing down the tree trunk (Rutter, 1963; Eaton *et al*, 1973). Rutter (1963) also recognised stem drip as being separate from stemflow and throughfall. Stem drip results from water dripping off branches just above their insertion to the main trunk. Rutter found that this form of water movement was restricted to a 15 cm diameter around the main stem and that stemdrip in terms of volume per unit area was negligible compared to other forms of water movement near the canopy. I did not find stemdrip to occur to any noticeable extent on

either species of *Acacia*. Stemdrip was therefore assumed to be negligible and no attempt was made to measure it.

Rutter (1963) found that stemflow in a *Pinus sylvestris* plantation varied greatly from tree to tree but that this was found to be a linear relation to the square of the trunk diameter at breast height. Rutter (1963) also found that stemflow in summer was half its winter value but this was balanced by an increase in throughfall. He found that the proportion of precipitation reaching the ground via throughfall or stemflow was a constant 68.3 % with the remaining 31.7 % being lost or absorbed as interception. Rutter (1963) found that interception was dependant on the nature of the rainfall. Showers of less than 3 mm had up to 60 % interception and generally higher interception resulted from numerous small showers than from larger or continuous showers. He further concluded that most of the water intercepted by the canopy was lost as evaporation. Pressland (1973) found that both stemflow and throughfall were independent of intensity of rainfall except at very low amounts (< 10 mm). He found that there was a tendency for throughfall and stemflow to decrease and interception to increase as basal stem area increased. Sollins *et al* (1980) found that only 1.8% of precipitation reached the soil as stemflow but nutrient concentrations were two to four times greater in stemflow than in throughflow.

Nitrogen in rainfall consists of ammonium, nitrite, nitrate

and organic nitrogen (Eriksson, 1952; Stock and Lewis, 1986). Although the amount of nitrogen present in precipitation is small, it may represent an important source for natural systems (Carlisle *et al*, 1966; Allen *et al*, 1968; Stock and Lewis, 1986). Nutrients washed out of the canopy in either stemflow or throughfall include nutrients in precipitation, nutrients washed from the canopy surface and nutrients leached from the canopy tissues (Eaton *et al*, 1973). Furthermore the presence of epiphytes may significantly modify the nutrient content of stemflow and throughfall. Nutrients can be absorbed by the canopy or microphytes on the canopy but there is usually a net gain of nutrients in precipitation under canopies.

Nitrogen in openfall sometimes showed a relation to amount of rainfall but also showed a high degree of inconsistency (Allen *et al*, 1968). Stock and Lewis (1986) found that 56 % of the total nitrogen in rain was inorganic nitrogen and the ammonium:nitrate ratio of this inorganic nitrogen was approximately one. Gore (1968) measured organic nitrogen for one year and found this to be equal to an additional 18.5 % of the total inorganic nitrogen. Carlisle, Brown and White (1966) recorded organic nitrogen to be an extra 52 % of total inorganic nitrogen.

Attiwill (1966) showed that the amount of nutrient leached per unit quantity of rain is greater during a low intensity rain than it is during a high intensity rain. This seems to be a

function of residence time of the water on the leaf. He also found that the decrease in nutrient loss from leaves is greatest in the initial hours of wetting. The amount of a nutrient leached from a leaf may be independant of the amount held within the leaf (Eaton *et al*, 1973). This is related to where and how the element is held in the leaf. Structural components such as nitrogen are less susceptible to leaching than elements held in the cell solution. The age of a leaf may also play a role in leaching susceptibility. Young leaves quickly metabolise nutrients which allows less exposure to leaching, but more mature leaves are slower to metabolise their nutrients making them more susceptible to leaching. Damaged leaves are also more susceptible to leaching (Eaton *et al*, 1973). Dry fallout of nitrogen containing substances from the atmosphere onto the canopy may confound determination of sources of nitrogen in throughflow and stemflow (Carlisle, White and Brown, 1967a). Eaton *et al* (1973) did not find that dry fallout was a major contributor to the nitrogen content of throughfall and stemflow.

Eaton *et al* (1973) found a significantly greater deposit of total nutrients per unit area in throughfall beneath larger trees. When they considered individual trees and species they found no significant difference between large and small trees. They further found that these effects differed between species for inorganic nitrogen. They found that losses of nitrogen in throughfall varied through summer but reached a maximum at senescence and declined thereafter (Eaton *et al*, 1973). They

found the concentrations in stemflow to be higher than those in throughfall but seasonally matched those of throughfall. Generally only a small percentage of precipitation becomes stemflow but because this is deposited at the tree base, it may be important to nutrient cycling (Eaton *et al*, 1973).

Acacia karroo and *A. nilotica* were the two species whose nitrogen dynamics in throughfall and stemflow was studied in the field. The important questions were:

a) how much nitrogen did the *Acacia* canopy contribute as stemflow or throughfall compared to that nitrogen in direct rainfall or openfall?

b) was there a difference in the proportions of ammonium and nitrate in stemflow and throughfall between the two species of *Acacia* growing in the field?

3.2.1 Materials and methods

Six rain gauges to gather openfall were erected approximately 140-190 cm above ground on poles situated away from canopy interference on the site. Glass wool was placed in the gauge to prevent particulate matter or organisms entering. Throughfall was collected using traps made from PVC guttering. These were 1 m long and 15 cm wide and covered in nylon stockings to prevent debris and litter from collecting in the trap. Water collected by these gutters was temporally stored in 2 l plastic bottles located under the gutter. One throughflow trap was constructed for each tree studied.

Samples of stemflow were collected using a specially constructed collar around the tree trunk. This collar was constructed from polypropylene sheeting to form an inverted cone around the tree trunk. These were situated 20 - 40cm above the ground and sealed with silicon seal. On multi-stemmed trees, as is common in *A. karroo*, stemflow traps were constructed on each stem and the samples pooled for analysis. A 1 cm diameter plastic pipe siphoned the water samples from the collar to a 2 l plastic storage bottle located below the collar. Sometimes it was necessary to bury the bottle to get the water siphoned passively from collar to bottle. Collars were covered with nylon stocking material to prevent litter and other debris from gathering. Samples were collected immediately after rainfall.

Samples were brought back to the laboratory and analyzed the next day for inorganic nitrogen content. Ammonium and nitrate concentrations were determined using steam distillation (Bremmer, 1965). Results were also examined for any relationship with tree size. Tree size and age was regarded as a function of basal stem area. For multi-stemmed trees, which are common for *A. karroo*, the basal area of individual stems was calculated and then each stem area added together to get a total basal stem area for the respective tree. *Acacia nilotica* is usually single-stemmed.

The volume of rainfall collected in each collecting apparatus was recorded. It was found that variability in canopy

structure, stem architecture and position of throughflow traps caused large variances in the volumes captured. Only rain gauges trapping openfall showed consistent volumes with small variability in rainfall volume. For this reason, comparisons were made on a parts per million basis rather than on actual volumes collected in the different collecting traps. Results were analyzed statistically to test for differences between species and openfall using an analysis of variance (ANOVA).

3.2.2 Results

Figure 3.2.1 illustrates the rainfall collected as stemflow, throughfall and openfall under *A. nilotica* (Figure 3.2.1A) and *A. karroo* (Figure 3.2.1B) as a percentage of the total collected. Openfall in both cases accounts for nearly fifty percent (49 and 48 % respectively) of the rainfall collected. Throughfall (29 %) was higher than stemflow (21 %) under *A. nilotica* but both were lower than openfall (Figure 3.2.1A). *Acacia karroo* showed a different pattern, with stemflow being marginally higher (27 %) than throughfall (25 %). This is probably related to the more upright stance of *A. karroo* and its habit of being multi-stemmed.

Figure 3.2.2A illustrates average concentrations (ppm) of ammonium and nitrate in stemflow, canopy throughflow and openflow for *A.nilotica* and *A. karroo*. Stemflow from *A. nilotica* has the highest nitrogen concentrations for both nitrate (4.8 ± 1.38 ppm) and ammonium (4.4 ± 0.64 ppm).

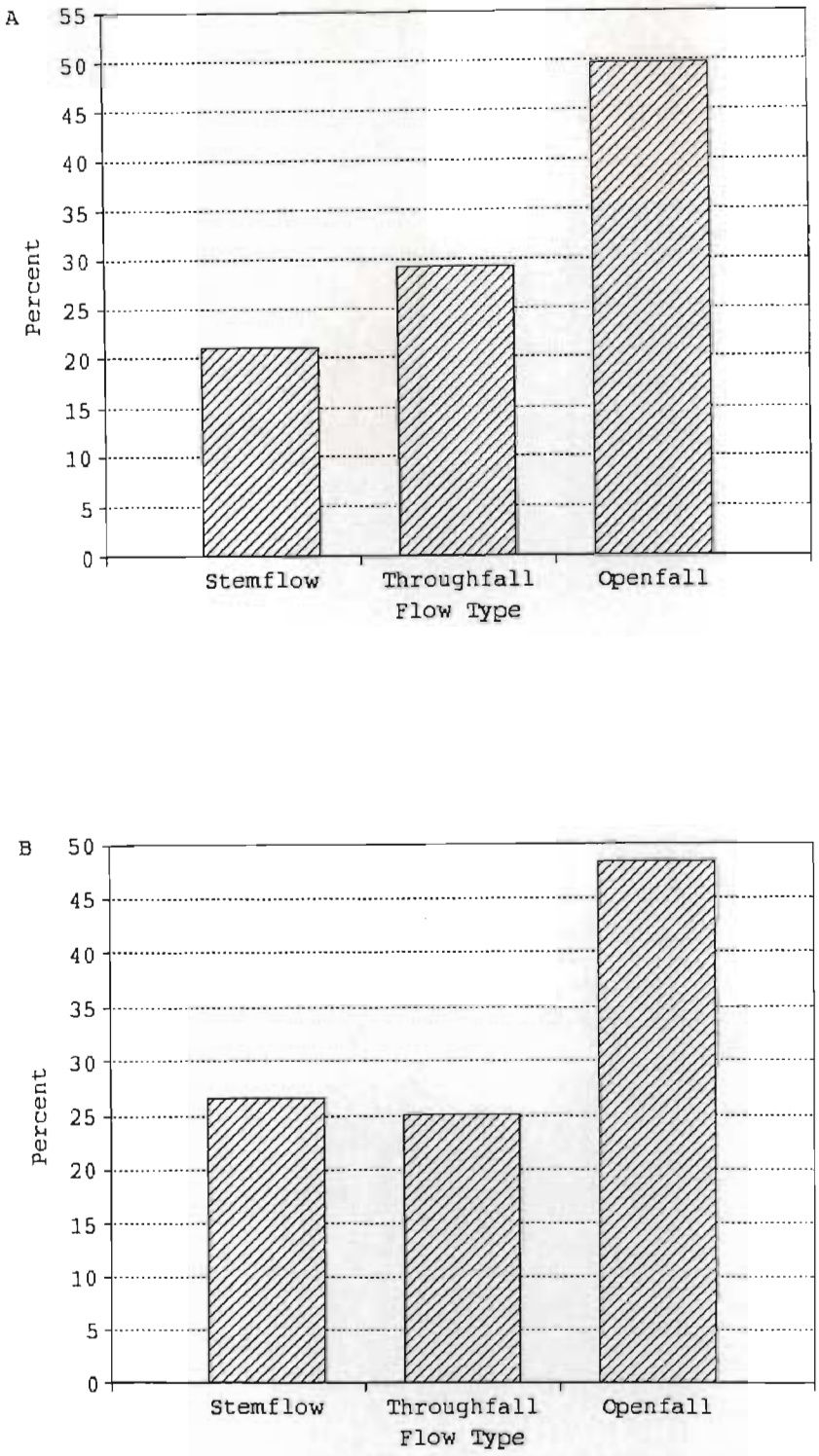
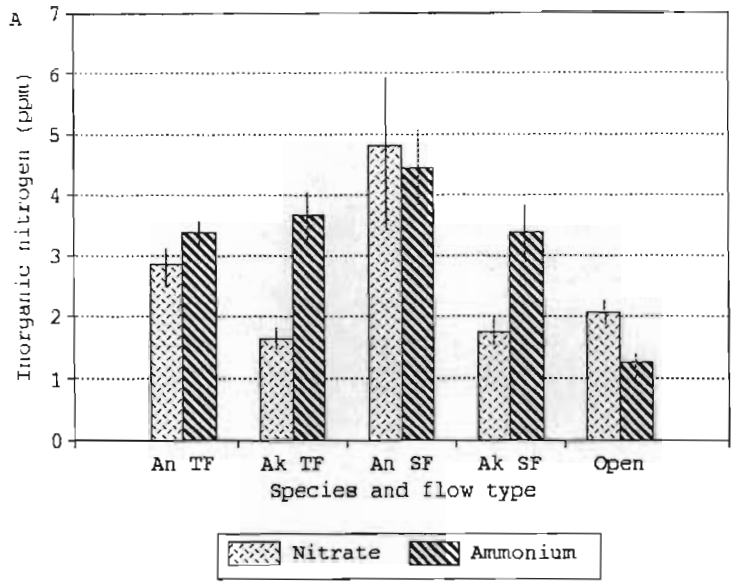


Figure 3.2.1 Rainfall collected as throughfall, stemflow and openfall under (A) *Acacia nilotica* and (B) *A. karroo* as a percentage of the total collected.



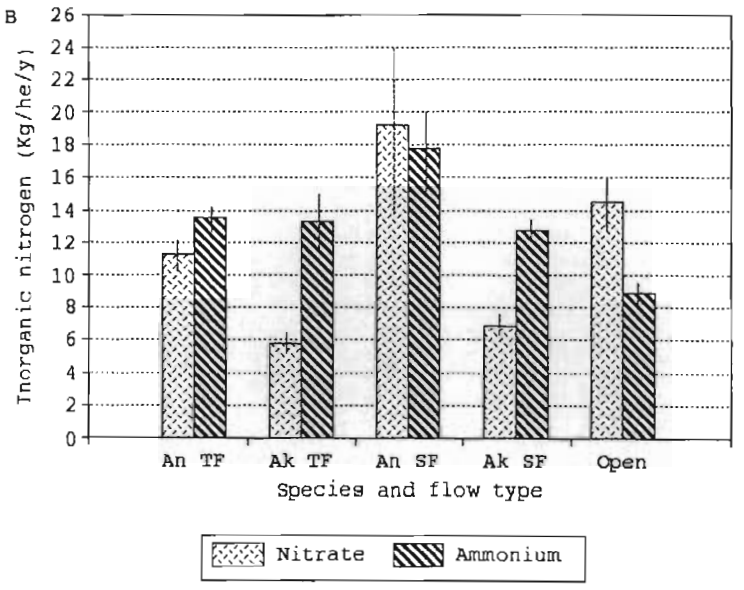


Figure 3.2.2 Average inorganic nitrogen (nitrate and ammonium) collected in rain as canopy throughfall, stemflow and openfall. (A) Nitrate and ammonium as parts per million. (B) Nitrate and ammonium in kilograms per hectare per year. Abbreviations on the X-axis are *Acacia nilotica* throughfall (An TF), *A. karroo* throughfall (Ak TF), *A. nilotica* stemflow (An SF), *A. karroo* stemflow (Ak SF) and openfall (Open).

Table 3.2.1 Nitrate (NO_3^-) and ammonium (NH_4^+) concentration (ppm) in rain collected as throughfall and stemflow under *A. karroo* and *A. nilotica* canopies, or as openfall. Treatments with different letters superscripted after mean values are significantly different ($P \leq 0.05$).

A Throughfall - nitrate

	Acacia karroo	*Acacia nilotica*	Openfall
n	7	8	6
mean	1.645^a	2.839^b	2.065^{ab}
SE	0.131	0.345	0.224

B Stemflow - nitrate

	Acacia karroo	*Acacia nilotica*	Openfall
n	6	5	6
mean	1.744^a	4.802^b	2.065^a
SE	0.135	1.379	0.224

C Throughfall - ammonium

	Acacia karroo	*Acacia nilotica*	Openfall
n	7	8	5
mean	3.675^b	3.386^b	1.26^a
SE	0.464	0.243	0.099

D Stemflow - ammonium

	Acacia karroo	*Acacia nilotica*	Openfall
n	6	5	5
mean	3.366	4.445	1.260
SE	0.459^b	0.642^b	0.099^a

Stemflow from *A. karroo* has the lowest nitrate concentration (1.7 ± 0.135 ppm) in its stemflow. Nitrate levels for throughflow are lower than those for stemflow for both species. *Acacia nilotica* had mean nitrate concentrations in throughflow of 2.839 ± 0.345 ppm while *A. karroo* was lower with a mean throughflow nitrate concentration of 1.64 ± 0.13 ppm. Openflow (rain) had a mean nitrate concentration of 2.065 ± 0.22 ppm, which was lower than either stemflow or throughflow from *A. nilotica*. Unexpectedly this was higher than nitrate concentrations in both stemflow (0.32 ppm higher) and throughflow (0.42 ppm higher) from *A. karroo*. Statistically, an ANOVA showed that nitrate levels in both throughflow and stemflow for *A. nilotica* were significantly greater ($P \leq 0.05$) than those in *A. karroo* but only nitrate concentration in stemflow under *A. nilotica* was significantly greater ($P \leq 0.05$) than values for openfall (Table 3.2.1).

Ammonium concentrations in throughfall, stemflow and openfall followed a slightly different pattern to those for nitrate. Openfall had the lowest ammonium concentrations (1.26 ± 0.09) *Acacia nilotica* had the highest ammonium concentration for stemflow (4.45 ± 0.64 ppm) and *A. nilotica* had the highest for throughflow (3.68 ± 0.46 ppm). Statistically no significant difference was found between species for either throughfall or stemflow (Table 3.2.1) but an ANOVA showed that ammonium concentration in openfall was significantly lower ($P \leq 0.05$) than either throughfall or stemflow concentrations under *A. nilotica* or *A.karroo* canopies.

Data illustrated in Figure 3.2.2B are estimates of nitrogen input in kilograms per hectare per year for nitrate and ammonium from throughfall, stemflow and openfall. Stemflow from *A. nilotica* contributes the highest nitrogen input to the soil with a mean of 19.15 ± 5.5 $kg.ha^{-1}.y^{-1}$ nitrate and a mean ammonium input of 17.73 ± 2.56 $kg.ha^{-1}.y^{-1}$. *A. karroo* has the lowest nitrate levels in both throughfall (5.82 ± 0.28 $kg.ha^{-1}.y^{-1}$) and stemflow (6.96 ± 0.54 $kg.ha^{-1}.y^{-1}$). Rainfall (openfall) has the lowest mean ammonium input of 8.85 ± 0.73 $kg.ha^{-1}.y^{-1}$ but the second highest mean nitrate input (14.51 ± 1.65 $kg.he^{-1}.y^{-1}$).

Figure 3.2.3 illustrates the concentration of inorganic nitrogen (ammonium and nitrate) as a function of basal stem area for *A. karroo*. Ammonium (Figure 3.2.3A) shows no clear pattern relating concentration in either stemflow or throughflow to size of *A. karroo*. A tree with a basal stem area of 113 cm^2 has 2.52 ppm and 2.17 ppm ammonium in its stemflow and throughflow respectively. This compares with a tree of basal stem area of 455 cm^2 with a stemflow of 2.38 ppm and a throughflow of 2.17 ppm. Nitrate concentrations in *A. karroo* (Figure 3.2.3B) show a similar pattern of general constancy in relation to tree basal stem size. Trees of basal stem areas of 113 cm^2 and 455 cm^2 have nitrate concentrations of 2.1 and 2.03 ppm in their stemflow respectively, and 1.4 and 1.4 ppm in their throughflow respectively. The data for *A. nilotica* (Figure 3.2.4) suggests that there maybe an

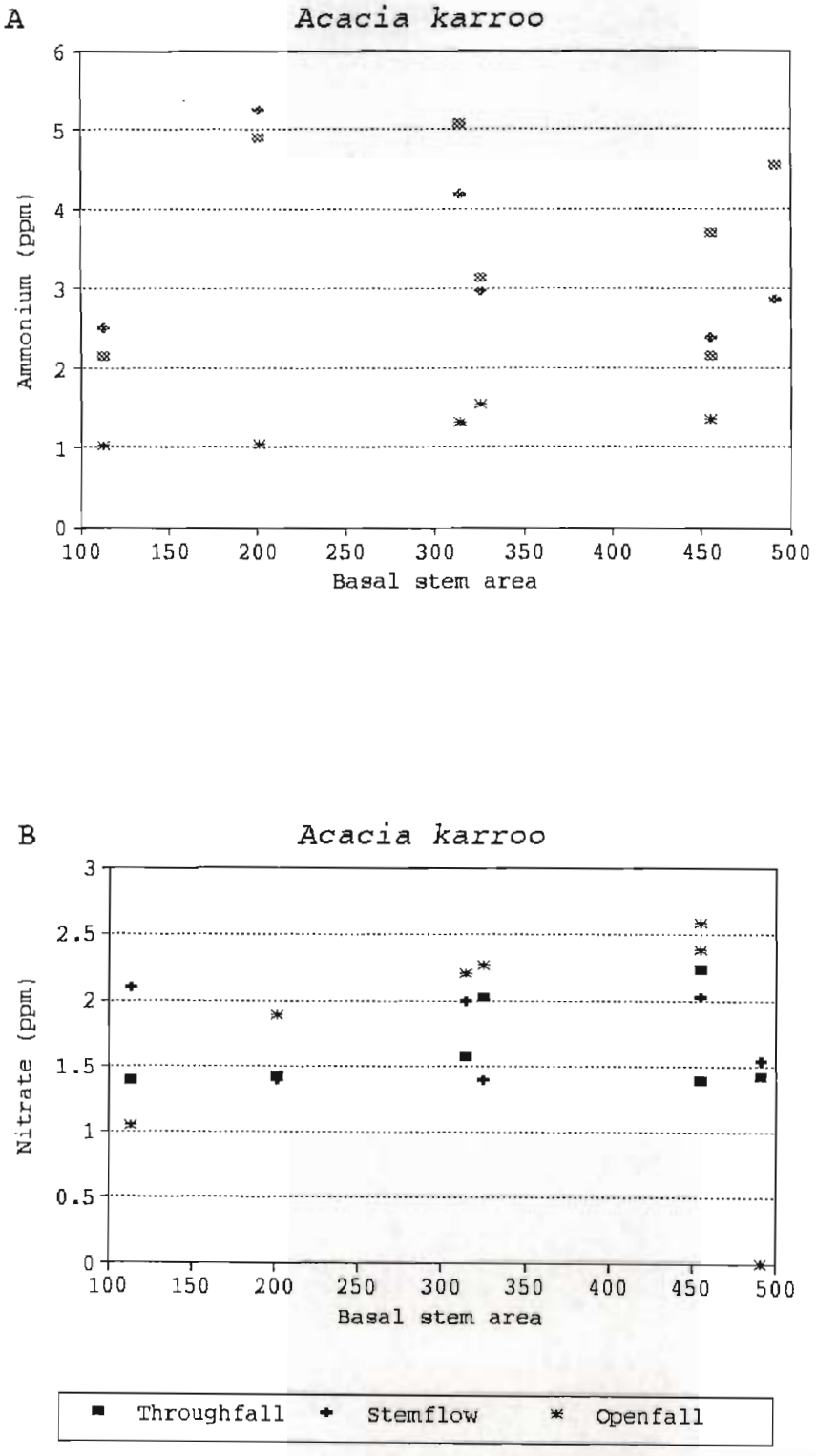


Figure 3.2.3 Inorganic nitrogen measured in rain collected as canopy throughfall, stemflow and openfall, as a function of the basal stem area of *Acacia karroo*. (A) Ammonium and (B) Nitrate.

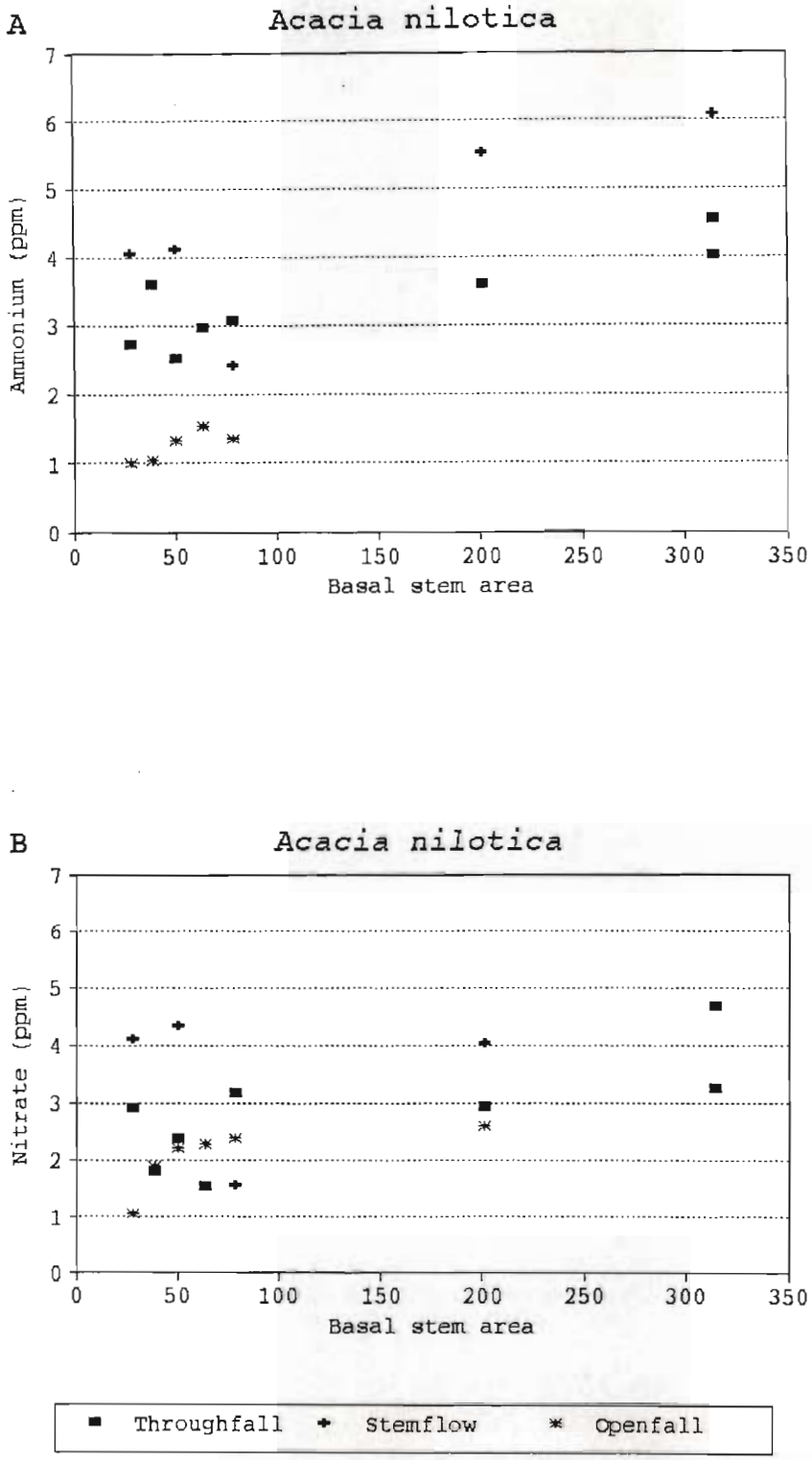


Figure 3.2.4 Inorganic nitrogen measured in rain collected as canopy throughfall, stemflow and openfall, as a function of the basal stem area of *Acacia nilotica*. (A) Ammonium and (B) Nitrate.

increase in nitrogen leached from canopies either as stemflow or as throughflow as basal stem area increases. A basal stem area of 28 cm^2 for *A.nilotica* has an ammonium concentration (Figure 3.2.3A) of 4.06 ppm in its stemflow and 2.73 ppm in throughflow compared to a tree with a basal stem area of 314 cm^2 which has concentrations of 6.09 and 4.55 ppm for stemflow and throughflow respectively. A regression was not performed on this data as basal stem areas for *A. nilotica* were either clumped in the smaller size category or had only one replicate for the large basal stem area.

3.2.3 Discussion

The increase in stemflow and decrease in throughfall in *A. karroo* compared to *A. nilotica* are believed to be a result of different tree canopy architecture. *A. nilotica* is a single stemmed, flat-crowned tree. *A. karroo* is an upright tree with a spherical-shaped canopy which is often multi-stemmed. Such architecture would give *A. karroo* a bigger surface area to intercept rainfall and better conduction of stemflow. *Acacia nilotica* also has a very rough, fissured bark which may reduce stemflow and increase throughfall. The volumes recorded as a percentage for stemflow and throughfall compare well with other studies. Rutter (1963) found that stemflow ranged from 15 - 30 % for *Pinus sylvestris* while this study shows 21 % for *A. nilotica* and 27 % for *A. karroo*. Pressland (1973) found that stemflow averaged 18 % for *A. aneura*.

The high inorganic nitrogen levels in *A. nilotica* compared to *A. karroo* and those in rainfall may also be related to the rough fissured bark of *A. nilotica*. These may harbour more microbes and particulate matter than the smooth bark of *A. karroo*. Openfall concentrations were low in nitrate and ammonium concentrations but nitrate input in kilograms per hectare per year was the second highest after *A. nilotica* stemflow input because of the high volume of openfall (double that of stemflow and throughflow). This high volume had little difference on ammonium inputs as openfall. The reasons for the very low amounts of nitrate in both stemflow and throughfall in *A. karroo* are unknown. These values may be the result of nitrogen uptake by the canopy. This effect was reported by Carlisle *et al* (1966) studying precipitation under a sessile canopy of *Quercus petraea*.

Inputs of inorganic nitrogen are noticeably higher than those recorded from other studies. In the Western Cape, Stock and Lewis (1986) recorded a much lower inorganic nitrogen in openfall of 1.12-1.79 kg.ha-*1*.yr-*1* (Stock and Lewis, 1986). Gore (1968) recorded a mean of 14.04 $kg.ha^{-1}.yr^{-1}$ for inorganic nitrogen in rainfall (openfall). Inorganic nitrogen input in openfall in this study was as high as 23.36 $kg.ha^{-1}.yr^{-1}$. Values obtained in this study for leaching are even more disparate than those of other studies. Langkamp *et al* (1982) recorded values of 2.6 $kg.ha^{-1}.yr^{-1}$ and Eaton *et al* (1973) recorded values of 6.59 $kg.ha^{-1}.yr^{-1}$. This study obtained values of inorganic nitrogen leached from canopies as high as

36.88 kg.ha^{-1}.yr^{-1} under *A. nilotica*. The reasons for this anomaly are not clear at this stage.

3.3 Conclusion

Other studies (Carlisle *et al*, 1966; Gosz *et al*, 1972) have shown that litterfall may contribute a large proportion of the nutrients being cycled. This study was unsuccessful in producing fresh litter in quantities allowing analysis and experimentation. A technique whereby sheets are placed below the canopy and the tree shaken is still to be tested.
Rainfall was sampled as openfall, throughfall and stemflow. These categories were then analysed for nitrate and ammonium content. It was found that *A. nilotica* stemflow contributed 19.15 kg.ha^{-1}.y^{-1} as nitrate and 17.73 kg.ha^{-1}.y^{-1} as ammonium. *A. karroo* contributed the least nitrate with 5.82 kg.ha^{-1}.y^{-1} in throughflow and 6.96 kg.ha^{-1}.y^{-1} in stemflow which was lower than that contributed by openfall. This anomally is difficult to explain but may be due to canopy uptake (Carlisle *et al*, 1966) or microphyte influence (Eaton *et al*, 1973). Ammonium levels were lowest in open rainfall with 8.85 kg.ha^{-1}.y^{-1}. No relationship was found between inorganic nitrogen leached from canopies as throughfall or stemflow and basal stem area.

Chapter 4 SOIL NITROGEN

4.1 Introduction

Soil nitrogen varies from less than 0.1% in desert and semi-desert soils to around 2.0% in highly organic soils (Stevenson, 1965). This nitrogen occurs either as combined nitrogen or as nitrate, nitrite and ammonium ions. The combined nitrogen in soils is bound to organic matter and mineral material and is unavailable to plants (Stevenson, 1965; Harmsen *et al*, 1965). Only a few kilograms per hectare will exist as ions (nitrate and ammonium) and it is these forms which are available for plant uptake. Nitrogen in organic form in soils may be considered the potential nitrogen reserve which, once mineralised, is available for plant uptake. Processes which supply nitrogen to the soil in an available form include decay of organic matter, rainfall and dry deposition (Scarsbrook, 1965).

Total nitrogen usually decreases with increasing depth (Jenny, 1962; Charley and West, 1977) and reflects patterns of root distribution and vertical diffusion of humus substances (Garcia-Moya and McKell, 1970). Lateral patterns of nitrogen distribution related to trees and shrubs have also been documented (Garcia-Moya *et al*, 1970; Charley *et al*, 1977; Boettcher and Kalisz, 1990). In grasslands, soil nitrate and ammonium levels are usually low (Risser and Parton, 1982) and constitute a very small portion of the total soil nitrogen.

This is apparently caused by rapid consumption and immobilization primarily by producers and decomposers. Risser and Parton (1982) found that greater than 98 % of the total nitrogen in a grassland system was in the soil.

Charley *et al* (1977) consider the nitrogen enrichment of surface soils under canopies as the single most important consequence of localization of litter fall. A legume, *Prosopis glandulosa*, growing in the Sonoran Desert has remarkably high soil nitrogen pools in the region of 1020 $g.m^{-2}$ (Rundel *et al*, 1982). Furthermore, these high total soil nitrogen levels under canopies had nitrate levels which comprised as much as 25 % of the total soil nitrogen. In comparison to nitrate levels, they found that ammonium levels were quiet low but still higher than most agricultural soils (Rundel *et al*, 1982). They further found that these high soil nitrogen values dropped considerably as one moved away from the centre of the canopy into adjacent open areas. Garcia-Moya *et al* (1970) found a similar effect under *Acacia gregii* in the Sonoran desert and Tiedemann and Klemmedson (1973) reported three times higher nitrogen concentrations in soil under *Prosopis juliflora* than in adjacent grassland soils. The aim of this research was to determine if any differences occured in the nitrogen content and type between soils from under *Acacia* canopies and soils from adjacent open grasslands.

4.2 Methods and materials

The study site is a savanna mosaic of *Acacia*-dominated thickets and clumps interspersed with grassland. Soil nitrogen levels were quantified from under *A. nilotica* and *A. karroo* canopies and from interspersed open grassland sites. Samples were taken under canopies from within one meter of the trunk and downslope from the base of the trunk. Sampling points in open areas were located more than one meter from the edge of the nearest canopy. One core was collected from each sampling point. At each sampling point the profile was sampled at depths of 0 - 5 cm, 10 cm and 20 cm. Samples where collected using an auger. Samples were stored in brown paper bags and air dried in the bags for 48 hrs. They were then ball milled and sieved through 60 mesh sieves. Total nitrogen was determined using a modified Kjeldahl analysis as described by Bremmer (1965) to incorporate nitrate nitrogen. Inorganic nitrogen in the form of available nitrate and ammonium were determined on sub-samples prior to analysis for total nitrogen. Inorganic ammonium and nitrate were extracted from these sub-samples using a 2 N KCl solution and the extractant analyzed using a steam distillation technique as outlined in Bremmer (1965). Organic nitrogen was determined as the difference between total nitrogen and the sum of ammonium and nitrate nitrogen. Results were analyzed for statistical trends by applying a one-way analysis of variance (Siegel and Castellan, 1988).

4.3 Results

Although higher organic and total nitrogen was associated with the presence of a canopy, no pattern is displayed that relates tree size to soil nitrogen. High levels of total and organic nitrogen with respect to canopy are particularly evident in the top 0-5 cm surface soil layers under both *A. karroo* (Figure 4.3.1A and 4.3.1B) and *A. nilotica* (Figure 4.3.2A and 4.3.2B). At 0 - 5 cm mean total nitrogen (Table 4.3.1) was 824.63 ± 109.28 ppm for *A. karroo* and 587.84 ± 44.89 ppm for *A. nilotica* compared to open sites which had 375.33 ± 33.32 ppm. An analysis of variance showed that total nitrogen in the 0 - 5 cm layer under *A. karroo* was significantly higher ($P \leq 0.05$) than total nitrogen under adjacent grassland soils, but was not significantly higher when compared to *A. nilotica* (Table 4.3.1). This pattern for total soil nitrogen was also apparent at ten centimetres ($P \leq 0.01$). In all cases, there was a decrease in total nitrogen with increasing depth in the soil profile.

Organic nitrogen closely followed patterns for total nitrogen (Table 4.3.1 and 4.3.2, Figure 4.3.1B and 4.3.2B). At 0 - 5 cm, soils under *A. karroo* had the highest soil organic nitrogen levels with a mean of 799.43 ± 109.76 ppm. *Acacia nilotica* had lower levels at this depth of 566.57 ± 44.11 ppm while adjacent open grassland had the lowest soil organic nitrogen (332.23 ± 30.08 ppm). An ANOVA showed the difference between *A. karroo* and adjacent open grassland sites was

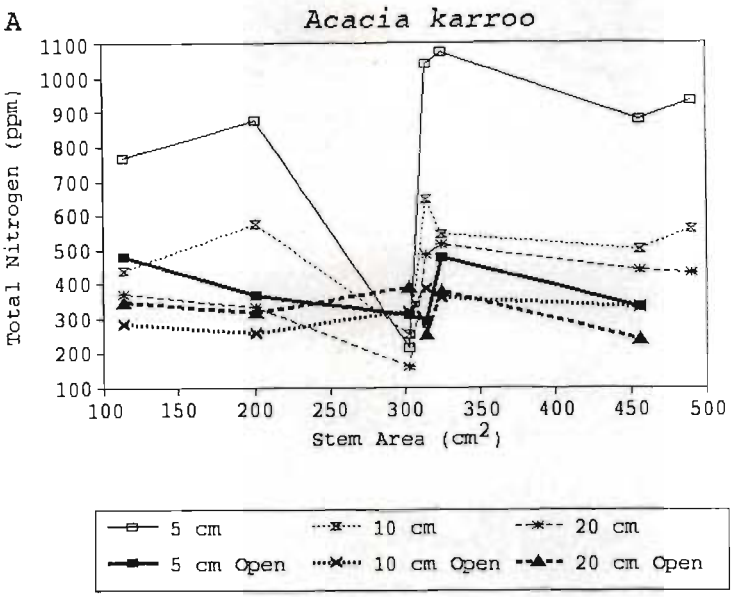
Table 4.3.1 Total nitrogen (TN), organic nitrogen (ON), ammonium (NH_4^+) and nitrate (NO_3^-) in soils at 5 cm and 10 cm depths under *Acacia nilotica*, *A. karroo* and adjacent open grassland sites. Lower case letters which are common denote no statistically significant differences at the probability indicated.

Depth	Type of nitrogen		*A. nilotica*	*A. karroo*	Grassland
5 cm	TN	n	8	7	6
		mean	587.84	824.63	375.33
		SE	44.89	109.28	33.32
		$P \leq 0.01$	ab	b	a
	ON	n	8	7	6
		mean	566.57	799.43	332.23
		SE	44.11	109.76	30.08
		$P \leq 0.01$	ab	b	a
	NH_4^+	n	8	7	6
		mean	8.125	9.853	12.158
		SE	1.147	0.685	0.152
		$P \leq 0.05$	a	ab	b
	NO_3^-	n	6	7	6
		mean	13.139	15.351	16.015
		SE	1.166	0.729	0.325
		$p \leq 0.05$	a	a	a
Depth	Type of nitrogen		*A. nilotica*	*A. karroo*	Grassland
10 cm	TN	n	8	7	6
		mean	405.66	500.38	322.54
		SE	31.4	47.89	18.73
		$p \leq 0.01$	ab	b	a
	ON	n	8	6	6
		mean	386.94	518.15	291.29
		SE	30.82	28.95	18.75
		$p \leq 0.01$	a	b	a
	NH_4^+	n	8	6	6
		mean	7.515	9.437	12.728
		SE	1.117	1.548	0.712
		$p \leq 0.05$	a	ab	b
	NO_3^-	n	8	6	6
		mean	11.206	13.993	17.061
		SE	0.581	1.362	0.672
		$p \leq 0.01$	a	ab	b

Table 4.3.2 Total nitrogen (TN), organic nitrogen (ON), ammonium (NH_4^+) and nitrate (NO_3^-) in soils at 20 cm deep under *Acacia nilotica*, *A. karroo* and adjacent open grassland sites. Lower case letters which are common denote no statistically significant differences at the probability indicated.

Depth	Type of nitrogen		*A. nilotica*	*A. karroo*	Grassland
20 cm	TN	n	8	7	6
		mean	365.41	388.99	319.55
		SE	26.27	45.06	25.87
		$p \leq 0.05$	a	a	a
	ON	n	8	7	6
		mean	344.65	366.98	319.55
		SE	25.41	45.85	25.87
		$p \leq$	a	a	a
	NH_4^+	n	8	7	6
		mean	8.677	9.548	11.612
		SE	1.042	0.799	0.868
		$p \leq 0.05$	a	a	a
	NO_3^-	n	8	7	6
		mean	12.085	12.475	14.666
		SE	0.756	0.979	0.319
		$p \leq 0.05$	a	a	a

statistically significant ($P \leq 0.01$). An increase in depth to 10 cm showed that organic nitrogen content of soil decreased in all cases. At 10 cm depths, mean soil organic nitrogen under *A. karroo* was still the highest but decreased from 0-5 cm values to a value of 518.15 ± 28.95 ppm. Mean soil organic nitrogen at 10 cm deep under *A. nilotica* had decreased to 386.94 ± 30.82 ppm and under adjacent open grassland to 291.29 ± 18.75 ppm. At 10 cm below the surface, both *A. nilotica* and adjacent open grasslands yielded significantly lower values than were found under *A. karroo* ($P \leq 0.01$). The general decrease in organic nitrogen and total nitrogen with depth



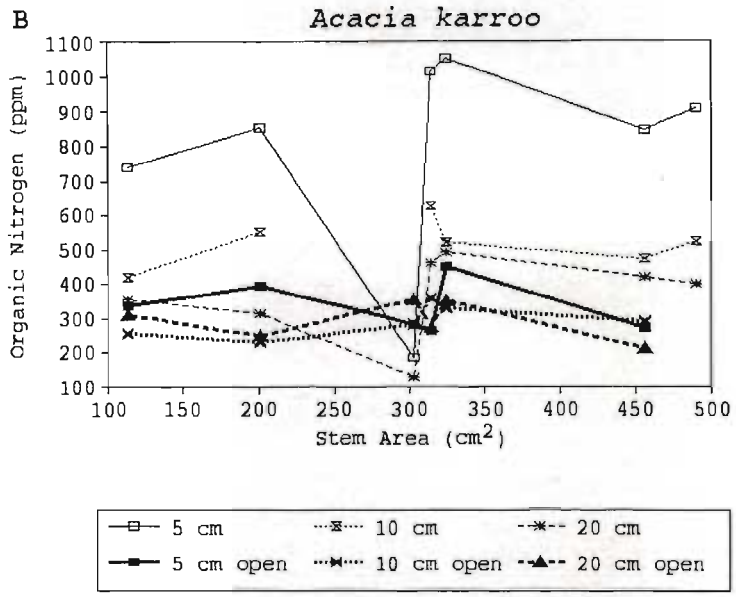


Figure 4.3.1 Total and organic soil nitrogen under canopies of *A. karroo* as a function of basal stem area compared to adjacent grassland sites. (A) Total soil nitrogen at 0-5 cm, 10 cm and 20 cm. (B) Organic soil nitrogen at 0-5 cm, 10 cm and 20 cm.

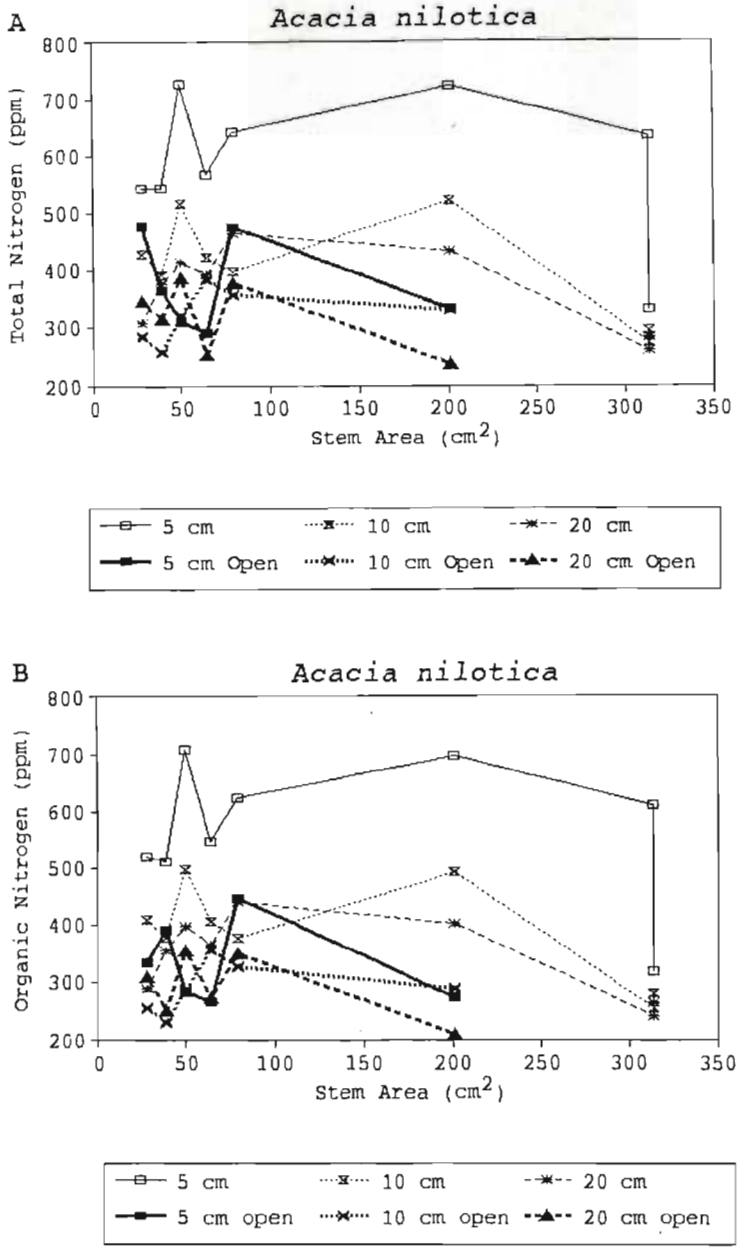


Figure 4.3.2 Total and organic soil nitrogen under canopies of *A. nilotica* as a function of basal stem area compared to adjacent grassland sites. (A) Total soil nitrogen at 0-5 cm, 10 cm and 20 cm. (B) Organic soil nitrogen at 0-5 cm, 10 cm and 20 cm.

continued to the point where at 20 cm deep all sites yielded their lowest mean values (Table 4.3.2). At this depth, no significant difference was found between soils under canopies of *A. karroo* or *A. nilotica* and those under adjacent open grasslands.

Soil ammonium and nitrate (Table 4.3.1 and 4.3.2) under *A. karroo* and *A. nilotica* follow a different pattern to that for total and organic nitrogen. At all depths mean soil ammonia values were higher in open grassland than corresponding values under canopies of either *A. karroo* or *A. nilotica*. There was no consistent decrease or increase in soil ammonium levels moving down the soil profile. *Acacia nilotica* had significantly lower soil ammonium levels compared to open grassland sites at 0-5 cm ($P \leq 0.05$) and 10 cm depth ($P \leq 0.05$), but not at 20 cm. Soil ammonium levels under *A. karroo* showed no significant difference between either open grassland sites (Figure 4.3.3A) or *A. nilotica* sites at any of these depths.

Soil nitrate at all depths, like ammonium, showed higher values in open grassland compared to corresponding values under either *A karroo* or *A. nilotica* canopies (Table 4.3.1 and Table 4.3.2). These differences were not statistically significant ($P \leq 0.05$) at 5 cm and 20 cm depths in the soil profile. Only the 10 cm depth showed that values of nitrate in grassland soils were significantly greater ($P \leq 0.01$) than values at corresponding depths under *A. nilotica*. There was

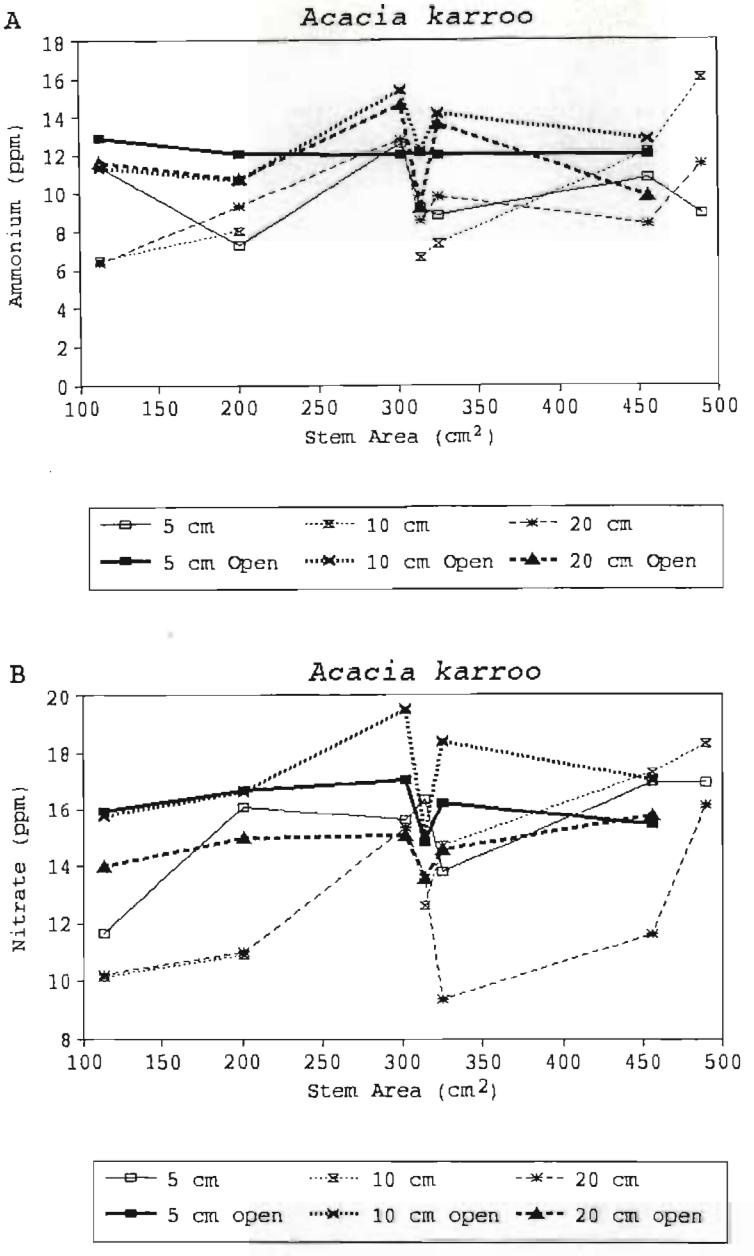


Figure 4.3.3 Inorganic soil nitrogen under canopies of *A. karroo* as a function of basal stem area compared to adjacent grassland sites. (A) Ammonium soil nitrogen at 0-5 cm, 10 cm and 20 cm. (B) Nitrate soil nitrogen at 0-5 cm, 10 cm and 20 cm.

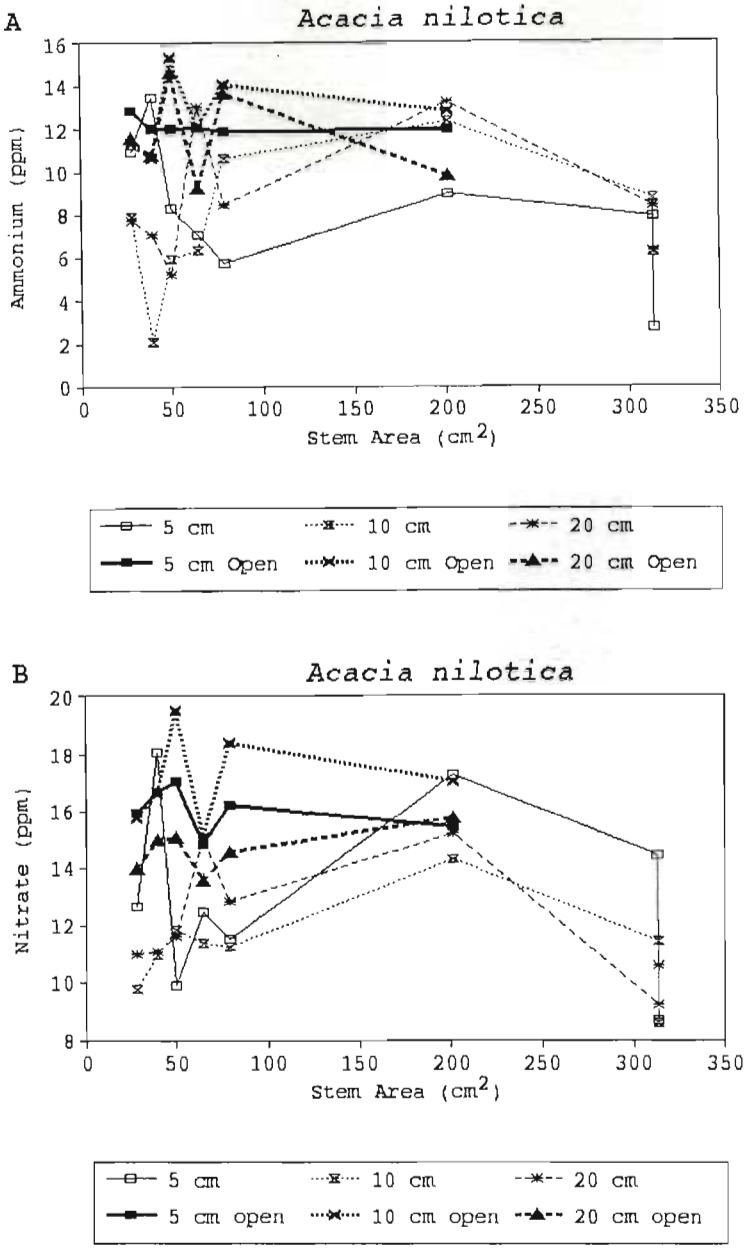


Figure 4.3.4 Inorganic soil nitrogen under canopies of *A. nilotica* as a function of basal stem area compared to adjacent grassland sites. (A) Ammonium soil nitrogen at 0-5 cm, 10 cm and 20 cm. (B) Nitrate soil nitrogen at 0-5 cm, 10cm and 20 cm.

no significant differences between nitrate values for soils at 10 cm deep under *A. karroo* or either open grassland sites or soils under *A. nilotica* canopies.

4.4 Discussion

No pattern could be discerned that related tree size as a function of basal stem area to soil nitrogen levels. This is contrary to expectations when one notes the large difference that exists between soil nitrogen under canopies and that for adjacent open grasslands (Garcia-Moya *et al*, 1970; Rundel *et al*, 1982). It was expected that as the canopy increased in size and leaf area, there would be a concomitant increase in soil organic or total nitrogen as a result of increased litterfall and canopy leaching.

Results obtained in this study of soil nitrogen concentrations under canopies of nitrogen fixing leguminous trees compare favourably with those of other researchers (Garcia-Moya *et al*, 1970; Rundel *et al*, 1982; Stock *et al*, 1995). Garcia-Moya *et al* (1970) found that surface soil nitrogen under shrubs of *Acacia gregii* decreased from 0.054 % (540 ppm) near the root to only 0.021% (210 ppm) at the edge of the canopy. Our results recorded values of 824 ppm for soil under *A. karroo* to 587 ppm under *A. nilotica*. Adjacent open grassland areas were much lower at 379 ppm total nitrogen in surface soils. Total soil nitrogen levels in 0 - 10 cm surface soils under invading exotic *A. cyclops* in the Western Cape Strandveld were 788 $\mu g.g^{-}$

[1] (788 ppm) dry soil (Stock, Weinand and Baker, 1995). Rundel *et al* (1982) found that soil under *Prosopis glandulosa* canopies, a nitrogen fixing legume, had a total nitrogen content of 1020 $g.m^{-2}$. This compared with areas between canopies which had total soil nitrogen levels of 160 $g.m^{-2}$ and adjacent open areas which had only 45 $g.m^{-2}$.

Garcia- Moya *et al* (1970) found that at 45 cm deep, values next to the root were 0.019 % (190 ppm) and at the canopy edge they were 0.013 % (130 ppm). At a distance of one canopy-radius beyond the canopy this dropped to 0.007 % (70 ppm). At 90 cm deep, they found no difference between total soil nitrogen at the root and one radius outside the canopy. This study also showed a decline in soil nitrogen content with increasing depth but the depth at which there was little difference between open sites and soil under canopies was only 20 cm deep compared to 90 cm recorded by Garcia-Moya *et al* (1970).

Garcia-Moya *et al* (1970), concluded that *A. gregii* has a deep root system and provided relatively high soil nitrogen to greater depths than the other two species in their study. They further concluded that *A. gregii* did not build up high soil nitrogen to the full extent of its canopy. I did not trace nitrogen gradients from the stem out, so no conclusions can be drawn about the area of influence the canopy exerts on soil nitrogen concentrations. Soils at the study site are relatively shallow (<40 cm) so most of the root mass is

concentrated close to the surface. This is supported by the rapid equilibrium in total and organic soil nitrogen for the different species at relatively shallow soil depths. It is noticeable that *A. karroo* has higher soil nitrogen levels at the 0 -10cm zone compared to *A. nilotica*. Whether this is a function of increased litterfall, quality of litter, increased fine root mass or increased nodulation and nitrogen fixation in the surface layers or to increased microbial immobilization under canopies of *A. karroo* is unknown.

Rundel *et al* (1982) found that soil under *Prosopis glandulosa*, a nitrogen fixing legume, had extremely high nitrate concentrations under these canopies with levels of 253 $g.m^{-2}$ compared to 55 $g.m^{-2}$ for soil nitrate between canopies. Ammonium concentrations were only 5 $g.m^{-2}$ under canopies with levels of 2 $g.m^{-2}$ between canopies (Rundel *et al*, 1982). This research found the opposite effect with higher nitrate and ammonium levels in adjacent open grassland soils than for soils from under *Acacia* canopies. Charley *et al* (1977) found that ammonium levels in the surface 2.5 cm of the soil profile were lower than nitrate levels, but deeper in the profile ammonium levels tended to be equal to or greater than nitrate levels. Ammonium concentrations in this study were consistently higher than corresponding nitrate concentrations at all soil depths whether in the open or under *Acacia* canopies. This is possibly a reflection of soil microbial patterns but there is no data from the study site to verify this. It has been known that grasslands tend to have higher

ammonium levels and forests higher nitrate concentrations (Scarsbrook, 1965) but whether this explains the differences in ammonium and nitrate concentrations is uncertain.

4.5 Conclusion

The study site is a savanna mosaic of *Acacia*-dominated thickets and clumps interspersed with grassland. Soil nitrogen levels were quantified from under *A. nilotica* and *A. karroo* canopies and from interspersed open grassland sites. These results on soil nitrogen concentrations under canopies of nitrogen fixing leguminous trees compares favourably with those of other researchers (Garcia-Moya *et al*, 1970; Rundel *et al*, 1982; Stock *et al*, 1995). Soil total nitrogen and organic nitrogen was higher under canopies of *A. karroo* and *A. nilotica* then under adjacent open grassland. There was a decline in soil nitrogen content with increasing depth but the depth at which there was little difference between open sites and soil under canopies was only 20 cm deep compared to 90 cm recorded by Garcia-Moya *et al* (1970). Soils at the study site are relatively shallow (<40 cm) so most of the root mass is concentrated close to the surface. *Acacia karroo* had higher soil nitrogen levels at the 0 -10cm zone compared to *A. nilotica*. Whether this was a function of increased litterfall, quality of litter, increased fine root mass, increased nodulation and nitrogen fixation in the surface layers or to increased microbial immobilization under canopies of *A. karroo* is unknown. No pattern could be discerned that

related tree size as a function of basal stem area to soil nitrogen levels.

Patterns for inorganic nitrogen were different to those reported from for other studies (Charley *et al*, 1977; Rundel *et al*, 1982). Ammonium concentrations in this study were consistently higher than corresponding nitrate concentrations at all soil depths whether in the open or under *Acacia* canopies. This is possibly a reflection of soil microbial patterns but there is no data from the study site to verify this.

Chapter 5 CONCLUSIONS OF STUDY

5.1 Introduction

At the beginning of this study, I formulated a number of research aims. These aims had two main thrusts. The first involved conducting a green house experiment in which five species of *Acacia* were inoculated with *Rhizobium* bacteria and grown in acid washed, nutrient free, quartz sand. The purpose of this experiment was to determine if inoculation of *Acacia* with cultured *Rhizobia* could be successfully achieved and if it could improve the nitrogen status of these species over uninoculated controls. The second major direction of this study was field related and involved quantifying the nitrogen in soil, water and plant components of the nitrogen cycle. This involved field sampling with laboratory analysis for nitrogen content.

5.2 Green house experiments

The results of these experiments showed that inoculation with cultured *Rhizobium* under controlled conditions significantly enhanced the growth and nitrogen status of all five species tested, namely *Acacia karroo*, *A. nilotica*, *A. robusta*, *A. sieberana* and *A. tortilis*. Root lengths in uninoculated controls were longer than that in corresponding inoculated treatments due probably to a greater need to find a nitrogen source. Inoculated plants had improved total plant mass, root

mass, shoot mass and length, acetylene reduction activity, nodule mass and number of nodules per plant compared to the uninoculated control plants. Furthermore, inoculated plants showed higher percentage nitrogen contents compared to their respective controls in both shoots and roots. These results are in agreement with results reported by other workers in different countries and continents working on different species of *Acacia* (e.g. Felker *et al*, 1980; Hansen *et al*, 1987; Umali-Garcia *et al*, 1988; Galiana *et al*, 1991; Ndoye *et al*, 1995). It was difficult to determine which species had the most improved growth after inoculation. This was a result of the difficulty in separating different natural patterns of growth between the five species of *Acacia* from the effects of inoculation.

5.3 Stem and leaf nitrogen from field plants

Two species, namely *Acacia karroo* and *A. nilotica*, were sampled in the field. Nitrogen levels in leaves showed little difference between the two species, but stem nitrogen in *A. karroo* was significantly higher than that in *A. nilotica*. Stem nitrogen values compared favourably with results from *Acacia gregii* in a Mojave desert wash (Garcia-Moya and McKell, 1970), but leaf nitrogen in the two Southern African species was considerably higher than corresponding values for *Acacia gregii*. A comparison of values obtained in this field study with values obtained in the greenhouse trials show that field plants of both species had values lower than inoculated

greenhouse plants but higher than uninoculated control plants from the greenhouse experiments. This suggests that levels of symbioses are lower in the field than those obtained in the green house experiments.

5.4 Nitrogen movement from the canopy of field plants to the soil surface.

Other studies (Carlisle *et al*, 1966; Gosz *et al*, 1972) have shown that litterfall may contribute a large proportion of the nutrients being cycled. My study was however unsuccessful in collecting fresh litter in quantities large enough to be able to carry out analysis and experimentation. As a result I focused on inorganic nitrogen input in rain as well as that in throughfall and stem flow for two species: *A. karroo* and *A. nilotica*. Openfall accounted for half the volume of rain fall collected. *Acacia karroo* had higher volumes of stemflow than throughfall and *A. nilotica* higher throughfall than stemflow. This is possibly due to differences in tree architecture and bark texture between the two species (see Chapter 3.2.3). No relationship was found between basal stem size and quantity of nitrogen leached from the canopy.

Acacia nilotica had higher quantities of ammonium and nitrate in both stemflow and throughfall than was found in openfall. The concentration of both ammonium and nitrate was higher in stemflow than in throughfall from *A. nilotica*. *Acacia karroo* showed a different pattern to that of *A. nilotica*.

Concentrations of ammonium in both stemflow and throughfall from *A. karroo* were lower than ammonium concentrations in openfall. Nitrate concentrations in stemflow and throughfall under *A. karroo* were greater than values in openfall but were similar to values for throughfall and lower than values for stemflow from *A. nilotica*. The reasons for the very low concentrations of ammonium under *A. karroo* are uncertain but may be a result of canopy uptake or microbe uptake on canopy surfaces. When concentrations were translated into an estimate of mass of nitrogen input per hectare per annum, it was found that results for this study were considerably higher than those obtained in other studies (Eaton *et al*, 1973; Langkamp *et al*, 1982). The reasons for this are presently unknown.

5.5 Soil nitrogen under two species of *Acacia*

Results showed that total soil nitrogen and organic nitrogen decreased significantly with increasing depth both under *Acacia* canopies and in soils from adjacent open grassland sites. Total soil nitrogen and organic nitrogen was significantly higher under canopies at all depths in the profile than at corresponding depths in adjacent open grassland sites. Furthermore, soils under *Acacia karroo* had higher total nitrogen and organic nitrogen at all depths than values found for *A. nilotica* at corresponding depths in the soil profile. These results compare well with some other studies (Garcia-Moya *et al*, 1970; Rundel *et al*, 1982; Stock *et*

al, 1995)

Patterns of ammonium and nitrate in the soil profile differed from those of total and organic soil nitrogen. Both ammonium and nitrate had similar values for all depths in the soil profile. Ammonium concentrations were higher than nitrate concentrations at most sites and all depths in the profile. Grasslands had higher concentrations of ammonium and nitrate at all depths compared to soils from under canopies of *A. nilotica* or *A. karroo*. Rundel *et al* (1982) found a different pattern of inorganic nitrogen distribution with very high levels of nitrate under canopies of *Prosopis glandulosa* compared to lower levels in adjacent open lands. Rundel's values for nitrate are much higher than values of soil nitrate measured in this study. Levels of ammonium were much lower than nitrate levels under *Prosopis* (Rundel *et al*, 1982).

5.6 Discussion

Results from the field site show that *Acacia* trees have a major impact on the nutrient economy of their environments. Soil pools of organic nitrogen under *Acacia* canopies are much higher than are levels in surrounding grasslands. Whether this is a result of symbiotic nitrogen fixation or a function of higher structural turnover (Leaves, stems, flowers and fruit) by tree canopies over grassland species is presently uncertain. The low incidence of nodules in the soil at the field site suggests that symbiotic nitrogen fixation may not

be important possibly due to higher levels of nitrogen in soils under canopies. The trees studied at the field site are of uncertain age but were all well established with basal stem sizes ranging from 3.5 cm diameter to greater than 25 cm diameter. The high infection rates and nitrogen fixing activity documented in the green house experiments suggest that symbiotic fixation may be important in seedling establishment (Hansen and Pate, 1987b; Hansen *et al*, 1987). The low levels of organic and total nitrogen in grasslands do perhaps support this hypothesis, although the higher levels of available inorganic nitrogen (ammonium and nitrate) found in grasslands in this study might confound this hypothesis by suppressing nitrogen fixation in the field (Hopmans *et al*, 1983; Hansen and Pate, 1987b).

5.7 Potential use of species of *Acacia* in soil and nutrient rehabilitation.

The high levels of shoot nitrogen documented for inoculated plants in the green house experiment suggest that these species may be useful in rehabilitating nutrient-depleted soils. Also, these species were grown in zero nitrogen conditions but still resulted in shoot nitrogen levels higher than those found in the field. Australian studies have produced good evidence that species of *Acacia* are useful in increasing soil nitrogen pools in nutrient depleted mine spoils (Langkamp, Swinden and Dalling, 1979; Barnet, Catt and Hearne, 1985). The rehabilitation time spans may be slower

using indigenous *Acacia* species rather than pasture legumes (Langkamp *et al*, 1979). This may be offset by indigenous *Acacia* species such as those in this study helping to re-establish natural successional patterns. Other evidence for the use of these *Acacia* species in rehabilitation include the increased levels of organic soil nitrogen under *Acacia* canopies when compared to adjacent open grasslands.

5.8 Future considerations

A number of authors have constructed nutrient budgets for various communities (Sollins *et al*, 1980; Langkamp *et al*, 1982; Risser *et al*, 1982; Rundel *et al*, 1982; Hansen *et al*, 1987). These have generally been for stands or communities of a relatively homogenous species composition with turnovers measured on a per hectare basis. In a typical-*Acacia* dominated vegetation type, this would be difficult because of high species and structural heterogeneity. As a result it would be better to construct nutrient budgets on a per plant basis with turnovers measured by square meters. A nutrient budget constructed in this way would be easier to adapt to different plant densities and age structures. In this study, different age trees have been identified on a basal stem area and the necessary sampling equipment to sample nitrogen movement between canopies and soil is already present. The techniques of sampling have been tested (apart from litter fall). More trees in the larger basal stem diameter sizes would have to be identified to suitably sample three main

size-age classes. Measurements of soil nitrogen have been successfully performed as well as plant nitrogen status. By increasing the data sets and sampling carefully over time, a nutrient budget could be constructed for nitrogen turnover around two species of *Acacia* tree. Such a nutrient budget would greatly aid in accurately predicting rehabilitation results when using these two species of *Acacia*.

5.9 Conclusion

Five species of *Acacia* were inoculated with cultured *Rhizobia* bacteria. It was found that this treatment significantly enhanced nitrogen content and growth rates compared to uninoculated controls. Plant nitrogen content was measured for two species of *Acacia* in the field. It was found that there were only small differences in stem nitrogen and leaf nitrogen between the two species in the field. Percentage nitrogen from field plants were lower than from corresponding species inoculated with *Rhizobia* and grown in a greenhouse, but higher than corresponding species grown in the greenhouse but not inoculated with nitrogen fixing bacteria.

Nitrogen inputs to soil pools as inorganic nitrogen in rainfall were measured. It was found that *A. nilotica* had higher values of nitrate and ammonium in throughfall and stemflow than in open fall. *Acacia karroo* had comparable levels of nitrate to *A. nilotica* but lower levels of ammonium than either *A. nilotica* or values in openfall. Soil nitrogen

pools showed significantly higher values of total and organic nitrogen under canopies of *Acacia* but lower values of nitrate and ammonium under canopies than in open adjacent grasslands. It was concluded that these species could be used for rehabilitating nutrient-depleted soils. Also, by increasing the size of the data sets, a nutrient budget for nitrogen flow around *Acacia* trees could be constructed. Such a nutrient budget would greatly enhance predictions related to rehabilitation of nutrient-depleted soils.

Chapter 6 APPENDIX

6.1 Modified Hoaglands nutrient solution

This nutrient solution was modified from Epstein (1972) so that no nitrogen was present.

Compound	Concentration of Stock Solution g/Liter	Element	Final concentration of element ppm
KH_2PO_4	136.09	K	235
		P	185
$CaCl_2$	110.98	Ca	160
$MgSO_4.7H_2O$	246.49	S	32
		Mg	24
KCl	3.728	Cl	143.6
H_3BO_3	1.546	B	0.27
$MnSO_4.H_2O$	0.338	Mn	0.11
$ZnSO_4.7H_2O$	0.575	Zn	0.131
$CuSO_4.5H_2O$	0.125	Cu	0.032
H_2MoO_4	0.081	Mo	0.05
Fe-EDTA	6.922	Fe	1.12

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